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IN VITRO PHYHOTOXICITY OF SILVER NANOPARTICLES IN COMMON FORAGE PLANT MEDICAGO SATIVA

HALA **M**UDHAFAR **H**AMID[*], **B**ELAN **M**OHAMMED **K**HALIL[**] and **A**THEEL **N**AJIB **Y**OUSEF[***]
[*]Ministry of Education, Kurdistan Region-Iraq
[**]Scientific Research Center, College of Science, University of Duhok, Kurdistan Region-Iraq
[***]Dept. of Biology, College of Science, University of Duhok, Kurdistan Region-Iraq

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ABSTRACT

Silver nanoparticles (AgNPs) are widely used in commercial products, and there are growing concerns about their impact onthe environment. the leakage of AgNPs in to water ecosystem could have consequential on plants through irrigation agricultural fields. Therefore, understanding some adverse effects of nanoparticles in forage crop plants is a matter of importancebecause nanoparticles are often released into soil environments. In current study, Medicago sativa, a common forage plant is used to investigate phytotoxic effect of AgNP on such plant. Preliminary results showed that higher concentrations (5, 10. 20 mg/L) of AgNP has stimulatory effect,compared to lower concentrations (0.2, 0.4, 0.8 mg/L),on several growth parameters. Among all, 10 mg/L of AgNPs shown to have most stimulatory effect on root weight, length and lateral root number. Shoot parameters appeared to be not affected by either high and low dosage of AgNP. Low concentration of AgNP combined with plant growth regulators (PGR) highly induced callus induction in either leaf or stem explants compared to control, while higher concentrations of AgNP showed induced regeneration capability in both leaf and stem explants with no or least callus induction. AgNO3 is used as a source of silver ion having two dimensions to compare with the three-dimensional AgNP. Therefore, the details regarding effect of AgNO3 is discussed in result section of this article.

***KEYWORDS**:* Silver nanoparticle, Medicago sativa, Phytotoxicity, Callus Induction

INTRODUCTION

Nanotechnology applies engineered nanomaterials (ENMs) of less than 100 nm (Prabhu and Poulose 2012). ENMs have been extensively studied due to their unique physiochemical properties (Murray, Kagan et al. 2000, Geisler-Lee, Qiang et al. 2013). These unique properties allow ENMs to become indispensable to society (Salata 2004). In the recent years, ENMs have been widely used in different sectors of industry; however, the concern of their safety to society and adverse effects in environments have been getting recognized (Barrena, Casals et al. 2009), especially these released ENMs (to society) may have different properties from their bulk counterparts (Geisler-Lee, Qiang et al. 2013). Among all ENMS, silver nanoparticles (AgNP) are one of the most commonly used in medicine, textiles, cosmetics, and electronics due to their versatility characteristics (Navarro, Piccapietra et al. 2008, Barrena, Casals et al. 2009, Gambardella, Costa et al. 2015). Nevertheless, these AgNPs have been found in waste water and biosolids and potentially affecting both aquatic (Navarro, Piccapietra et al. 2008, Gambardella, Costa et al. 2015) and terrestrial life (Choi and Hu 2008, Ahamed, AlSalhi et al. 2010, Singh and Kumar 2015). Thus, understanding toxicological effects and underlying mechanisms of AgNPs and how to balance their versatility properties and potential downstream adverse effects are critical in the ecosystem with plants, animals and microbes.

The increased applications of AgNPs lead to increased risks of releasing such particle in to the environment, which differ from their bulk counterpart (Geisler-Lee, Qiang et al. 2013). Studying the interaction of AgNPs with autotrophic compartment of food chain is critical, due to uncertainty of the aggregates and

agglomerates, unlike the ionic form of Ag (Mirzajani, Askari et al. 2013). As the bactericidal properties of AgNPs were size, shape and concentration dependent, the same aspect is applying for their phytotoxic properties. (Geisler-Lee, Qiang et al. 2013) reported AgNPs sizes 20 and 40 nm showed increased toxicity to Arabidopsis roots than 80 nm Ag NPs in lower concentrations. However, in high concentrations, 267.73 and 534.72 mg/L, all AgNPs sizes exhibited similar phytotoxic effect. Another study by (Lee, Kwak et al. 2012) demonstrated increased phytotoxicity of AgNP in *Phaseolus radiatus* and *Sorghum bicolo*r in a dose dependent manner in agar medium. Interestingly, accumulation of AgNPs in root tissues were increased rapidly as the exposure concentration raised, however, accumulation of AgNPs in shoot were not significant. The authors revealed sensitivity of *P. radiatus* to AgNPs to greater degree than *S. bicolor*. Seedling growth of P. radiatus decreased down to 20%, and 47% in S. bicolor, when seedlings treated with 40 mg/L, indicating that concentration dependent manner could be species dependent too. The results shown in this study confirms the importance of the type of media and its effect on toxicity of AgNP on plants, and it recommends, unequivocally, to use real soil in studying phytotoxic effects of AgNP to have real understanding about terrestrial toxicity of AgNPs.

Shape dependent phytotoxicity of AgNPs were also recorded.(Syu, Hung et al. 2014) demonstrated differential phytotoxic effects of morphologically differed AgNPs. The authors used decahedral, triangular and spherical AgNPs with different concentrations. The results shown that triangular and decahedral AgNPs enhanced seedling growth with decahedral one resulting highest degree of root growth promotion, and lowest degree oxidative stress. On the other hand, spherical AgNPs showed no change in seedling growth, however, inhibited cotyledon growth and promoted anthocyanin accumulation in root tissue a sign of abiotic stress existence, therefore, causing highest degree of oxidative stress.

In current study we investigate the effect ofAgNP on certain growth parameters, biomass formation, callus induction and plant regeneration of Medicago sativa commonly known as alfalfa, a fast-growing common forage plant used as fodder for livestocks.

MATERIALS AND METHODS

Silver nanoparticles and characterization

Silver nanoparticles were obtained from Ted Pella Inc., Reading, CA, USA (NanoXactTM line, average size 20 nm, 4.50x 10^{11} particles/mL and zeta potential at -50 mV range). The silver colloid was kept at 4 C in total darkness (covered by aluminum foil).

Plant material

One year old Seeds of Medicago sativa (alfalfa) were donated by School of Agriculture at University of Sulaymaniyah was used. The seeds were tested for viability and germination rate in Petri dishes by sowing 20-25 seeds on filter paper saturated with deionized distilled water. The filter paper maintained humid enough to promote seed germination and avoid desiccation. Seeds were superficially sterilized with sodium hypochlorite (bleach) (5%) solution. (1957, Fahraeus) for 8-10 minutes then washed with sterile distilled water three times to get rid of traces of the bleach and therefore the water stuck thereto was disposed of by putting it on a sterile filter paper.

Media preparation

In current study two kinds of media were used, nitrogen free (NF) medium (Fraues, 1957) and Murashige and Skoog medium (MS 1965). The constituent of each media is mentioned in Appendix 1.0. NF media constituents prepared separately as stock solutions. The pH was adjusted at 6.5 (except for callus culture) since Medicago sativa prefer alkali environment as an optimum growth condition. MS media used as powder (4.45 g/ L) the pH is adjusted at 5.6-5.8. the media were autoclaved at 121C for 15 min on 1.5 Pi. Either medium distributed on Petri dishes and kept tilted at 30° to form a slope which promotes root growth and elongation. In case of callus culture experiments, the latter step canceled.

Plant exposure to silver nanoparticles and silver nitrate

Prior to use, AgNP solution were vortexed to ensure uniform distribution of nanoparticle then spread on the plates with MS or NF media in different concentrations (0.0, 0.2, 0.4,0.8, 5.0, 10.0, and 20.0 mg/L). to compare it with non-nano

scale AgNP, AgNO3 was used as an Ag ion source with the same concentrations of AgNP. The plates kept in room temperature to allow complete absorption of AgNP by the media. After that, seeds of *Medicago sativa* sterilized as mentioned in previous section. five seeds were sown in each plate, with three replicates for each concentration of AgNP and AgNO3. The plates maintained at 16/8 h light/dark condition at growth chamber at 24 ± 2 C for 10 days. Three biological replications were maintained for the control and each of the AgNPs concentration and the experiment was repeated twice. MS agar medium devoid of AgNPs was used as the control.

Callus induction and Plant regeneration

Twenty-one days old plantlets grown on MS media were used to induce callus cultures leaf and stem tissue of *Medicago sativa.* the above-mentioned concentration of AgNP and AgNO3 was applied on both MS and NF media supplemented with different concentration of 2,4-D and Kinetin in Petri dishes. Healthy leaves and stems were excised from the germinated plant. For each plate 6-9 explants were placed, followed sealing with parafilm to prevent contamination. Plates were maintained in growth room in total darkness, or intermittent dark and light period at 24±2 C°.

Statistical analysis

The raw data submitted to SPSS program (SPSS, 2019), and the descriptive statistics, correlation, one-way and two-way ANOVA were applied to illustrate the relationship between the studied characteristics, and also to appear the effect of different treatments and different levels on the studied characters. The means differences were separated using Duncan's multiple range test (Duncan, 1955). All curves were drawn using Excel software.

RESULT AND DISCUSSION

Effect on Seed Germination

The effect of AgNP and AgNO3 on seed germination were studied. Exposure of alfalfa seeds to different concentration of either kinds of Ag showed dose independent pathway. As shown in Fig. 1 the germination rate was negatively affected only in case of using 5 mg/l of a AgNP and AgNO3. The reduced germination rate in lowest dose was significantly differentiated only to 10 mg/L only.In another word, both AgNP and AgNO3, at all concentrations affected seed germination, however, it was only statistically significant at 10 mg/L. in the latter concentration the AgNP not only did not adversely affected seed living process, indeed it enhanced see germination. Enhancement of seed germination could be due the formation of nano holes on seed coat causing improving germination conditions. Another reason is might due to the slow-release rate of Ag ions from AgNPs which minimize the toxic effects of Ag (Parveen and Rao 2015). The germination rate parameter is not considered as critical factor to evaluate AgNP toxicity in plants.AgNP possesses hermetic properties having both positive and negative effect on living organisms(Cox, Venkatachalam et al. 2016). Arabidopsis seeds exposed to different concentrations and diameter of AgNPs were not influenced significantly at all (Geisler-Lee, Wang et al. 2013).On the other hand oat and berseem showed improved germination rate significantly compared to control treatment (Maity, Natarajan et al. 2018).For this reason, germination rate could not be suitable as a parameter to determine the phytotoxic effect of AgNP.

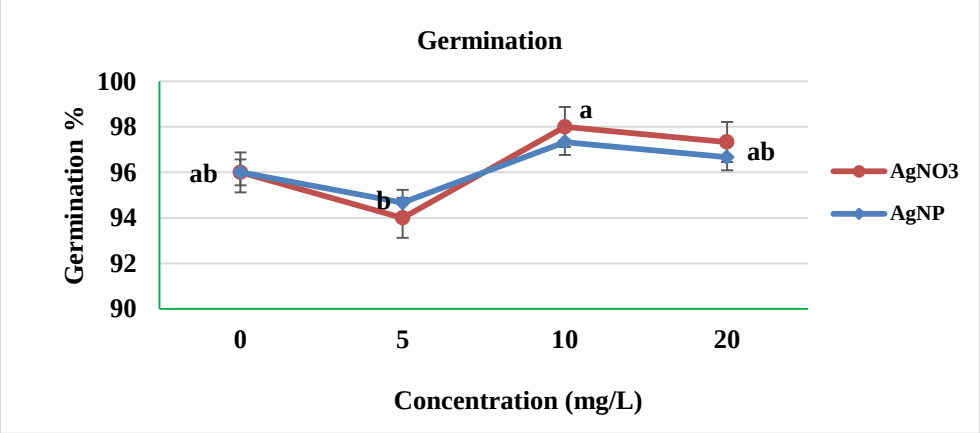


Fig. (1):- The effect of AgNP and AgNO3 on germination rate of *Medicago sativa*. Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different (P≤0.05)

EFFECT ON PLANT GROWTH PARAMETERS

Root Growth

Root is the first organ to come in contact with medium or soil, therefore the presence of any contaminant in the medium or soil directly affects its growth. In current study, root fresh and dry weight, length and lateral roots were measured to determine the effect of AgNP on root growth. Seeds exposed to AgNP showed increased root fresh weight in a dose dependent manner. The latter means increasing AgNP concentration resulted in increased fresh weight compared to control. As mentioned previously two sets of concentrations were used in this study, the low concentration (0.2- 0.8 mg/L) and high concentration sets (5-20 mg/L). As illustrated in Fig. 2, the low concentration (0.2-0.8 mg/L)of AgNP increased gradually until it reached highest point at 0.8 mg/L which was statistically significant from all other doses.While plants exposed to AgNO3 showed different pattern. Highest fresh weight was observed in plants treated with 0.8 mg/L, however, it was statistically significant only to 0.2 mg/L. Unlike low Ag concentrations, plant response to higher concentration of Ag was fluctuated (Fig. 3). The results in both treatmentswere reverse in all concentrations. While 5 mg/L of AgNP showed low fresh weight, the same concentration of AgNO3 recorded the otherwise, and this is true for the rest of the concentrations (Fig. 3). Increasing fresh weight due to exposure to AgNPs were also recorded in zucchini, corn and watermelon (Almutairi and Alharbi 2015), Indian mustard (Pandey, Khan et al. 2014)and common bean , *Phaseolus vulgaris*(Salama 2012).In contrary, in the aquatic plant *Lemnaminor* the fresh weight was negatively affected byAgNP even in very low concentration (µg/L). on the other hand, in mung bean plant *Vigna radiata* exposure of seedling to up to 10 mg/L did not show any significant effect on root growth. The deleterious effect of AgNP occurred only when the concentration raised to 20 and 50 mg/L.

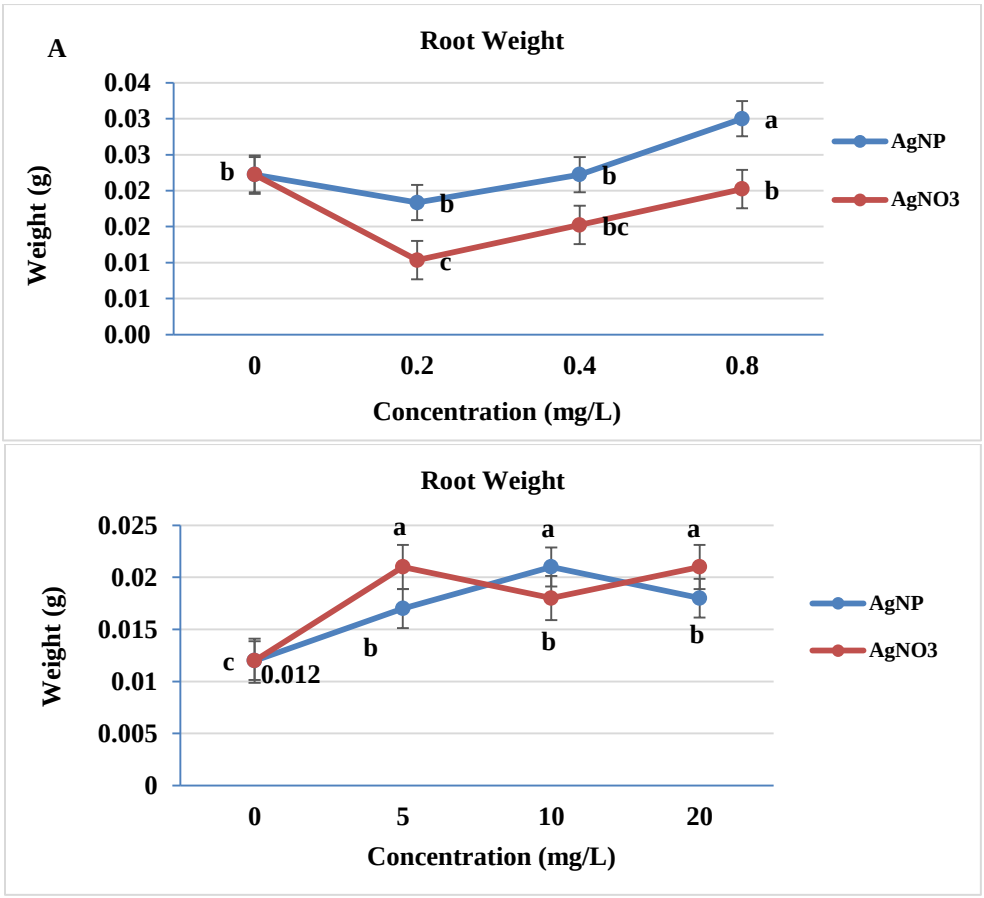


Fig.(2):- The effect of AgNP and AgNO3 on root fresh weight of *Medicago sativa***.** A) Low concentration, B) Hight concentration. Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different (P<0.05)

Root length as another criteria of root growth showed different response toward AgNP and AgNO3. Low concentration of both Ag exerted inhibitory effect on root length compared to control, and (fig. 4). In contrast, high doses of Ag stimulated root length reaching maximum length at 10 mg/L of AgNP which was statistically significant to all other treatments (fig. 5). In addition to weight and length, Plants exposed to Ag demonstrated, least to describe, different pattern of root system through inducing or suppressing lateral root formation. Low concentration of AgNPs exhibited slight change in lateral root number in all concentrations (0.2- 0.8 mg/L), which was statistically non-significant. On the other hand, root system severely affected by AgNO3. The least root numbers were recorded in plants exposed to 0.2 mg/L AgNO3 and it was statistically significant to all other treatments (Figure 6). Increasing AgNO3 concentration resulted in culminating in root numbers until it reached highest level in plants treated with 0.8 mg/L similar to that of control plants. The situation was varied during exposing seedlings to higher concentrations of Ag.All concentrations exhibited significant increase in root number compared to control. The highest lateral root number was observed in plants treated with 5 mg/L AgNO3, while its counter part in AgNP showed least root number (Figure 7), while the highest LRN in AgNP treated plants was recorded at 10 mg/L. Eventually, the LRN declined in either Ag type treated plants at 20 mg/L. the fluctuation of growth response is common in AgNP dependent studies, and it varies according to plant species, dose, size and shape of AgNP. Medicago sativa, the case of current project, is well known as common forage for cattle, therefore, the resistance of such plants toward adverse environmental conditions or pollutants might exceed or greater than those of certain crops, especially annual crops. Furthermore, the high protein content of the

seed might have additional resistant mechanism surpassing heavy metal stress like Ag. Increasing root fresh weight, length and lateral root number, despite being non significance, are observed in certain plants. *Vigna radiata* (mung bean) previously known as *Phaseolus radiatus* like *Medicago sativa* is member of legume family, showed resistance against AgNP up to 10 mg/L(Nair and Chung 2015). Since the root is in direct contact with the medium, the possibility of being affected by AgNP is greater than other parts of the plant either negatively or positively. In current study, AgNP is observed to have stimulatory rather than inhibitory effect and it was more obvious in higher concentrations. In contrast, AgNO3 is tend to have neutral or slight inhibitory effect, except in certain concentrations it is shown to have growth stimulation-like effect. This distinctive effect and changes in growth due AgNP and AgNO3 was molecularly confirmed through monitoring induction and suppression of stress proteins in either AgNP and AgNO3 (Vannini, Domingo et al. 2013). Among 22 and 43 proteins expressed by AgNP and AgNO3, only 4 proteins were overlapped between both Ag types, whereas 18 and 39 proteins were specifically expressed in each AgNP and AgNO3 treatments respectively.In both Ag types, proteins related to sulfur metabolism were significantly induced and accumulated in root tissues,particularly the jacalin lectin protein family (JAC) (Vannini, Domingo et al. 2013). These proteins are seed-specific and catalyze the hydrolysis of glucosinolates and release nitrile and sulfate (Kissen and Bones 2009). Since nitrile possesses an auxin like effect and can be converted to indole-3- acetic acid(Mithen 2001) it may stimulate root growth. Therefore, the stimulatory effect of either Ag types is due to increased levels of nitrile.

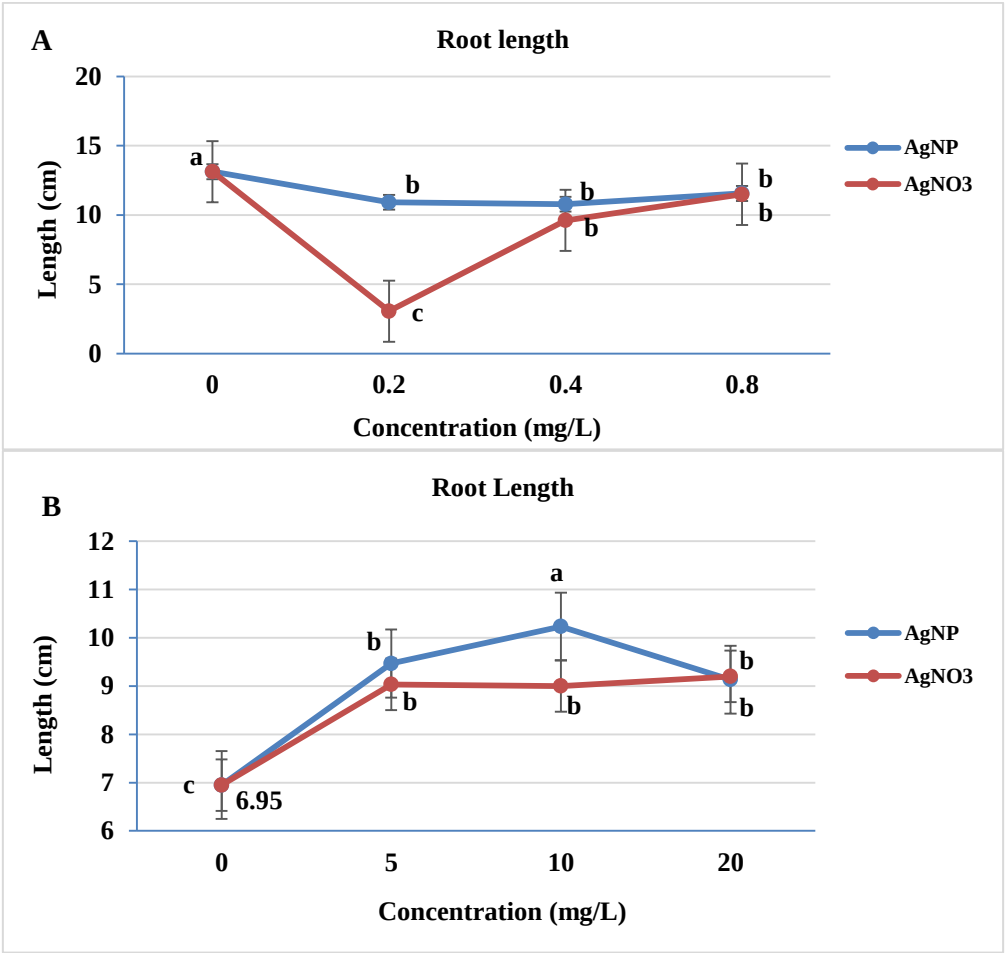


Fig.(3):- Effect of high concentration of AgNP and AgNO3 on root length of *Medicago sativa*. A) low concentration, B) high concentration of AgNP and AgNO3Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different (P<0.05).

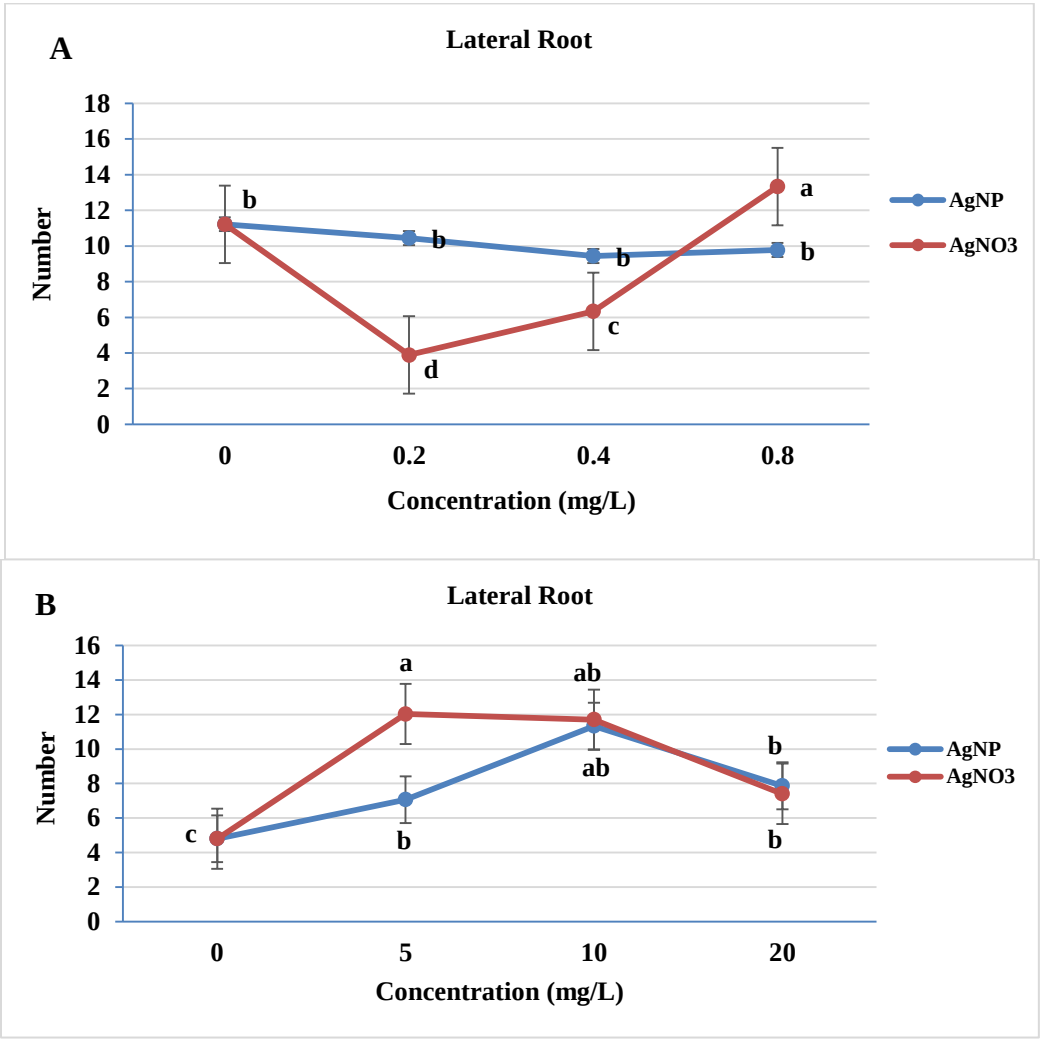


Fig. (4):- Effect of AgNP and AgNO3 on lateral root number of *Medicago sativa*. A) low concentration, B) high concentration of AgNP and AgNO3 Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different($P<0.05$)

Shoot Growth

Shoot growth parameters, which comprises both stem and leaf, were also recorded.The parameters included shoot weight and leaf number, while shoot height is not recorded since the experiment is conducted in vitro and the period of the cultivation was no more than ten days.Unlike root, not being in direct contact with the media and been covered with a cuticle layer the effect of both Ag types on shoot is expected to be comparably lower. The low concentration of both Ag types showed no significance effect on shoot weight compared to control (Fig. 5, a). As shown in the figure, there is a considerable gap between shoot weight in AgNP and AgNO3 treated plants, however, it is statistically non-significant. This due to the large standard error value in each concentration point because of the differences in shoot weight among the replication in each concentration.In higher levels of both Ag types the shoot weight was significantly increased compared to control. Furthermore, highest shoot weight was recorded in plants treated with 5 mg/L and 10 mg/L of AgNO3 and AgNP respectively (Fig 5, b). leaf number, as another parameter, showed no significance difference in either low and high doses of both Ag types (Fig 6, a and b). The minimized effect of nano and ionic silver on shoot tissue strongly correlated with chlorophyl content. In current study the chlorophyl content was not

measured, since adverse effect of either Ag types require higher concentrations as indicated in literature. In Vigna radiata, a legume plant, chlorophyll content showed no significant change in seedlings exposed up to 20mg/L. However, chlorophyll content was significantly declined upon exposure to 50 mg/l(Nair and Chung 2015). Therefore, we did not expect alterations in chlorophyll content because of the low concentration of AgNP and AgNO3 compared to previous study, and stable and increased shoot fresh weight in all concentration of either Ag types.

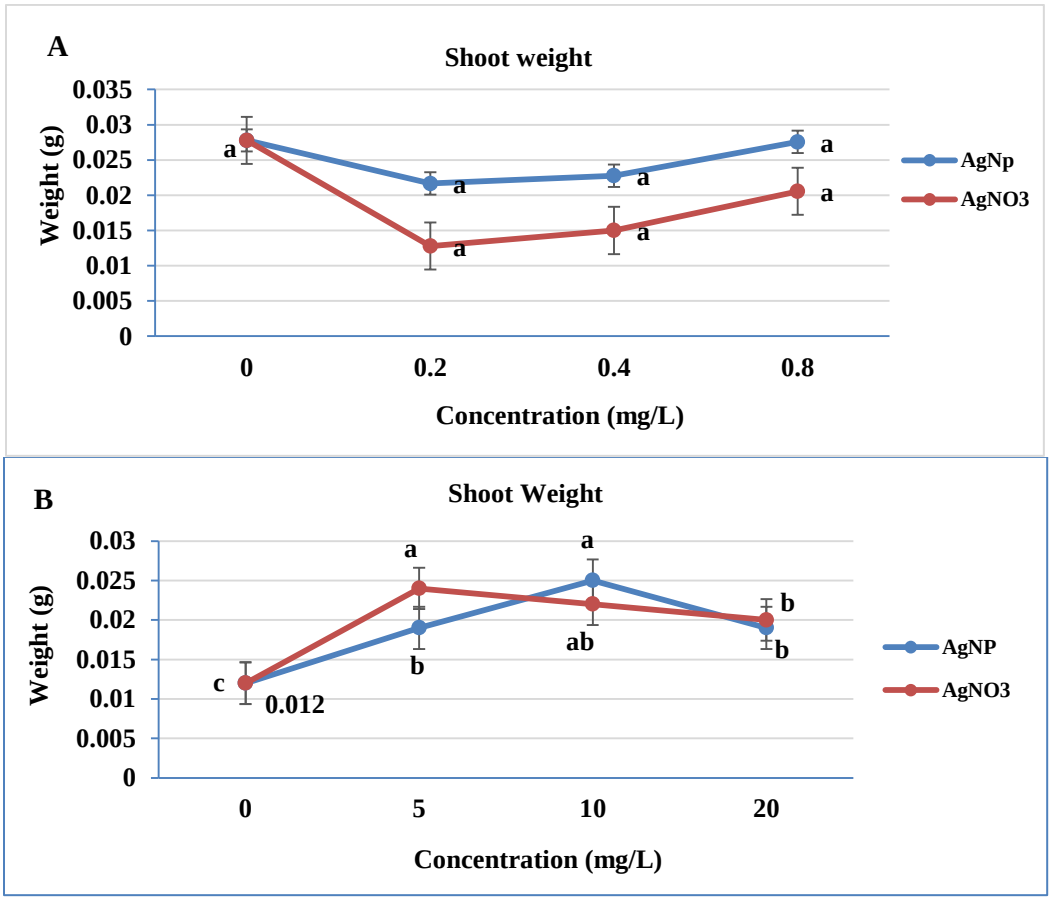


Fig.(5):- The effect of AgNP and AgNO3 on shoot fresh weight of *Medicago sativa*. A) low concentration, B) high concentration of AgNP and AgNO3.Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different (P<0.05)

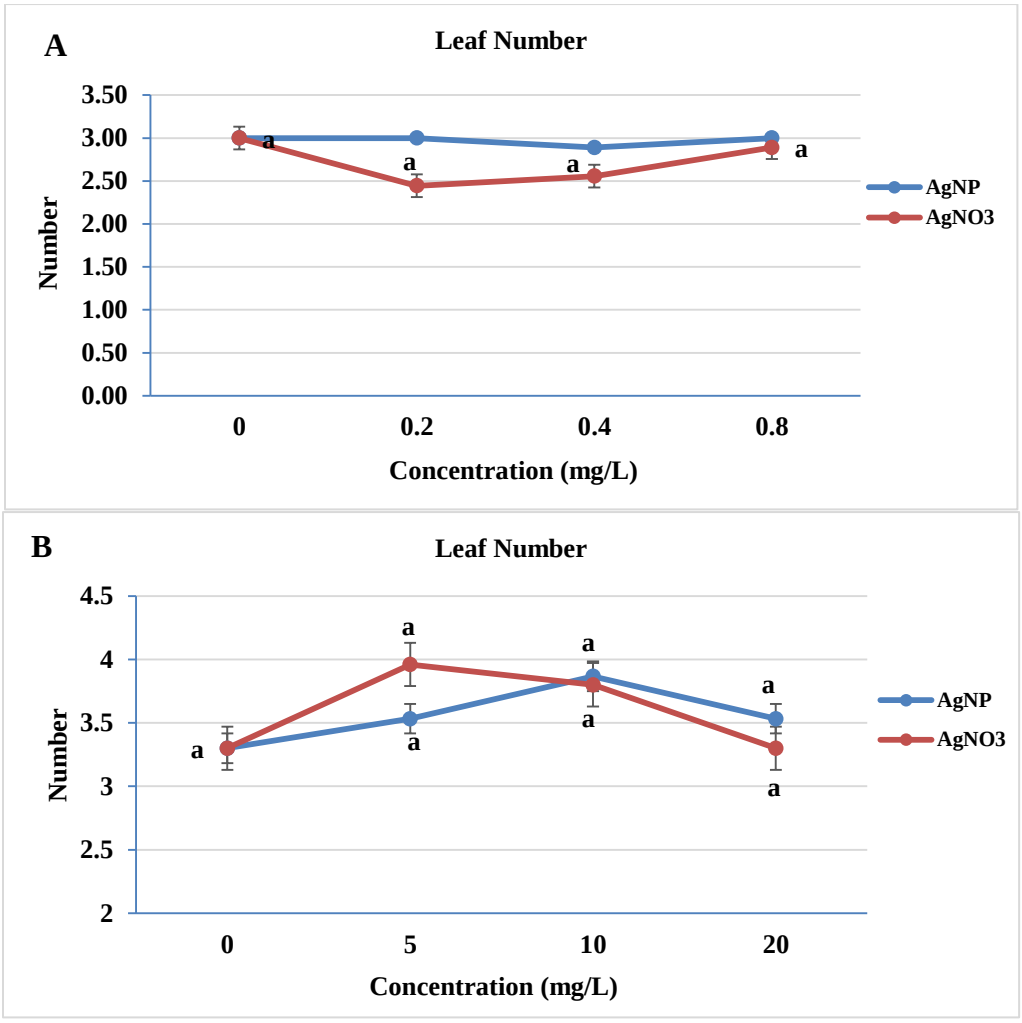
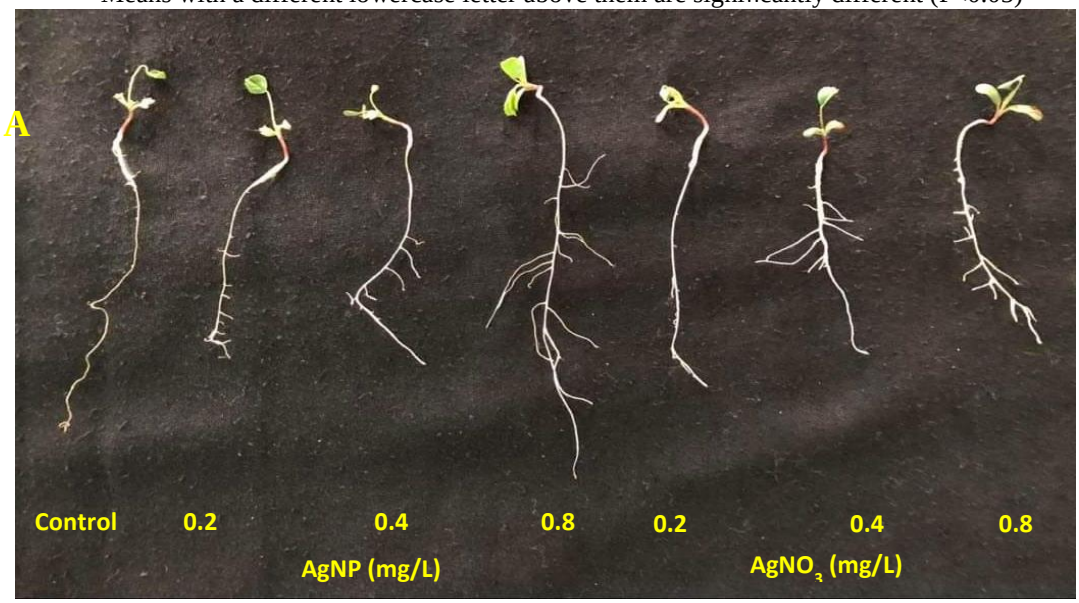


Fig.(6):- The effect of high concentration of AgNP and AgNO3 on leaf number of *Medicago sativa*. A) low concentration, B) high concentration of AgNP and AgNO3. Data are shown as the mean± SE. Means with a different lowercase letter above them are significantly different (P<0.05)



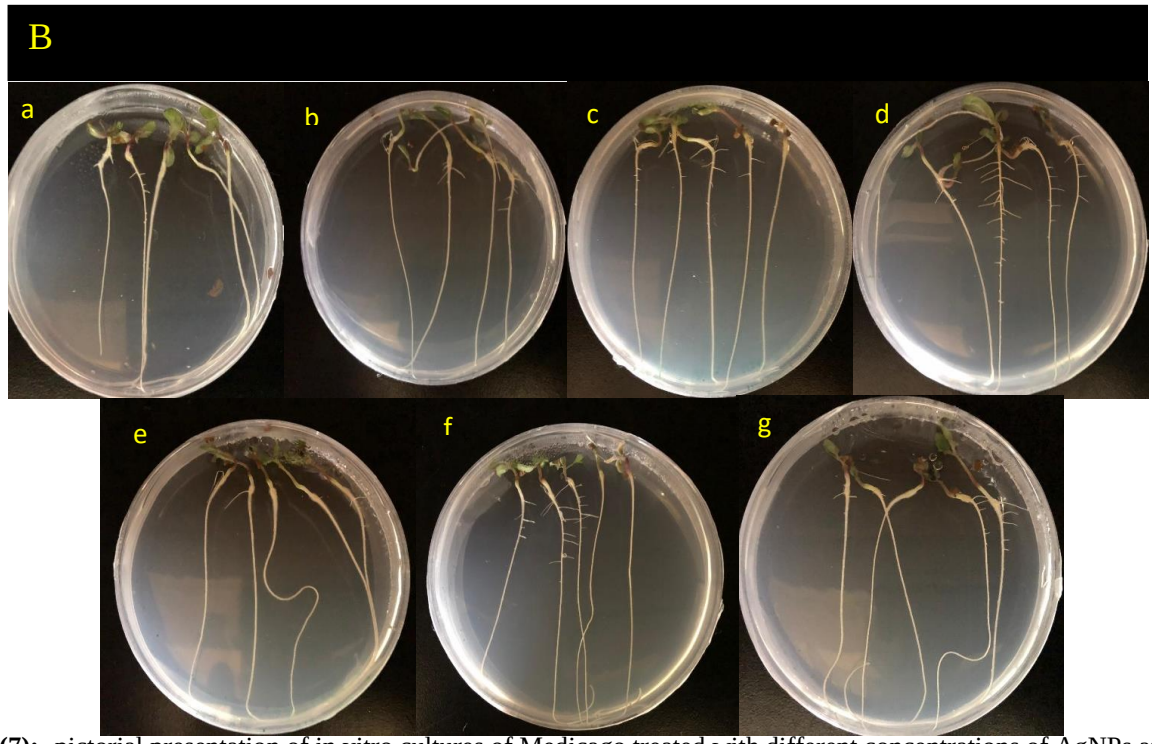


Fig.(7):- pictorial presentation of in vitro cultures of Medicago treated with different concentrations of AgNPs and AgNO3.A) seedlings treated with low concentration of AgNPs and AgNO3, and B) seedlings treated with high concentration of AgNPs and AgNO3 (a=0.0 mg/L, b-d= 5, 10, 20 mg/L AgNP, e-g= 5, 10, 20 mg/L AgNO3).

Effect on callus induction and plant regeneration

Callus is a mass of undifferentiated rapidly proliferating cells(He, Tanner et al. 1986) that can be used to study various aspects in plant biotechnology and plant genetics. In current study the effect of AgNP and AgNO3 were investigated in shoot and leaf tissue in *Medicago sativa*in MS and NF media. Same concentrations of AgNP and AgNO3 are used as in plant growth parameter experiments. Explants treated with low concentrations of both Ag types were cultivated in dark condition for four weeks then transferred to 16/8 light condition. As shown in figure 7,A, in stem the highest callus induction was recorded in explants grown in MS media supplemented with 2.0 mg/L 2,4-D and 1.0 mg/L Kin and treated with 0.2 mg/L of AgNP and it was statistically significant from all other treatments. On the other hand, the lowest callus induction was observed in explants grown MS media supplemented with 2.0 mg/L of 2,4-D treated with 0.8 mg/L of AgNP. Furthermore,explants grown in media supplementedwith the aforementioned plant growth regulators combination (2,4-D+ Kin) showed higher callus induction rate compared to the rest shoot explants grown in various 2,4-D or/and Kin combinations, regardless of AgNP concentration.This phenomenon was not observed in explants treated with AgNO3. Unlike stem, leaf tissue showed highest callus induction rate in explants treated with 0.4 mg/L AgNO3 and in identical PGR concentration (2.0 mg/L 2,4-D and 1.0 mg/L Kin) as in AgNP treated stem explants (Figure 7,B). a topographical difference between AgNP treatment in either stem and leaf callus tissue, is the presence of consistent decrease or increase in fresh weight in leaf callus tissue treated with different PGR. In another word there were no considerable gab amongcallus weight in leaf explants differentially treated with PGR (a, b, c, and d treatment as described in figure legend). In stem callus tissue, the situation was entirely distinct so that this consistency approach was observed in AgNO3 treated stem explants. Plant regeneration was not detected in either Ag treatment despite transferring callus tissues to 16/8 h light condition. This might indicate that low concentration nano and ionic silver unable to promote plant regeneration regardless to type and

concentration of PGR. In higher concentration of both Ag types, there were no signs of callus induction regardless of PGR concentration and light condition. In contrast, nano and ionic silver promoted direct plant regeneration from both stem and leaf explants. To ensure if the regeneration is due to light condition, stem and leaf explants were cultivated in two and four weeks of dark ness, then transferred to four week and two week 16/8 h light condition respectively. In either case, the results were identical and showed noticeable plant regeneration and callus induction inhibition. This indicates that either Ag type had inhibitory effect on callus induction in high concentrations.

It must be clarified that PGRs play an important role in callus induction with or withoutapplication of Ag. The auxin like growth regulator 2,4-D is widely used to induce callus initiation in various plant species (Mikami and Kinoshita 1988, Bregitzer, Somers et al. 1989).As phytotoxicity of AgNP was sufficiently investigated in different plant species, its effect on callus tissue and plant regeneration has drawn attention. AgNP is shown to induce callus initiation in 60 µg/L in *Carallumatuberculata*in combination with0.5 mg/l 2,4-D plus 3.0 mg/l BA(Ali, Mohammad et al. 2019). to concentrations in current study, is considered very low, however this might due to the nature of the plant since the *Caralluma tuberculate* is a medicinal plant. In Phaseolus vulgaris, which belongs to Leguminosae family like Medicago sativa, maximum callus fresh weight recorded using 50 mg/L(Mustafa, Oraibi et al. 2017). Fine tuning of desirable and optimal concentration of nanoparticle to induce plant cell growth to maximum level is very critical to prevent the nanoparticle toxicity related to frequent application of nanomaterials in plants (Ali, Mohammad et al. 2019). As mentioned earlier, PGR plays critical role in callus induction, the mechanism of interaction between AgNPs and PGR to enhance callus induction is not yet explored clearly.The studies about the absorption,translocation, accumulation, biotransformation and toxicity ofNPs are mostly contradictory(Ma, Geiser-Lee et al. 2010).However, the speculation is that AgNPs mutilates the cell wall leading to enhanced nutrient and water uptake by plant cells form culture medium(Ali, Khan et al. 2018). Another reason is may due to alteration in gene expression of certain genes that regulate auxin level which might induce or suppress callus induction and plant regeneration. Transcriptomic study revealed an overlap of 15 upregulated and 29 downregulated genes due to AgNP and AgNO3 respectively(Kaveh, Li et al. 2013). This observation may suggest that the effect of AgNP on callus induction is partially due to release of Ag ion from AgNP as was investigated by other scholars (Dimkpa, McLean et al. 2013, Wang, Koo et al. 2013). However, certain effects of AgNP is due to its three dimensional form since it enhance the expression of a gene encodes for MLP that responds to wounding (gene ID:AT2G01520) (Kaveh, Li et al. 2013).

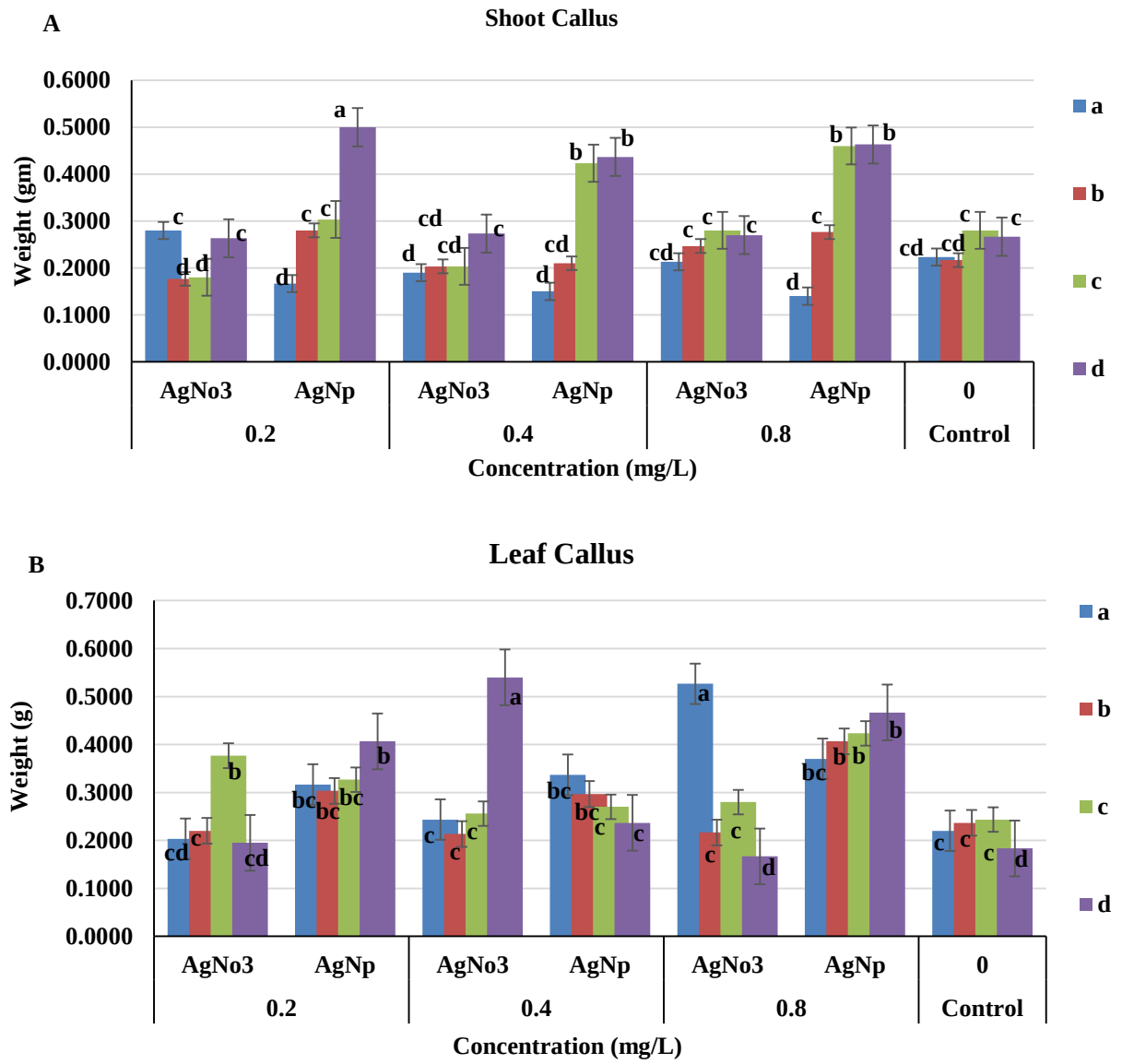


Fig.(7):- Effect of AgNP and AgNO3 on callus induction in *Medicago sativa*. A) Shoot callus, B) Leaf callus.Blue barsrepresent treatments treated with 2.0 mg/L 2,4-D, orange, gray, and yellow bars represent treatments treated with 2.0 mg/L 2,4-D+ 0.25 mg/L Kin, 2.0 mg/L 2,4-D + 0.5 mg/L Kin, and 2.0 mg/L 2,4-D+ 1.0 mg/L. Data are shown as the mean± SE and are derived from five independent trials. Means with a different lowercase letter above them are significantly different (P<0.05)

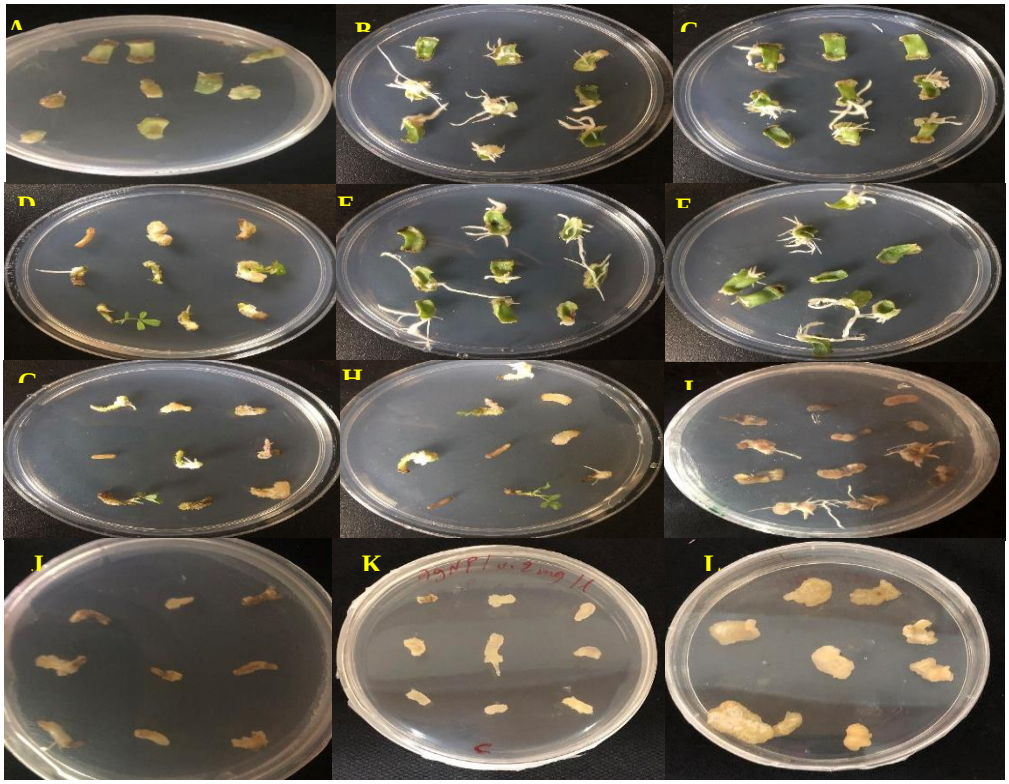


Fig.(8):- Pictorial presentation of Callus induction and plant regeneration in leaf and stem explants treated with AgNP and AgNO3. A-F, explants grown on MS media supplemented with 2.0 mg/L 2,4-D+1 mg/L Kinand treated with high concentration (0, 5, 10, 20 mg/L) of AgNP and $AgNO_3$ and cultivated underdark and 16/8 h light conditionsfor two and four weeks respectively.
G-I explants cultivated underdark and 16/8 light conditions for four and two weeks respectively.
J-L explants grown on MSmedia supplemented with 2.0 mg/L 2,4-D+ 1 mg/L Kin and treated with low concentrations AgNP and AgNO3.

CONCLUSION

Medicago sativa is an important forage crop plant used as fodder for cattle due to high protein content. Understanding the effect of AgNPs which considered as novel stress in critical. The spherical 20 nm AgNP showed stimulatory effect on the majority of growth parameter of the plant. Furthermore, AgNP induce callus induction and plant regeneration depending on the concentration of nano silver. Theincreased growth parameter upon exposure to AgNP cannot be considered as a positive effect, since AgNP is toxic to living organisms. The increase in root weight or length might be considered as a strategy to overcome the stress caused by AgNP. Therefore, future investigations in required to test the toxicity effect on subsequent generations *in vivo* to mimic the natural environment of the plant.

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