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investigation of effects of phytosynthesized and mycosynthesized based silver nanoparticles on the growth of maize

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ABSTRACT

Effects of phytosynthesized and mycosynthesized based silver nanoparticles on plant growth parameters such as shoot and root lengths, leaf surface area, chlorophyll, RWC, MSI, SSC, carotenoid, SOD and POD contents of corn (*Zea mays* L.) was probed in the present research. The study was carried out with three replications. 4 dose of silver nanoparticles (20, 40, 60 and 80 ppm) were used. After germination, daily supply with 15 ml from each concentration was carried out for 12 days during plant growth. The results showed that small dose of silver nanoparticles enhanced the growth, while the higher dose induced the inhibitory effect. However, 40ppm of phytosynthesized NPs showed best results on increasing the shoot and root lengths, leaf length, chlorophyll, carotenoids SOD and POD contents of the tested crop plants while mycosynthesized showed best results at 60ppm dose on all the growth parameters as compared to the control. The present study illustrate the effects of phytosynthesized and mycosynthesized silver nanoparticles on maize growth parameters.

Keywords: Phytosynthesized NPs, mycosynthesized NPs, silver NPs, dose.

INTRODUCTION: Agriculture provides human food, bio-energy and pharmaceuticals and 40% of the earth's land surface (Power, 2010). The current agriculture situation in Pakistan presents pressing challenges and effects economic infrastructure especially in poor economies, the increasing population gives rise to food security issues, its safety and security infrastructure should be designed and implemented to provide food more securely (Akhtar, 2015). Agriculture needs beneficial ties to increase its productivity using modern technology (Hussain *et al.*, 2015). Nanotechnology being modern technique has proved its area in agricultural sciences and related industries, as an interdisciplinary generation and a pioneer in resolving problems. The use of nanotechnology in agriculture and forestry will probably have environmental benefits. Nanotechnology has supplied new solutions to troubles in vegetation and food technology and gives new methods to the rational selection of uncooked substances, or the processing of such substances to beautify the best of plant products (Mousavi and Rezaei, 2011). Nanobiotechnology is a exceedingly interdisciplinary discipline of research and is based on the cooperative efforts of chemists, physicists, biologists, scientific doctors and engineers (Prasanna and Hossain, 2007). Maize (*Zea mays*), also known as corn belong to the family Poaceae (Esen, 1987). It is one of the world's leading cereal grains along with rice and wheat. It is a tall annual diploid plant with 10 chromosomes (x = 10, 2n = 20). Maize is highly photosynthetic-efficient C4 grass. It is estimated through genetic analysis that maize has been cultivated about 9000 years ago (Tenaillon and Charcosset, 2011). Silver is a metal known for its broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, fungi, protozoa, and certain viruses. Generally, nanoparticles are prepared by a variety of chemical and physical methods, which are not environmentally friendly. Nowadays, green chemistry procedures using plant extracts for the synthesis of nanoparticles are commonly used (Muthuraman *et al.*, 2019). Silver nanoparticles (AgNPs) have been recently used as promising agents against multiple-drug resistant microorganisms. Silver nanoparticles are obtained via a Phytosynthesis approach. AgNPs are an efficient antimicrobial mediator acts against a variety of harmful pathogens, as well as a variety of chemical and biochemical methods being surveyed for its production (Madakka *et al.*, 2018). Fungi are more advantageous because the fungal mycelial mesh can withstand flow pressure, agitation and other conditions in bio-reactors or other chambers compared to plant material sand bacteria. They are fastidious to grow, easy to handle and synthesize easily (Balakumaran *et al.*, 2016). Mycosynthesis deal with an energy-saving and eco-friendly process intended for extracellular synthesis of AgNPs, by means of cell-free filtrates of fungi *Aspergillus niger* and *Fusarium semitectum* as reducing agents. When this process is being proceed then there will be the reduction of metal during the reaction of the formation of the nanoparticles (Madakka *et al.*, 2018).

OBJECTIVES: The present study was planned to study the effects of phytosynthesized and mycosynthesized based Ag nanoparticles on maize.

MATERIALS AND METHODS: The present study was conducted in Plant Biotechnology Lab, Department of Botany, PMAS-Arid Agriculture University Rawalpindi, to check the effects of phytosynthesized and mycosynthesized silver nanoparticles on maize. Seeds of maize variety i-e Sargoda 2005 were obtained from National agriculture Research Council (NARC) Islamabad. Silver nanoparticles were synthesized in Plant Biotechnology Lab, Department of Botany, PMAS-Arid Agriculture University Rawalpindi, by using silver nitrate ($AgNO_3$) which is the most frequently used salt in the synthesis of AgNPs. Phytosynthesis of silver nanoparticles was done by using the extracts of fresh and healthy leaves of lemon grass (*Cymbopogon citratus*). where as, mycosynthesis of silver

nanoparticles was done by using fungus *Aspergilus falvus.* The extracts were utilized for the successful formation of silver nanoparticles. Extracts of fungus and plant act as reducing and stabilizing agent for synthesis of AgNPs. For the preparation of plant extract we took 20g of fresh and healthy leaves of lemon grass were taken from the university field in a beaker. Then the leaves of lemon grass were cut into small pieces and rinsed with tap water twice then followed with distilled water in order to remove all the remains of unwanted particles such as dust and dried leaves. Transferred the washed leaves into 100ml of distilled water in a 500ml beaker and boil it for 10-20 minutes in oven. After that let the extract cooled down and filtered it through Whatman no.1 filter paper thrice to obtain the clear solution and then was refrigerated at 4°C in measuring flask for further use. These optimum conditions were maintained at each step to prevent contamination in the experiment for accuracy in the results (Ajayi and Afolayan, 2017). For the successful formation of silver nanoparticles different parameters were optimized i.e. pH, temperature, salt concentration and extract concentration. Best suited pH for AgNPs is 7 (neutral). AgNPs synthesis can be carried out above 40°C and even below 25°C. Different concentrations of salt can be utilized ranging from 1-10mM. Among different concentration 1mM salt concentration showed the best response for the synthesis of silver nanoparticles. Prepared 1mM silver nitrate salt solution for which 0.17 g of silver nitrate salt was dissolved in 1 liter of distilled water. Prepared plant extract was then treated with salt solution at room temperature in the ratio 1:4 to obtain the maximum number of nanoparticles. pH was maintained upto 8. The solution was kept in incubator at 37°C for 24 hours. Colour of solution changed from transparent to light yellow then to dark brown was the indication of formation of silver nanoparticles (Masurkar *et al.*, 2011). The solution was then filled in the falcon tubes. 50ml of solution was prepared in each falcon tube and was centrifuged at about 3000 rpm for 10 minutes. Supernatant was discarded and pellets were collected in petri plate and washed with methanol to remove the impurities from the nanoparticles then dry the nanoparticles at 100°C for 24 hours. The dried nanoparticles were in kept in appendrof tubes for further use. The resulting AgNPs were characterized as well as used for the assessment of germination and growth of maize plant (Ajayi and Afolayan, 2017).

For mycosynthesis silver nanoparticles were also synthesized in Plant Biotechnology Lab, Department of Botany, PMAS-Arid Agriculture University Rawalpindi. Silver nitrate salt was used for synthesis of AgNPs. Fresh culture of *Aspergilus falvus* was used for the mycosynthesis of silver nanoparticles. Cell-free filtrate was treated with silver nitrate salt solution. Cell free filtrate act as reducing and stabilizing agent for mycosynthesis of AgNPs. Fungal biomass was prepared by using five to seven days old colonies of *Aspergilus falvus* were used for the synthesis of fungal biomass. PD broth was used for biomass synthesis. For 1 liter broth 3 petri plates of fully growth of fungus was used. PD broth was prepared by heating a 1 liter distilled water and then was added 20g of starch and 20g of dextrose and mixed it well to avoid clumps formation and was autoclaved. Sterilize the spatula on flame until red hot then let it cool meanwhile open the petri plate containing fungus. Chop the fungus with media into small pieces and this mass was then added to broth and was shaked well. All this was done in laminar flow hood. Poured the broth with fungus in the media bottle and was kept it in the shaking incubator for 72 hours, 150rpm and 28°C temperature. After 72 hour cell free filtrate was prepared from fungal biomass then filtered it via whatsmann filter paper no. 1 and using plastic sieve and medium chunks were removed with hand. Weight of the biomass with filter paper was measured. 20g of fungal mass was dissolved in 100ml of distilled water. Filter paper containing fungal biomass was then washed in distilled water then kept in the shaking incubator for 48-72hours, 28°C and 150rpm. After 72 hours took the biomass out of the shaking incubator filtered it again and the filtrate at that stage was known as cell free filtrate which was then ready for its treatment with silver nitrate solution (Ingle *et al.*, 2008). For the preparation of mycosynthesized nanoparticles parameters i.e pH, salt concentration, extract concentration and most importantly temperature were considered. Silver nitrate salt with concentration of 1mM was used for the purpose of silver nanoparticles formation. For 1mM salt solution preparation 0.17g of $AgNO_3$ salt was dissolved in 1 liter of distilled water. The synthesis of nanoparticles involved the treatment of cell free filtrate with 1mM solution of silver nitrate in 1:4. After two hour color change was observed from light brown to dark brown. Color changed was the indication of silver nanoparticles formation (Fatima *et al.*, 2016).

RESULTS: Phytosynthesis of silver nanoparticles was carried out by using the leaves of lemon grass *(Cymbopogon citratus)* and silver nitrate ($AgNO_3$) salt solution. Characteristic of silver nanoparticles was done by UV-visible spectroscopy, scanning electron microscopy (SEM) and energy dispersion X-ray spectroscopy (EDX). Characterization of Silver nanoparticles was conducted in institute of space technology, Islamabad, usually different characterizations peaks occur between 410-470nm for the synthesis of AgNPs. Different wavelengths attribute different shapes and sizes of AgNPs. The synthesis of AgNPs was monitored by UV-visible spectrum (figure 1). UV-visible spectra of green synthesized silver nanoparticles AgNPs showed broad peak at 435nm wavelength and peak value was (2.243) that confirmed formation of silver nanoparticles. The SEM micro graph showed the green synthesized silver nanoparticles were rectangular and it also indicated the average particle size of AgNPs falls in the range between 80 and 90nm (figure 2). The (EDX) analysis of green synthesized silver nanoparticles confirmed the presence of silver nanoparticles by showing the energy peak at 3keV (figure 3). FTIR showed the biomolecules involved in the mycosynthesized silver nanoparticles. There are total 14 peaks in the spectrum at 427.58, 532.37, 669.32, 800.49, 875.71, 1026.16, 1072.46, 1261.49, 1384.94, 1618.33, 2848.96, 2916.47, 2962.76 and 3373.61 cm^{-1}. 427.58cm^{-1} and 532.37cm^{-1} peaks showed the C-Cl stretch absorbation of alkyl halides, 669.32cm^{-1}represent the peak of alkynes, 800.49cm^{-1} showed the C-Br stretch peak of alkyl halides, 875.71cm^{-1} showed the absorbation of aromatic compounds, 1026.16cm^{-1} and 1072.46cm^{-1} showed the peak of aliphatic amines, 1261.49cm^{-1} showed C-H wag functional group, 1384.94cm^{-1} showed alkanes functional group, 1618.33cm^{-1} showed 1 amines, 2848.96cm^{-1} showed aldehydes group, 2916.47cm^{-1} and 2962.76cm^{-1} showed alkanes peak and 3373.61cm^{-1} showed the peak of 1,2 amines and amides functional group (figure 4).

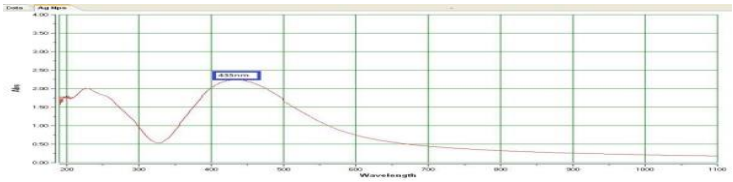

Figure 1: The UV-visible spectroscopy of the phytosynthesized nanoparticles.

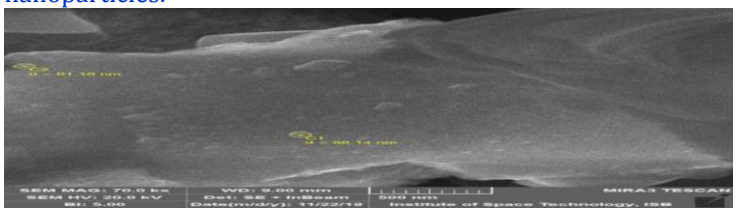

Figure 2: SEM micro graph of phytosynthesized silver nanoparticles.

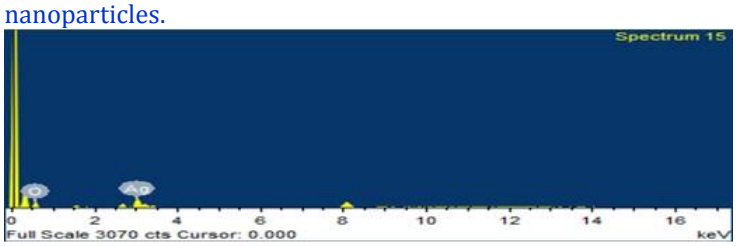

Figure 3: SEM micro graph of phytosynthesized silver nanoparticles.

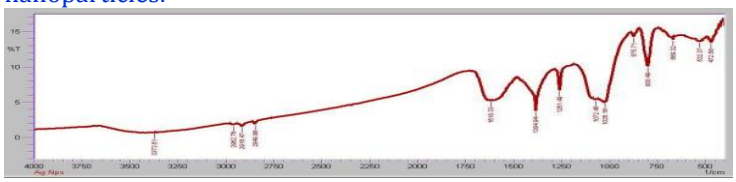

Figure 4: FTIR spectrum of phytosynthesized silver nanoparticles.

Mycosynthesized silver nanoparticles: The mycosynthesis of silver nanoparticles was carried out by using young culture of fungus *Aspergilus falvus* and silver nitrate ($AgNO_3$) salt solution. Characteristic of silver nanoparticles was done by UV-visible spectroscopy and Fourier Transform Infra Red Spectroscopy (FT-IR). Characterization of Silver nanoparticles was done in Fatima Jinnah women university, Rawalpindi. Different characterizations peaks usually occur between 410-470nm is obvious for the synthesis of AgNPs. Different wavelengths may attribute different shapes and sizes of AgNPs. UV-visible spectra of green synthesized silver nanoparticles AgNPs showed broad peak at 441nm wavelength and peak value was 1.232 and it confirm silver nanoparticles (figure 5).

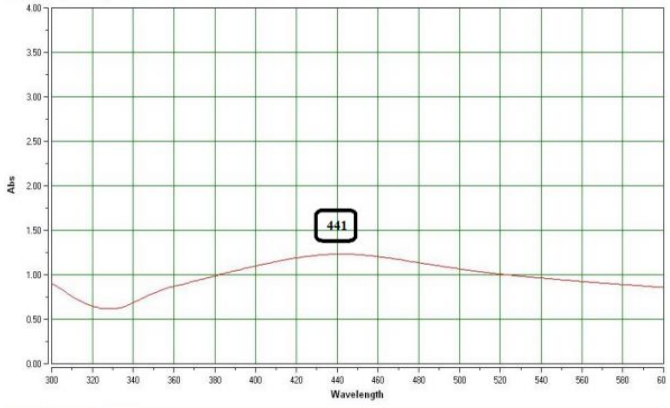

Figure 5: UV-visible spectroscopy of the mycosynthesized silver nanoparticles.

FTIR showed the biomolecules involved in the mycosynthesized silver nanoparticles. There were 9 peaks in the spectrum at 3462.34, 2962.76, 2378.31, 1261.49, 1097.53, 1022.31, 864.14, 800.49 and 592.17 cm^{-1}. 3462.34 cm^{-1} peak showed the presence of alcohols and phenols, 2962.76 cm^{-1} for caboxylic acids, 2378.31 cm^{-1} for nitriles, 1261.49 cm^{-1} for aromatic amines, 1097.53 cm^{-1} and 1022.31 cm^{-1} for C-O sretch that may either be Alcohols, Carboxylic Acids, Esters or Ethers, 864.14 cm^{-1} and 800.49 cm^{-1} for alkenes and 592.17 cm^{-1} peak for C-Cl stretch that may be alkyl halides functional group (figure 6).

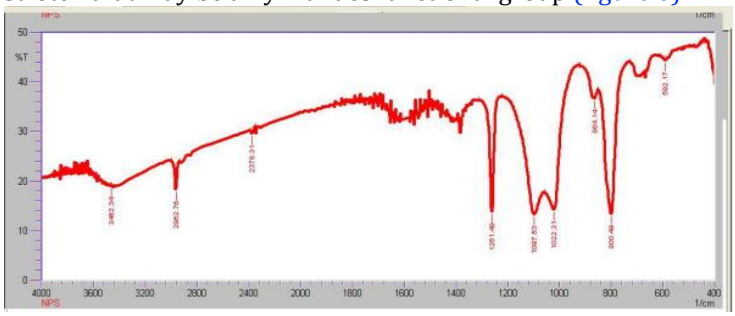

Figure 6: FTIR of the mycosynthesized silver nanoparticles.

The SEM micro graph showed that the mycosynthesized silver nanoparticles were spherical in size and it also indicated the average particle size of AgNPs falls in the rang between 50 and 60nm (figure 7). The (EDX) analysis of green synthesized silver nanoparticles confirmed the presence of silver nanoparticles by showing the energy peak at 3keV.

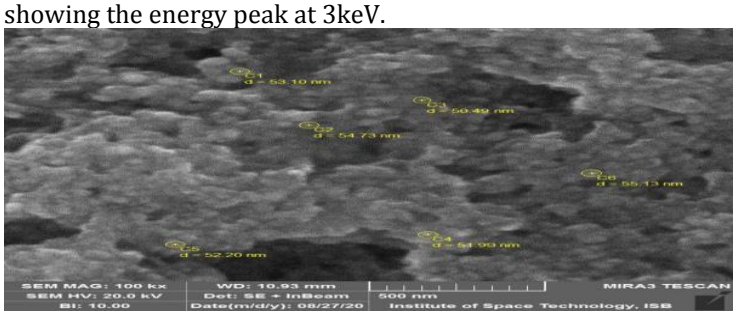

Figure 7: SEM of the mycosynthesized silver nanoparticles.

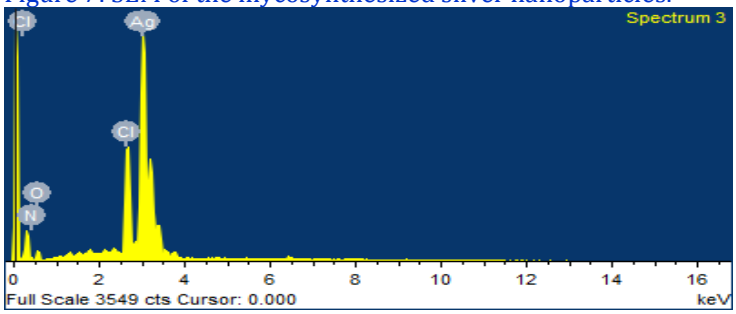

Figure 8: EDX spectrum of mycosynthesized nanoparticles.

Germination responses: Silver nanoparticles showed effect on germination parameters ($p \leq 0.05$) and its application enhances germination percentage by 6.2% while mycosynthesized nanoparticles by 7.7%. Phytosynthesized silver nanoparticles shows the highest increase in the germination percentage at 40ppm concentration while mycosynthesized nanoparticles at 60ppm. Overall lower dose of silver nanoparticles cause an increase in the germination percentage while the higher dose cause decline in the germination percentage (figure 9). However, phytosynthesized silver nanoparticles showed significant results in maize plants. Germination index of maize plant were increased by 11% and 16% for phytosynthesized and mycosynthesized nanoparticles respectively (table 1).

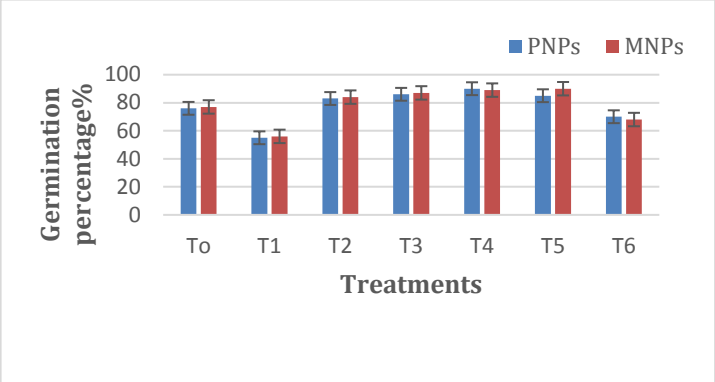

Figure 9: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the germination percentage of maize plant.

Where, To= untreated seeds, T_1 = $AgNO_3$, T_2= Plant extract and fungal extracts T_3= seeds treated to 20ppm dose AgNPs, T_4=Seeds treated to 40ppm dose of AgNPs, T_5=Seeds treated to 60ppm dose of AgNPs, T_6=Seeds treated to 80ppm dose of AgNPs and PNPs= phytosynthesized nanoparticles, MNPs= mycosynthesized nanoparticles.

AgNPs conc.		G%	GI	SVI
control		75±0.55	1.71±0.02	121±0.88
20ppm	P	85±2.02	1.52±0.014	104±0.58
40ppm	N	91.67±0.88	1.96±0.032	145±2.88
60ppm	P	84.67±0.88	1.82±0.145	129±0.58
80ppm	s	71±2.08	1.70±0.031	83.67±0.88
20ppm	M	71±2.08	1.60±0.028	80.33±0.88
40ppm	N	85±0.88	1.68±0.015	128±1.67
60ppm	P	91.33±0.88	1.94±0.032	148±4.40
80ppm	s	70±1.15	1.74±0.021	82±1.15
Salt soln		54±0.59	1.03±0.021	96.67±0.88
Plant extract		77.3±1.20	1.28±0.015	111±3.51
Fungal extract		78±1.52	1.41±0.019	102±1.45

Table 1: Effect of phytosynthesized and mycosynthesized silver nanoparticles on germination parameters of maize crop.

Experiment was conducted in triplicate and mean ± standard deviation was calculated. Where as , PNPs = Phytosynthesized nanoparticles. MNPs =Mycosynthesized nanoparticles.

Lesser dose of silver nanoparticles increased the germination index while higher dose reduced the germination index. Phytosynthesized silver nanoparticles shows the highest increase in the germination index at 40ppm concentration while mycosynthesized nanoparticles at 60ppm. Overall, phytosynthesized silver nanoparticles showed significant results in maize plants. Results of germination index were shown in figure 10.

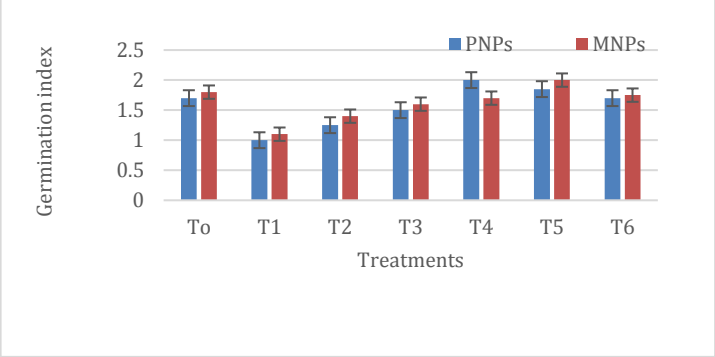

Figure 10: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the germination index of maize plant.

Seedling vigor index results were shown in figure 11.

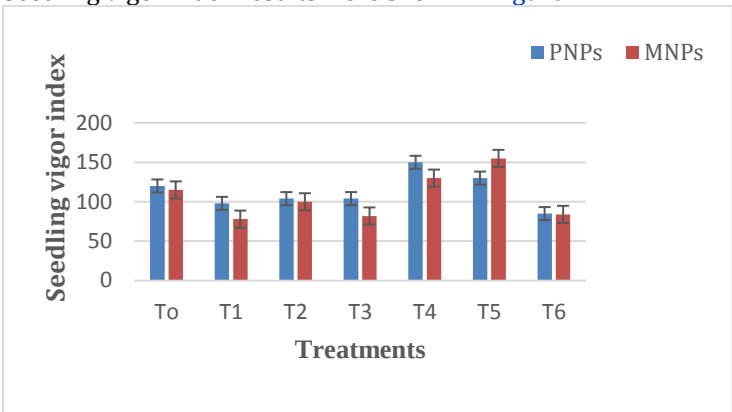

Figure 11: : Effect of phytosynthesized and mycosynthesized silver nanoparticles on the seedling vigour index of maize plant.

Exposure to the high dose of silver nanoparticles there was decline in the seedling vigor index (25% and 34.7% of phytosynthesized nanoparticles and mycosynthesized nanoparticles). Lower dose of silver nanoparticles increase the seedling vigor index while higher dose decrease the seedling vigor index. Phytosynthesized silver nanoparticles shows the highest increase in the seedling vigor index at 40ppm concentration while mycosynthesized nanoparticles at 60ppm. Overall phytosynthesized silver nanoparticles showed more significant results.

Morphological parameters: The effects of phytosynthesized and mycosynthesized silver nanoparticles on morphological parameters has shown significant effects ($p \leq 0.05$) on maize plants. Both types of Nanoparticles application increase the growth of plants as compared to the control. Adverse results were observed in growth parameters of maize crop through the application of silver nanoparticles (table 2) treatments. Phytosynthesized and mycosynthesized silver nanoparticles application increases the shoot length (figure 12).

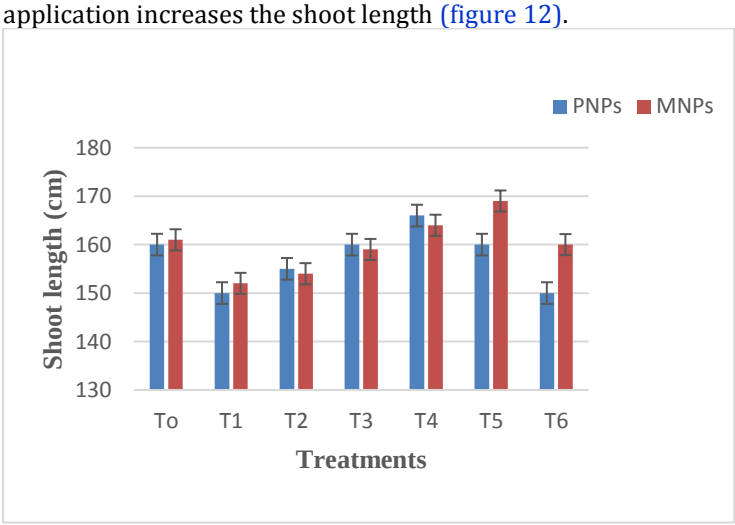

Figure 12: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the shoot length of maize plant.

Plant extract and fungal extract have similar effect to control. The best results are obtained at lesser dose of nanoparticles application. Phytosynthesized nanoparticles showed best results at 40ppm concentration and caused an increase in shoot length by 5%. While mycosynthesized silver nanoparticles best results were observed in 60ppm and increase in shoot length by 4.9%. Data obtained for root length of maize in figure 13

showed the variation in root growth in silver nanoparticles application.

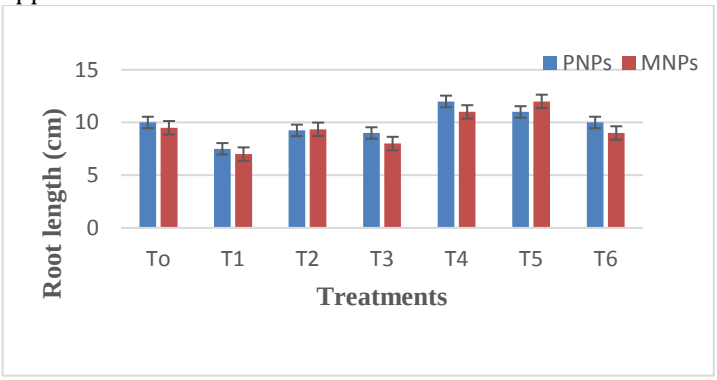

Figure 13: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the root length of maize plant.

There is increase in root length growth in silver nanoparticles application. Extracts and control have same effects to control. Phytosynthesized silver nanoparticles showed best result in 40ppm and cause an increase in the root length by 20% while mycosynthesized silver nanoparticles also showed increase in the root length by 26% at 60ppm. However significant results were showed by soil application. Nanoparticles treatments increased the plant height in maize plant as showed in figure 14 Extracts have growth impact equal to control.

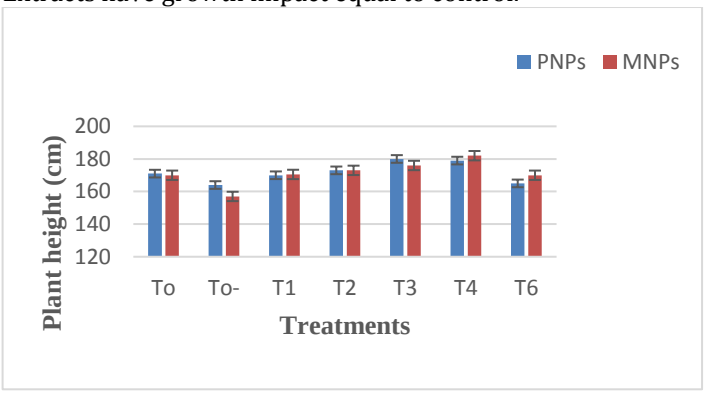

Figure 14: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the plant height of maize plant.

Phytosynthesized silver nanoparticles showed an increase in plant height by 5.2% at 40ppm. While mycosynthesized silver nanoparticals caused an increase in plant height by 7% at 60ppm. Silver nitrate application reduces the plant growth by 2.3%. However non-significant results were shown in both application Remarkable increase in the plant fresh weight was observed as shown in figure 15.

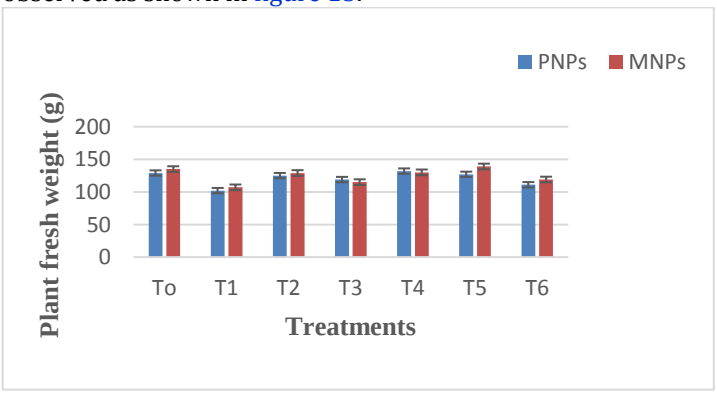

Figure 15: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the plant fresh weight of maize plant.

Phytosynthesized silver nanoparticles showed an increase in plant fresh weight by 2.3% at 40ppm dose. While mycosynthesized silver nanoparticles by 2.9%. No major effect is caused by the extracts (fungal/plants). Silver nitrate application reduced the fresh weight by 19%. higher concentration of nanoparticles application had reduced the fresh weight of plants. Data regarding plant dry weight of maize in figure 16 showed variability in regard to silver nanoparticles treatments. Silver nitrate reduced the plant dry weight by 25%. Phytosynthesized silver nanoparticles caused an increase in plant dry weight at 40ppm by 3.1% while mycosynthesized silver nanoparticles at 60ppm by 6%. Higher dose of silver nanoparticles caused the reduction in plant dry weight. Silver nanoparticles treatments adversely effects the leaves length as shown in figure 17.

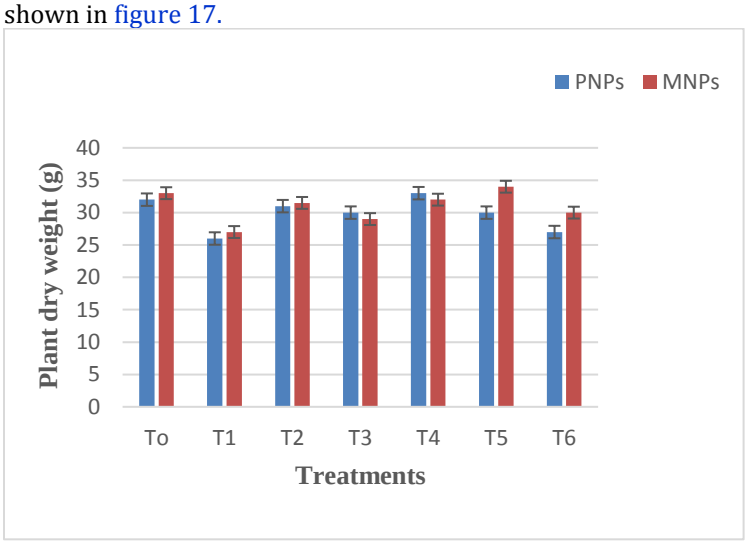

Figure 16: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the plant dry weight of maize plant.

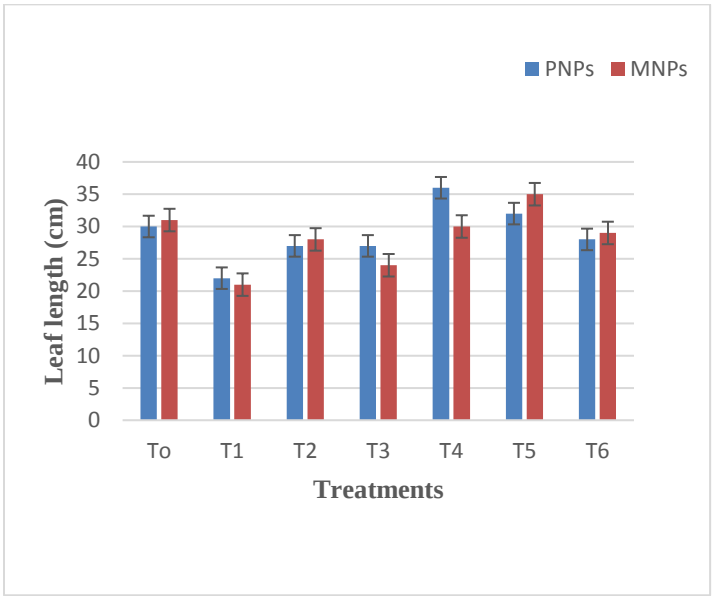

Figure 17: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the leaf length of maize plant.

Silver nitrate application reduced the leaf length by 36%. Phytosynthesized silver nanoparticles caused an increase in the leaf length by 20% at 40ppm dose. While mycosynthesized silver nanoparticles at 60ppm dose by 12%. A pronounced effect in leaf numbers was observed as shown in figure 18.

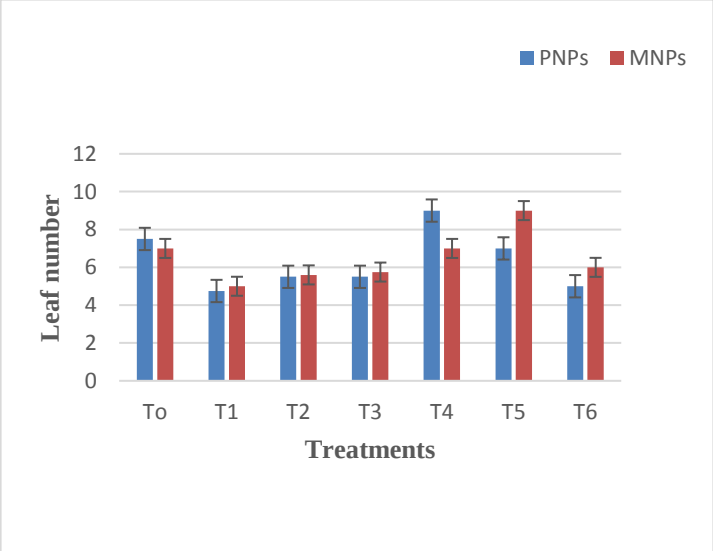

Figure 18: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the leaf number of maize plant.

No remarkable effect is shown by plant and fungal extracts. Phytosynthesized silver nanoparticles caused an increase in the leaf number by 20% at 40ppm. While mycosynthesized silver nanoparticles by 28% at 60ppm.

Physiological Parameters: Comparative effects of phytosynthesized and mycosynthesized silver nanoparticles has shown significant effects ($p \leq 0.05$) on the physiological parameters of maize plants. Both types of silver nanoparticles increases the growth attributes as compared to the controls in plants (Table 3). Increased dose of AgNPs increased the RWC. Soil application increased the RWC by 31% in PNPs while 28% in foliar application of MNPs. Salt application reduced the RWC of maize plants by 17%. Plant extract improved the RWC by 6% while fungal extract increased RWC by 1% (figure 19). Increased concentration of AgNPs increased the MSI. Soil application increased the MSI by 74% for PNPs while in case of MNPs soil application increased MSI by 58%. Salt application reduced MSI by 25%.

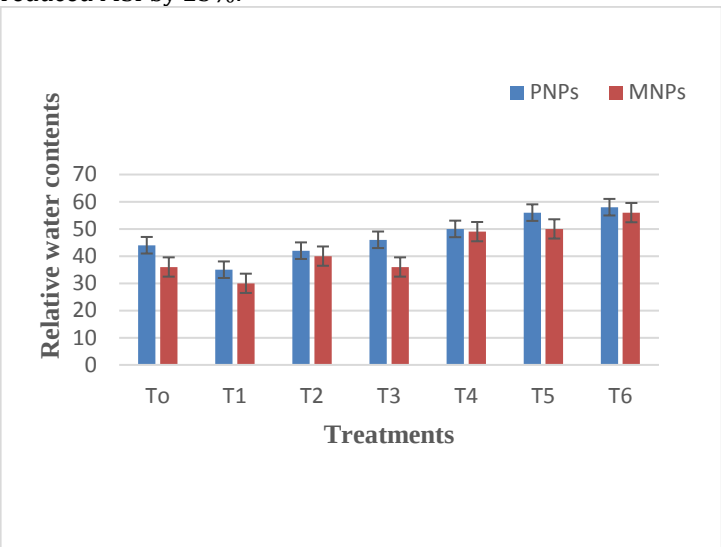

Figure 19: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the relative water contents of maize plant.

Plant extract increased MSI in soil treatments by 1.6%. Fungal extract increased MSI by 3.7% (figure 20).

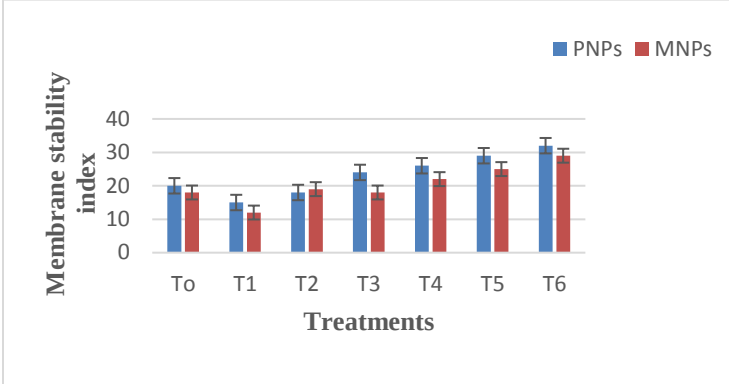

Figure 20: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the membrane stability index of maize plant.

Chlorophyll a content is maximum at 40ppm dose of PNPs by 47% while at 60ppm for MNPs by 39%. Salt application decreased the chlorophyll contents by 11%. Plant extract increased the chlorophyll contents in soil application by 2.5%. Fungal extract decreased the chlorophyll a by 4% (figure 21).

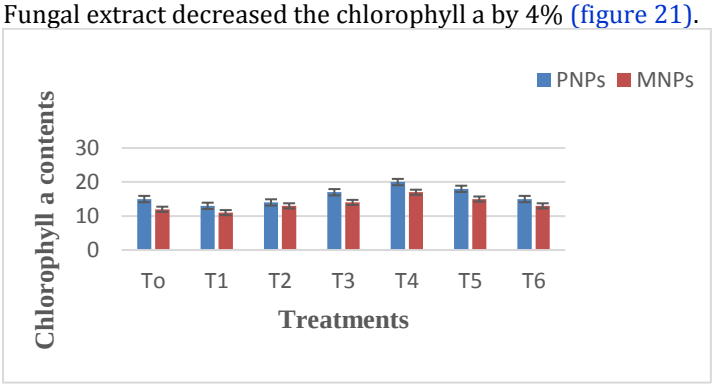

Figure 21: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the chlorophyll a contents of maize plant.

Nanoparticles application increased the chlorophyll b contents but higher dose decreased it. Chlorophyll b content is highest at 40ppm in PNPs and increased by 25% while MNPs at 60ppm increased by 19%. Salt application decreased the chlorophyll b contents by 22%. Plant extract increased the chlorophyll b contents by 13% in both applications while fungal extract by 7.7% (figure 22). Increased dose of nanoparticles decreased the total chlorophyll contents TCC. Salt application reduced the TCC by 19%. TCC is highest by 19% at 40ppm for PNPs while for MNPs at 60ppm by 17%. Plant extract increased TCC by 4.6% while fungal extract increased TCC by 2.1% figure 23.

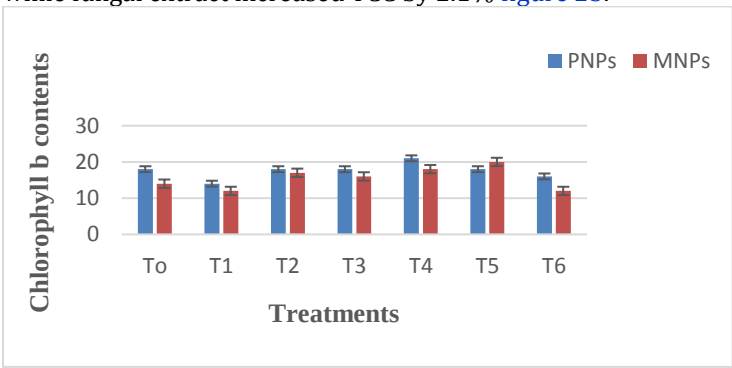

Figure 22: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the chlorophyll b contents of maize plant.

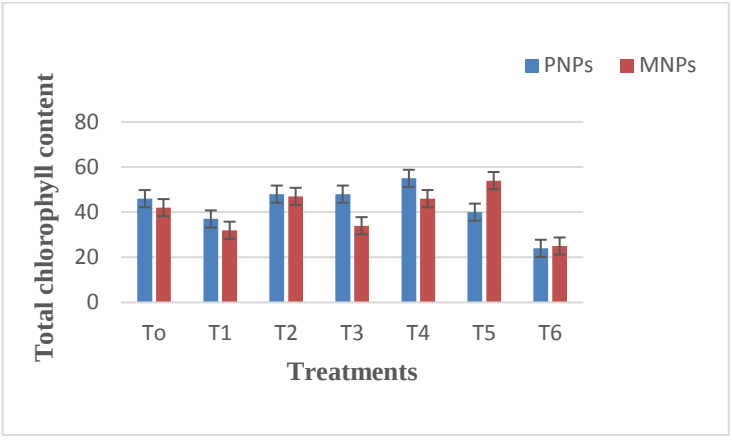

Figure 23: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the total chlorophyll contents of maize plant.

Biochemical Parameters: Significant effect ($p \leq 0.05$) was showed by the application of PNPs and MNPs on biochemical parameters of maize crop. Proline is an essential compatible solute and its accumulation and production in plant system totally depends (table 4) upon the environmental conditions. In normal growth of maize plants proline production is not too high but was increased upon the application of NPs and was maximum at 40ppm by 35% for PNPs while in MNPs at 60ppm by 28%. Salt application decreased the proline contents by 22%. Plant extract increased the proline contents by 14%. Fungal extract have same effect as the control in soil application (figure 24).

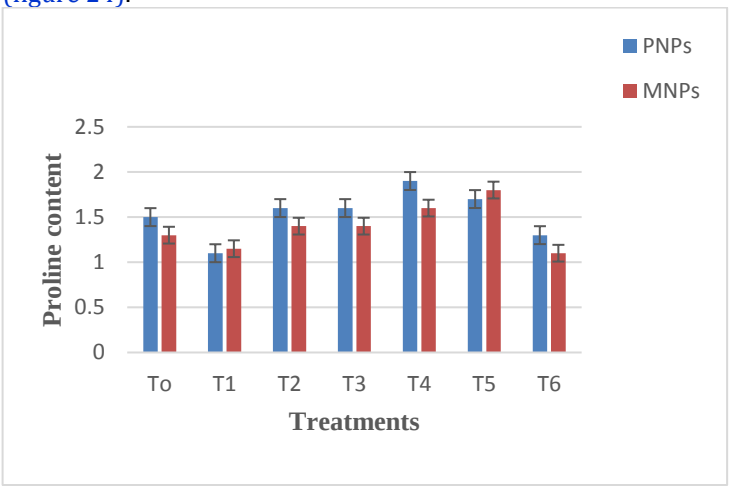

Figure 24: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the soluble sugar contents of maize plant.

Present study showed that soluble sugar content was higher in low dose application of NPs but with increased dose of NPs it was reduced. In case of PNPs SSC increased by 45% at 40ppm while in MNPs SSC was increased by 40% at 60ppm of concentration. Salt application reduced the SSC by 25%. Plant extract increased by 5%. Fungal extract treatment do not effect the SSC level in soil treatment (figure 25).

Results of carotenoid contents were in figure 24. Increasing dose of silver nanoparticles increased the carotenoid contents in maize crop. Both PNPs and MNPs showed significant results in carotenoid contents. PNPs increased carotenoid contents by 85% while MNPs by 89%. silver nitrate 10ppm concentration reduced the carotenoid contents by 16 %. Plant extract showed increased carotenoid contents by 8.3% while fungal extract decreased by 30% (figure 26).

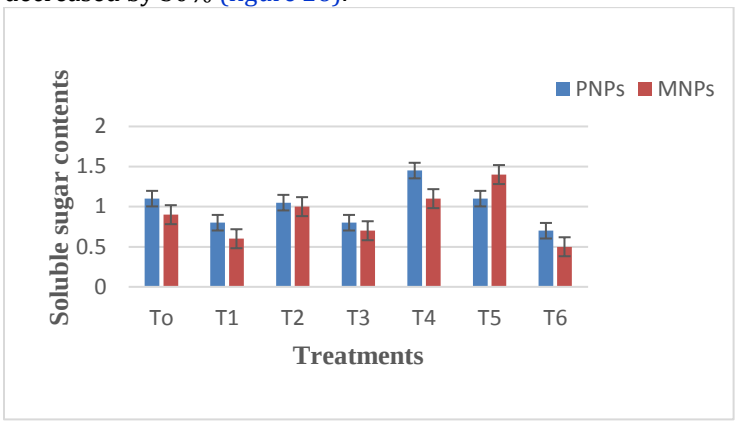

Figure 25: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the soluble sugar contents of maize plant.

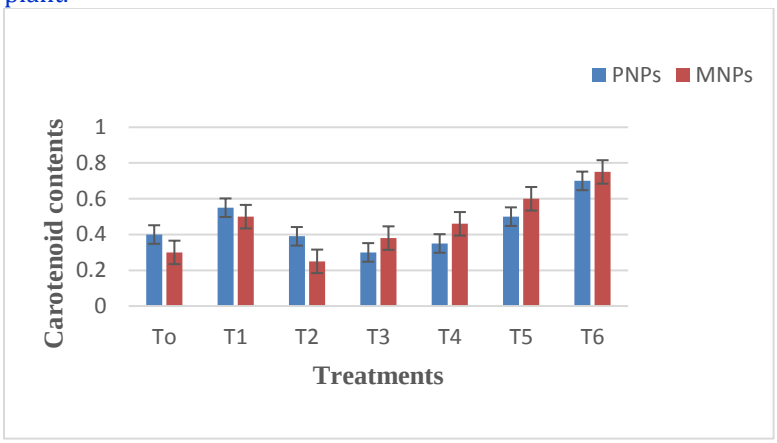

Figure 26: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the Carotenoid contents of maize plant.

It was observed that TPC was increased with the NPs dose no matter of MNPs or PNPs. In case of PNPs TPC was increased by 93% while in MNPs it was increased by 70%. Salt application reduced TPC by 18%. Plant extract did not effect the level of TPC. fungal extract treatment decreased the TPC by 31% respectively (figure 27).

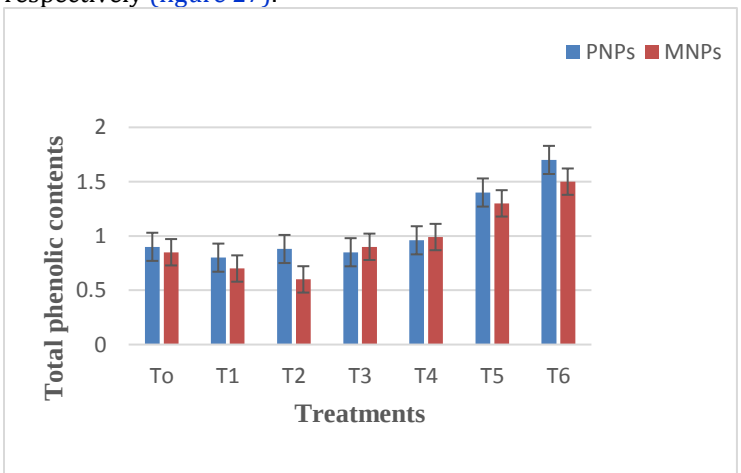

Figure 27: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the total phenolic contents of maize plant.

Maize plants showed increased in antioxidant activity with increased dose of NPs (figure 28).

AgNPs conc.		Root length (cm)	Shoot length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Leaf length(cm)	Leaf number
control		9.86±0.18	160±2.88	129±0.33	32±0.57	30.3±0.33	7.33±0.33
	P	9.18±0.09	160±1.73	119±0.57	30±0.28	27±0.57	6±0.57
40ppm	N	12.0±0.06	166.6±1.2	131±0.57	33±1.15	36±1.15	9±1.15
60ppm	P	11.0±0.08	160±2.88	127±0.57	30±1.15	32±1.15	6.66±0.88
80ppm	s	10.0±0.03	150±3.46	110±3.17	27±0.57	28.6±1.20	5±1.15
20ppm	M	8.00±0.14	159±0.57	115±2.88	29±0.57	24±0.57	4.66±0.88
40ppm	N	11.1±0.07	165±0.57	130±2.30	31±0.57	30±1.15	6.66±0.33
60ppm	P	12.0±0.14	169±0.57	138±0.88	34.16±0.73	35±2.88	8.66±1.45
80ppm	s	9.00±0.14	155±2.88	119±0.57	30±2.30	29±0.57	5.66±0.33
Salt solution		7.31±0.15	150±2.88	102±1.45	26.3±0.33	21±0.57	5±0.00
Plant extract		9.17±0.04	155±2.88	122±1.45	30.5±0.28	27.16±0.44	5±0.00
Fungal extract		9.28±0.14	153±0.88	128±0.57	31.5±0.28	28.56±0.29	5.33±0.33

Table 2: Effect of phytosynthesized and mycosynthesized silver nanoparticles on morphological parameters of maize crop. Experiment was conducted in triplicate and mean ± standard deviation was calculated. Where as , PNPs = Phytosynthesized nanoparticles. MNPs =Mycosynthesized nanoparticles.

AgNPs Conc.		Relative water content	Membrane stability index	Chlorophyll a contents	Chlorophyll b contents	Total chlorophyll contents
control		39.67±2.33	18.33±0.88	13.67±0.88	16.67±0.88	45±0.57
20ppm	P	45±0.57	23±0.57	16±0.57	17.33±0.67	47.67±2.18
40ppm	N	50.3±0.33	27±0.57	20.3±0.33	21.33±0.88	55±0.57
60ppm	P	55±0.57	27±1.15	18.3±0.33	16.67±0.88	40±4.62
80ppm	s	57.3±0.67	31±0.57	15.3±0.33	15.67±0.33	28.67±0.33
20ppm	M	35.3±0.67	17±1.00	14±0.57	16.33±0.67	34.33±2.33
40ppm	N	48±0.57	21±0.57	16.3±0.67	19±0.57	50±2.88
60ppm	P	51±0.57	24±0.57	16.3±1.33	15±0.57	52.67±3.84
80ppm	s	55.67±0.88	29±0.57	13±0.57	12.33±0.33	24±0.57
Salt solution		32.67±1.45	13.67±0.88	12±0.57	13.33±0.67	37.67±2.33
Plant extract		42±1.15	17±0.57	14±0.57	17.67±0.33	48±0.57
Fungal extract		41±0.57	14±0.57	13±0.57	16±0.57	44.67±0.88

Table 3:Effect of phytosynthesized and mycosynthesized silver nanoparticles on physiological parameters of maize crop. Experiment was conducted in triplicate and mean ± standard deviation was calculated. Where as , PNPs = Phytosynthesized nanoparticles. MNPs =Mycosynthesized nanoparticles.

AgNPs Conc.		Proline contents	Soluble sugar contents	Carotenoid contents	TPC	SOD	POD
control		1.4±0.05	1.00±0.06	0.36±0.03	0.88±0.02	0.79±0.02	0.48±0.02
20ppm	P	1.55±0.03	0.86±0.03	0.29±0.008	0.87±0.01	0.54±0.02	0.56±0.01
40ppm	N	1.86±0.03	1.44±0.03	0.35±0.02	0.95±0.02	0.94±0.03	0.67±0.01
60ppm	P	1.72±0.02	1.15±0.03	0.55±0.03	1.45±0.03	1.15±0.02	0.65±0.02
80ppm	s	1.34±0.02	0.73±0.24	0.74±0.02	1.73±0.02	1.34±0.03	0.83±0.02
20ppm	M	1.45±0.03	0.74±0.03	0.35±0.01	0.92±0.02	0.45±0.03	0.53±0.03
40ppm	N	1.64±0.03	1.14±0.03	0.45±0.03	0.96±0.02	0.84±0.03	0.55±0.02
60ppm	P	1.83±0.03	1.45±0.03	0.65±0.03	1.35±0.03	1.09±0.05	0.61±0.01
80ppm	s	1.14±0.02	0.54±0.03	0.75±0.02	1.54±0.03	1.24±0.02	0.75±0.02
Salt solution		1.08±0.04	0.75±0.03	0.30±0.10	0.72±0.03	0.37±0.02	0.35±0.03
Plant extract		1.5±0.57	1.08±0.44	0.35±0.02	0.84±0.02	0.72±0.01	0.46±0.01
Fungal extract		1.36±0.03	0.95±0.03	0.28±0.015	0.65±0.03	0.65±0.03	0.44±0.03
Blank		-	-	-	-	-	0.42±0.008

Table 4: Effect of phytosynthesized and mycosynthesized silver nanoparticles on biochemical parameters of maize crop. Experiment was conducted in triplicate and mean ± standard deviation was calculated. Where as , PNPs = Phytosynthesized nanoparticles. MNPs =Mycosynthesized nanoparticles.

Maximum increased in the production of SOD was observed at 80ppm for both PNPs and MNPs. 54% increased in SOD for PNPs application to maize plants while 50% for MNPs application. Silver nitrate application reduced the SOD by 53%. Plant extract showed decreased in SOD by 12% While fungal extract by 18%.

Antioxidant activity POD in maize plants was shown in figure 29. POD was increased with the increased in NPs dose application and was maximum at 80ppm. PNPs showed increased POD by 70% while MNPs by 61%. Salt treatment reduced the POD by 36% . Plant extract application to maize plants increased SOD by 4.20% while fungal extract reduced SOD by 14%.

DISCUSSION: The use of inexpensive, sustainable and eco-friendly modern technology i-e nanotechnology which increase the agricultural productivity are feasible option. The use of green synthesized silver NPs is reasonable example of acceptable agricultural practices. For viable farming systems there has been increase in demand of biochemically synthesized nanoparticles. Because of importance of silver NPs as growth promotor, present work was performed to investigate the effects on germination and growth parameters

of maize plants. Different concentration of silver NPs are applied on maize variety Sargoda 2005 and there response was known by various experiments carried out in laboratory.

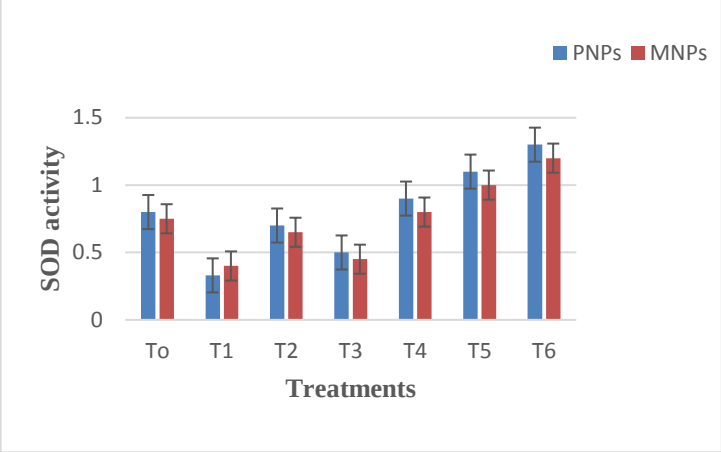

Figure 28: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the SOD activity of maize plant.

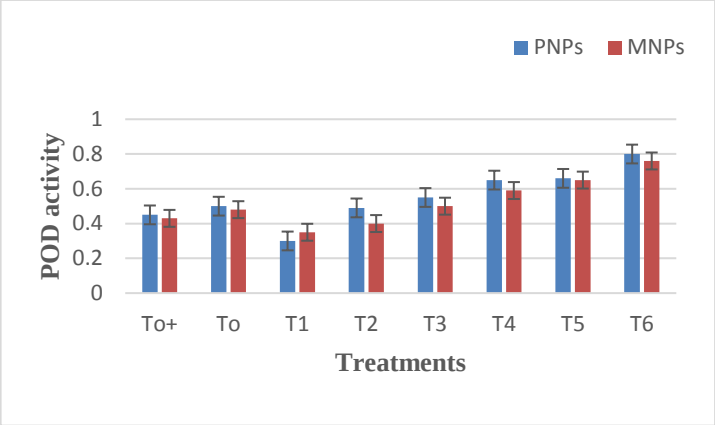

Figure 29: Effect of phytosynthesized and mycosynthesized silver nanoparticles on the POD activity of maize plant.

Krishnaraj *et al.* (2010) findings were contrary to our results that phytosynthesized silver nanoparticles had not effected seed germination at the maximum concentration of 100ppm even AgNPs and silver salt had not effected seed germination at (10ppb and 100ppb) of concentration. In the literature AgNPs do not effect the germination vigor even at the highest concentration of 100ppm. Whereas, $AgNO_3$ showed 100% growth retardation at 100ppm and 40% at 10ppm dose. Tremendous increase in the germination rate of corn crop seeds were observed with the increase in the concentration of AgNPs, however no effect on the germination % of corn (Almutairi and Alharbi, 2015). It was contrary to our research that silver nitrate had no impact on the seedling germination % even at the highest dose. Other sources showed that it is not effected at dose of 0.0005-0.01% (Barabanov *et al.*, 2018). Change in the plant biomass is the sensitive biomark. AgNPs had significant inhibitory effects on root length and fresh weight of Arabidopsis thaliana as compared to the Ag+. Ag+ had stronger inhibitory effects on the chlorophyll contents than AgNPs. As the concentration increases inhibitory effects are more. AgNPs toxicity is conc-dependent, toxicity increases with increase in the concentration of AgNPs (Qian *et al.*, 2013).

Corn seedling were severely infected with the AgNPs. Decline in the Root length was observed at all concentrations of AgNPs specially at the dose of 1.5 & 2 mg/L. Fresh weight of seedling was higher at certain dose of AgNPs mainly at 2mg/L than control while significant effect on seedling dry weight was not observed with AgNPs dose. While in case of watermelon and zucchini significant results of root length were observed with increased in the AgNPs dose, whereas zucchini root length is higher at low dose of AgNPs and opposite in the watermelons seedling. Seedling fresh weight of watermelon increased with increased in AgNPs dose while dry weight was reduced at certain dose of NPs. Fresh and dry weight of zucchini seedling increased at certain values of AgNPs dose (Almutairi and Alharbi, 2015). Contrary to our findings that nano CeO_2 had not significantly affected the biomass of root, stem and leaf of sunflower at all concentration. None of the treatments showed toxicity on leaves and roots. Even at 400mg/kg no visible symptoms of phytotoxicity was observed on roots and leaves while the level of SOD was increased (Tassi *et al.*, 2017). It was demonstrated that significant effect of silica NPs on maize and found that growth % and germination rate was improved while decline in growth potential, leaf area and biomass are the best parameters for calculating the toxicity of NPs in plants. It was observed that NPs of TiO_2 improved the growth of leaves in maize even at dose of 30 and 1000mg/L but reduced the lateral root growth in pea plants (Tripathi *et al.*, 2017). Chlorophyll an essential parameter for calculating stressers toxicity in plants. TCC was significantly declined by 50-70% with comparison to control for exposure of 4days (Ke et al., 2017). Study showed that silver NPs induced phytotoxicity at the physiological parameters. It decreased the chlorophyll b and disturbed the equilibrium of essential contents in leafy gametophytes (Liang et al., 2018). Toxicity of silver NPs in plants effect the level of chlorophyll contents, alteration of hormones and reduction in the transpiration rate. AgNPs can disrupt the photosynthetic system of various plant that would directly affect the synthesis of chlorophyll contents (Tripathi *et al.*, 2017). Nair & Chung studied that one week exposure of silver NPs of 0.5mg/L significantly reduced the biomass of root and shoot, leaf surface area, level of chlorophyll and carotenoid in the seedling of rice in a dose dependent fashion (Nair and Chung, 2014). Fluidity and permeability of the membrane consequently water and nutrient was also effected by the silver NPs exposure. Recent studies showed that Radish sprout when exposed to silver NPs reduction in water contents and nutrient uptake (Zn, Mg, B, Cu, Mn and Ca) was observed in the dose dependent pattern which suggested that silver NPs exposure directly affected the plant growth by altering the level of water and nutrient in plants (Zuverza-Mena *et al.*, 2017). It was reported that NPs produced ROS that affected the lipid peroxidation that significantly affected fluidity and permeability of membrane and acquisition kinetics of the nutrients (Cabiscol Català *et al.*, 2000).

Another research that agreed with our finding demonstrated that a decline in the TCC was observed in the fresh and marine water micro alga at the exposure of silver NPs dose from 0.01-10 mg/L. Chlorophyll contents in fresh water alga was reduced at 1 and 10mg/L dose of silver NPs by 34% and 51% with comparison to control while in case of marine micro alga it was reduced by 44% and 75% at dose of 1 and 10mg/L respectively (Oukarroum *et al.*, 2012). It was demonstrated that ZnO NPs decline the chlorophyll contents in peas at dose of 125, 250 and 500mg/L (Mukherjee *et al.*, 2014). Similarly reduction in the chlorophyll level by 60% and 85% at the exposure of 1000 and

2000ppm dose of NPsCeO_2 while total chlorophyll contents was unaffected by the exposure of In2O3NPs (Ma *et al.*, 2013). It was observed that titanium NPs cause alteration in the cucumber and Phaseolus vulgaris while in case of tobacco aluminium NPs caused decline in activity of enzymes of dehydrogenase and oxireductase. Similarly chlorophyll level was reduced by ZnO nanoparticles in green pea while in cowpea it caused hindrance in translocation process (Tripathi *et al.*, 2017). Total Proline contents increased with increased in the concentration of CuO nanoparticles but maximum accumulation was at 1.5mM. Leaf carotenoid contents was decreased with the increased in the concentration of CuO nanoparticles and increased at 1.5mM concentration at 7th days but as the time passed to 14th day the carotenoid level reduced by 1.5 fold as compared to control. CuO nanoparticles were less effective to SOD activity. It was increased by 1.4fold at 1.0mM dose at 7th day but at 14th day SOD activity was maximum for 1.5mM dose. AgNPs treatment increased the level of protein and carbohydrates and lowered the level of POX, TPC and CAT in plants as compared to AgNO3 treatments (Krishnaraj *et al.*, 2010). It was reported that nickel nanoparticles increased the SOD and catalase activity and contents of glutathione and lipid peroxidation was increased in tomato similarly ZnO in green pea caused oxidative stress at the exposure of 500ppm dose. Titanium NPs damaged chloroplast that reduced the photosynthetic activity due to generation of antioxidant stress in spinach crop (Tripathi *et al.*, 2017).

CONCLUSION: From the present study it is concluded that phytosynthesized silver nanoparticles have positive effect on maize crop at 40ppm concentration while mycosynthesized silver NPs at 60ppm concentration. It has improved germination and growth parameters of maize variety i.e. Sargoda 2005. In general, this modern technique can be helpful for enhancement of crop germination, development and growth of maize. However there is need of application of this technology at commercial level.

CONFLICT OF INTERES: The authors declare no conflicts of interests.

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