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PHYTOTOXIC ASSESSMENT OF $AGNO_3$ AND $ZNSO_4$ *VIS À VIS* AGNPS AND ZNONPS IN *TAGETES ERECTA* L. AND *ZINNIA ELEGANS* JACQ.

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ABSTRACT

The present study was intended to assess the comparative responses of the two asteraceous plants, Mexican marigold (*Tagetes erecta* L.) and zinniasp. (*Zinnia elegans* Jacq.) against silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) and their respective salts, silver nitrate ($AgNO_3$) and zinc sulphate ($ZnSO_4$) by assessing different growth parameters, oxidative stress biomarkersand anti-oxidative defense system. Metal salts were found to be more toxic than their nanoparticles, which was manifested in terms of more reductions in growth parameters and more enhancements in reactive oxygen species along with MDA equivalents content. Further, various antioxidant enzymes *viz.*superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione-*S*-transferase (GST) enhanced significantly but not at the level that minimizes the oxidative stress.

Keywords: Antioxidants, Marigold, Nanoparticles, ROS, Zinnia

Introduction

Nanotechnology is emerging as one of the most promising techniques of the modern technology based world. It has so dramatically affected all the fields of science that it would be far appropriate to say the contemporary era of science is the 'era of nanoscience' or 'era of nanotechnology'. Nanoparticles are those particles that exist on a nanometer scale (i.e. below 100 nm in at least one dimension). Several metal NPs have been synthesized which have profound applications in several sectors and among them the silver and zinc oxide nanoparticles (Ag NPs and ZnO NPs) were found to have significant position due to their tremendous applications in drug-delivery systems, cosmetics and so forth (Burdusel*et al.,* 2018).Ag NPs have been reported to cause toxic effects on the physiology and biochemistry of several plants (Jiang *et al.,* 2017 and Tripathi *et al.,* 2017). Ag NPs release ionic silver into cells' *milleuinterna* and generate reactive oxygen species (ROS) by hindering respiratory and photosynthetic electron transport chains and enzymes (Jiang *et al.,* 2017). Even after no known metabolic function of silver, Ag^+ containing salts like $AgNO_3$, has been reported to have a major role in influencing somatic embryogenesis, root and shoot formation (Bais *et al.,* 2000).

Likewise AgNPs, ZnONPs easily dissolve in soil and are taken up by plants. ZnO NPs also inhibit biomass accumulation in *Arabidopsis*perhaps due to induction of ROS (Wang *et al.,* 2016).There are certain disadvantages of soluble salt of zinc i.e. $ZnSO_4$ over ZnO NP, as $ZnSO_4$ can cause damage to the leaf, it can undergo speciation within the plantand it is costlier (Doolette *et al.,* 2018). At the same time the ZnO NPs are considered a bio-secure material for biological species, since their efficiency has been demonstrated to promote the germination of seeds and the growth of plants, as well as in the suppression of disease and the protection of plants for their antimicrobial activity (Pethakamsetty *et al.,* 2017). Thus, the study about the impact of manufactured NPs on plant, animal and human health becomes imperative in order to assess the extent of toxicity and environmental risk.

Contrary to Ag, zinc (Zn) is an important component of a large number of enzymes. Zinc is considered as an essential plant nutrient and it plays a pivotal role in maintaining normal plant cellular metabolism. Zn is associated with the protein biosynthesis, carbohydrate and phosphate metabolism, gene expression and regulation related to environmental stress, RNA polymerase and DNA-binding proteins ribosome's structural integrity (Broadley *et al.,* 2007 and Sadeghzadeh and Rengel, 2011). Moreover, Zn plays critical roles in the defense system of cells against ROS, and thus represents an excellent protective agent against the oxidation of several vital cell components such as membrane lipid, chlorophyll and –SH groups of protein (Cakmak, 2000). However, zinc accumulation may delay or diminish the growth and root development and causes leaf chlorosis (Wang *et al.,* 2009). Excess Zn could cause the excess formation of ROS in plant cells, which results in cellular damage and membrane lipid peroxidation. Wang *et al.* (2009) have demonstrated the effects of excess Zn on the activity of many antioxidative enzymes like SOD, POD, CAT, APX and non-enzymatic antioxidants contents, ascorbate and glutathione in plants.

Present piece of study is an exclusive attempt to explore the relative behavior of biosynthesized Ag NPs and ZnO NPs

versus their precursor metal salts, $AgNO_3$ and $ZnSO_4$, respectively on the seedlings of Mexican marigold (*Tagetes erecta* L.; Asteraceae)and zinnia (*Zinnia elegans* Jacq.; Asteraceae) by analyzing seedling growth, morphology appearance, oxidative stressand enzymatic antioxidants activity which are more or less unexplored. Marigold and zinnia are the most widely grown flowers in gardens, terrace and balcony and used in garlands and decoration for weddings, festivals and religious events throughout India.

Materials and Methods

Synthesis and characterization of silver nanoparticlesand zinc oxide nanoparticles

The silver nanoparticles (AgNPs) were synthesized by using green technology as per the method of Tripathi *et al.* (2017) whereas; thezinc oxide nanoparticleswere prepared byadapting the chemical method. The characterization of prepared Ag NPs and ZnO NPs was performed by taking the absorption spectrum of the solution in the range of 360-700 nm with the help of double beam UV-Visible Spectrophotometer (Systronics, PC Based Double Beam Spectrophotometer 2202). Similarly the size and shape of synthesized nanoparticles was also confirmed by using the dynamic light scattering (DLS) instrument (Beckman Coulter, DelsaNano C).

Plant material and culture conditions

The seeds of Mexican marigold (*Tagetes erecta* L.) and zinnia (*Zinnia elegans* Jacq.) were procured from the National Seed Corporation, New Delhi. Consistent sized seeds of marigoldand zinnia were disinfected with 2% (v/v) sodium hypochlorite (NaOCl) for 5 min, thereafter they were rinsed carefully by distilled water and left for the dousing for 4 h. Further, healthy looking uniform sized seeds were kept in Whatman No.1 filter papers moistened with 0.5×Hoagland's medium. The temperature was maintained at 25±2ºC to facilitate germination in dark. After 3 days seedling stage was attained. Thereafter, seedlings were transferred in plastic cups (surface area 20 cm^2; volume 150 ml) and grown under a photon flux density (PFD) of 250 µmol photons m^{-2} s^{-1} with relative humidity of 50-60%. Light/dark regime of 12/12 h was maintained in a growth chamber at 25±2ºC. After 15 days of growth, root and shoot samples from both of the test seedlings were harvested and different parameters were analyzed.

Silver andzinc metalsandtheir nanoparticles treatments

In the present study, only two concentrations for each treatment was taken, i.e. 50 µM and 100 µM of $AgNO_3$ and AgNPs, and $ZnSO_4$ and ZnONPs along with the control (i.e. only 0.5×Hoagland's solution). Each treatment contained five healthy and uniform sized seedlings. For each treatment three such cups were arranged and each experiment was performed in triplicate. During the course of treatment i.e. plant growing period (15 days) only the nutrient solutions whether control or treated were changed at every 3rd day and the solution was aerated every day to avoid root anoxia under aseptic condition.

Estimation of growth

Growth was measured in terms of root length and shoot length along with plant fresh mass. For this, at the end of the experiment seedlings from each setup were uprooted and washed gently with distilled water and blotted to remove the surface water. The length of root and shoot was measured with the help of meter scale. The fresh mass of the test seedlings was recorded in single pan digital electronic balance (Model CA 223, Contech, India).

Estimation of reactive oxygen species and indices of damage

The superoxide radical ($O_2^{\bullet-}$) was estimated by determining the formation of NO_2^- from hydroxylamine in the presence of $O_2^{\bullet-}$ as per the method of Elstner and Heupel (1976). The H_2O_2 formation in the leaves of control and treated seedlings was estimated by the method of Velikova *et al.* (2000). Indices of oxidative damage were assayed by quantifying MDA equivalents contents as per the method of Heath and Packer (1968) and then calculated by measuring the difference between absorbance at 532 and 600 nm and taking the extinction coefficient as 155 mM^{-1} cm^{-1}.

Estimation of Enzymatic Antioxidants: SOD, POD, CAT and GST

SOD activity was determined by monitoring the changes in the reduction of nitrobluetetrazolium chloride (NBT) as per the method of Giannopolitis and Ries (1977). One unit of SOD is justified as the amount of enzyme needed to cause decline in NBT reduction by 50% under the defined conditions. POD activity in the leaves of each test seedlings was quantified as per the method of Zhang (1992) using the extinction coefficient 25.5 $mM^{-1}cm^{-1}$. Enzyme activity was calculated in the terms of Unit g^{-1}FM.One unit of POD activity is the amount of enzyme oxidizing 1 nMguaiacolmin^{-1}.CAT activity was monitored in terms of reduction in absorbance at 240 nm due to depletion of H_2O_2 as per the method of Aebi (1984) by using the extinction coefficient of 39.4mM^{-1} cm^{-1}. One unit of CAT activity is justified as the amount of enzyme causing the dissociation of 1 nmol H_2O_2 min^{-1}.Glutathione-S-transferase activity was quantified as per the method of Habig *et al.* (1974) by using the substrate CDNB (1-chloro-2,4-dinitro benzene). The GST activity was figured by using the extinction coefficient 9.6 mM^{-1} cm^{-1}. One unit of GST activity is justified as 1 nmol CDNB conjugates min^{-1}.

Statistical analysis

The results presented are means ± standard error of three independent experiments with three replicates in each experiment to confirm the reproducibility of the data (n = 3). Since the results showed normal distribution, comparison between control and treatment's means was carried out by using one-way ANOVA to test significance level (Duncan's multiple range test, DMRT) at $P< 0.05$.

Results

Characterization of silver and zinc oxide nanoparticles

The optical properties of prepared Ag NPs and ZnO NPs were monitored in the range of 360-700 nm by using double beam UV-Visible Spectrophotometer. UV-Vis spectrum of synthesized Ag NPs was exhibited absorption peak at 439 nm however the absorption peak for ZnO NPs was 350 nm (Fig. 1a and b).Moreover, the size of synthesized nanoparticle was also testified by using the dynamic light scattering (DLS) instrument and the results of DLS exhibited the average particle size of synthesized Ag NPs and ZnO NPs was 79.5 nm and 82.6 nm respectively (Fig. 1c and d.).

Effect of silver and zinc metals and their nanoparticles on growth

Root and shoot length as well as plants fresh massof marigold and zinnia seedlings subjected to 50 µM and 100 µM of $AgNO_3$ and AgNPs, and $ZnSO_4$ and ZnONPs was measured (Fig. 2). Increasing concentration of either metal salts or metal nanoparticles caused severe decline ($P<0.05$) in all the growth parameters in comparison to the control (Fig. 2). However, the reductions in these parameters were found more intense under $AgNO_3$ (50 and 100 µM) treatment in both test seedlings (marigold and zinnia) as compared to $ZnSO_4$ metal. More or less similar trends were obtained in case of their nanoparticles too, where Ag NPs found more toxic as compared to ZnO NPs. The relative decline in these growth parameters was lesser for zinnia for both the treatments of the metal salts as well as metal nanoparticles.

Effect of silver and zinc metals and their nanoparticleson oxidative stress biomarkers

SOR and H_2O_2 contents increased significantly ($P<0.05$) by 78 and 99% and 67 and 88%, respectively under 50 and 100 µM treatment of $AgNO_3$ in the case of marigold, while it was 68 and 84%, and 48 and 79%, respectively in case of zinnia over the value of control (Table 1). Under similar doses of treatment of $ZnSO_4$, the SOR and H_2O_2 contents rose again significantly and reached up to 61 and 76%, and 54 and 70%, respectively in case of marigold, while in case of zinnia it was increased up to 40 and 64%, and 33 and 55% respectively over the value of control. Similar trend was obtained for malondialdehyde (MDA), a key indicator of injury to lipids (Table 1). Overall the results showed the acute toxic effects of metallic salts of silver and zinc in both the test plants as compared to their nano forms.

Effect of silver and zinc metals and their nanoparticleson enzymatic antioxidants

After 50 and 100 µM of $AgNO_3$ treatment, the SOD activity increased significantly ($P<0.05$) by 32 and 60 % in marigold and 58 and 95% in zinnia respectively (Table 2). In case of $ZnSO_4$, the increment was even more and reached 48 and 90% in marigold and 70 and 108% in zinnia, respectively, over the value of control. Further, under 50 and 100 µM of AgNPs and ZnONPs treatments, marigold showed 55 and 77%, and 68 and 106% enhancement in SOD activity, respectively while it was 89 and 126% and 74 and 110% respectively, in zinnia (Table 2). The POD, CAT and GST activities also tracked more or less similar fashion as recorded for SOD in both the test seedlings under metal salts as well as their nanoparticles (Table 2).

Discussion

The decline in various growth parameters of marigold and zinnia seedlings after the treatments of metal salts, $AgNO_3$ and $ZnSO_4$ as well as the respective nanoparticles, AgNPs and ZnONPs (Fig. 2) can be associated with the higher accumulation of reactive oxygen species (ROS) (Table 1).The probable reason for growth reduction after $AgNO_3$ treatment may be attributed to the increased uptake of Ag^+ by plants (Qian *et al.,* 2013). The above observations suggest that Ag in both forms, i.e. bulk silver and nano silver can interact with proteins and components of the plant system. Our results are in conformity with the findings of Pandey *et al.* (2014) who have also reported the significant reduction in growth of *Brassica juncea* L. seedling after $AgNO_3$ treatment. Our results are also in conformity with the findings of Cherif *et al.* (2011) where the growth of tomato seedlings was found to be reduced due to oxidative stress caused by higher concentration of Zn. More toxic symptoms of $ZnSO_4$in comparison to ZnO NPsis in agreement with the findings of Burman *et al.* (2013) where chickpea seedlings revealed lesser toxic responses with ZnONPs due to lower accumulation of ROS and MDA equivalent contents.

The phytotoxicity due to metal salts as well as nanoparticles is the manifestation of disturb equilibrium of ROS versus anti-oxidative defense system. Besides damaging impact on growth parameters the metal salts and their respective nanoparticles can also induce oxidative stress in the cellular arrangement via forming distinct oxidizing markers as SOR and H_2O_2 (Tripathi *et al.,* 2017). These oxidizing markers are byproducts of aerobic metabolism (Gill and Tuteja, 2010) and being highly reactive, they have potential to damage various biologically active molecules including lipids, proteins, DNA and nucleic acid leading to altered metabolism of stressed plants. Our results pertinent to SOR, $H_2O_2$and MDA substantiate these notions (Table 1). Moreover, our results are in consonance with the findings of Burman *et al.* (2013) and Jiang *et al.* (2017).

Increments in all the estimated enzymatic antioxidants *viz.* SOD, POD, CAT and GST in both the seedlings after the metal salts and their respective nanoparticles' treatment are the testimony of the defence response of the organisms against oxidative stress due to prevailing oxidizing environment inside them (Gill and Tuteja, 2010). In case of marigold, the exposure of $AgNO_3$ significantly enhances SOD activity (Table 2), but since the level of SOR in cells is not mitigated by SOD in particular or the orchestration of enzymatic antioxidants in whole, the amount of SOR is still higher. Under such circumstances, lipid peroxidation also reached at its peak level that has been manifested in increased MDA equivalents (Table 1). Upsurge in SOD activity provided protection to the cell by neutralizing SOR thereby reducing the risk of oxidative burst in tobacco plants (Cvjetko *et al.,* 2018) after $AgNO_3$ and Ag NPs, and in chickpea seedlings (Burman *et al.,* 2013) after $ZnSO_4$ and ZnO NPs treatments. In another finding, Bharti *et al.* (2014) have reported an appreciable rise in SOD activity in wheat genotype after $ZnSO_4$ treatment and in chickpea seedlings after ZnO NP treatment (Burman *et al.,* 2013). Likewise SOD, POD and CAT also effectively scavenged the subsequent formed H_2O_2 after the action of SOD upon SOR in the cellular system of the test seedlings (Table 2). CAT (a tetramericheme enzyme) bearing the highest turnover rate in cells plays a vital role in breakdown of H_2O_2 into H_2O and O_2 (Gill and Tuteja, 2010). In other words, CAT does not require reductant while working upon H_2O_2. Table 2 shows more synchronous activity of POD and CAT enzymes in zinnia in case of $ZnSO_4$andits nanoparticles. It successfully ameliorates the negative consequences of H_2O_2 whereas, the least activities of POD and CAT in marigold in case of $AgNO_3$ and AgNPs have also verified by the results of oxidative markers (Table1). Similar results were also obtained by Song *et al.* (2013) in *Lycopersicum esculentum* seedlings after AgNPs treatment. Similar studies performed by Sharma *et al.* (2012), Torabian*et al.* (2016) and Ali *et al.* (2019) also reported the increased activities of SOD, CAT and POD enzymes in *Brassica juncea,* sunflower and *Caralluma tuberculata* seedling after AgNPs and ZnONPs

exposures, respectively. Moreover, least per cent increment in studied enzymes activities under $AgNO_3$ treatment could be correlated with the interaction of Ag^+ with proteins present in cytosol and lipid bilayer thereby shifting the configuration and damaging the antioxidant defence systems of the test plants (McShan *et al.,* 2014).

GST also followed more or less similar trend as observed for rest of the enzymes (SOD, POD and CAT) in marigold and zinnia under both the metal salts as well as their nanoparticles treatment (Table 2). The highest percent activity of GST enzyme in zinnia under ZnONPs could be elucidated on the basis of its function in the proficient alleviation of most reactive oxygen derivatives along with the products of lipid peroxides through reduced glutathione. However, in the case of marigold under similar tested doses of both the metal salts and nanoparticles the least GST activity represented that products of lipid peroxidation were beyond the scavenging limit. This may further lead to set off the chain reactions along with free radicals, which may have detrimental effects on growth of both the test seedlings. Similar results were also reported by Sanjay *et al.* (2015) who also correlated the increased GST activity with the increased concentration (and size) of the $ZnSO_4$ and ZnO NPs particles in *Solanum melongena* plants.

Conclusion

The present study reveals, based on growth attributes and well defined relationship between reactive oxygen species and anti-oxidative defence system along with the anatomical analysis that the nanoparticles has potential to be toxic at the early growth and development stages of marigold and zinnia. However, the ionic form of both metals $AgNO_3$ and $ZnSO_4$ had severe effects in both the test seedlings that were clear manifested in enhanced SOR and H_2O_2 contents along with the MDA equivalents content. The studied antioxidant enzymes also found to be incompatible in both seedlings under metal treatments conditions. Contrary to this, nanoparticles especially the zinc (ZnONPs) shows better response in zinnia and has least damage that could be correlated with their essentiality for (1) biomass production (2) important for functioning of enzymes, and (3) plays pivotal role in membrane integrity and maintenance. Taking influences of the both selected metals and nanoparticles, our study suggests that the metal forms are more toxic than its nanoform while potential risks of both forms is critical on the growth and development of plants which is an agriculturally significant plant species worldwide.

Table 1: Impact of $AgNO_3$, AgNPs, $ZnSO_4$ and ZnONPs treatments on SOR, H_2O_2 and MDA equivalents contents of marigold and zinnia. Data are mean ± standard error of three independent experiments with three replicates (n=3).Values with different superscripts within same column show significant differences at $p<0.05$ level between treatments according to the Duccan's multiple range test.

Treatments (µM)	Oxidative stress biomarkers (nmol g^{-1} Fresh weight)					
	Marigold			Zinnia		
	SOR	H_2O_2	MDA	SOR	H_2O_2	MDA
$AgNO_3$						
0	189.99±5.40^{f}	681.81±16.89^{f}	13.57±0.34^{g}	279.18±7.43^{e}	639.19±17.23^{g}	16.36±0.46^{g}
50	338.62±8.82^{bc}	1136.84±25.73^{c}	26.64±0.63^{c}	468.21±9.43^{bc}	946.53±20.06^{d}	30.61±0.70^{c}
100	378.64±8.98^{a}	1281.35±26.11^{a}	33.11±0.69^{a}	514.25±8.37^{a}	1143.66±20.80^{a}	32.61±0.54^{b}
AgNPs						
0	194.85±5.26^{f}	684.29±19.95^{f}	13.54±0.36^{g}	278.58±7.38^{e}	612.68±16.94^{g}	16.38±0.43^{g}
50	298.61±7.24^{e}	971.39±27.03^{e}	24.64±0.60^{d}	399.56±8.81^{cd}	834.57±18.60^{f}	27.22±0.56^{e}
100	352.25±8.09^{b}	1212.36±23.03^{b}	29.22±0.64^{b}	472.16±9.49^{b}	1002.96±17.83^{b}	30.53±0.47^{c}
$ZnSO_4$						
0	199.49±5.67^{f}	696.48±17.25^{f}	13.27±0.33^{g}	285.36±7.60^{e}	623.09±16.80^{g}	16.21±0.46^{g}
50	321.12±8.36^{cd}	1071.28±24.25^{d}	23.69±0.56^{e}	399.67±8.05^{cd}	826.35±17.51^{f}	27.72±0.64^{e}
100	351.54±8.34^{b}	1178.31±24.01^{bc}	29.88±0.62^{b}	469.59±7.65^{bc}	967.11±17.59^{c}	34.48±0.58^{a}
ZnONPs						
0	197.15±5.33^{f}	694.79±20.26^{f}	13.24±0.35^{g}	282.78±7.49^{e}	624.18±17.26^{g}	16.18±0.43^{g}
50	282.21±6.84^{e}	924.91±25.74^{e}	21.97±0.53^{f}	363.68±8.02^{d}	752.12±16.76^{c}	24.16±0.50^{f}
100	301.59±6.93^{de}	1102.16±20.94^{cd}	25.89±0.57^{cd}	402.54±8.09^{c}	872.26±15.51^{e}	28.23±0.44^{d}

Table 2: Impact of $AgNO_3$, AgNPs, $ZnSO_4$ and ZnONPs treatments on SOD, POD, CAT and GST activities of marigold and zinnia. Data are mean ± standard error of three independent experiments with three replicates (n=3).Values with different superscripts within same column show significant differences at $p<0.05$ level between treatments according to the Duccan's multiple range test.

Treatments (µM)	Antioxidants enzyme activity (U g^{-1} Fresh weight)							
	Marigold				Zinnia			
	SOD	POD	CAT	GST	SOD	POD	CAT	GST
$AgNO_3$								
0	36.91±1.03^{f}	1506.97±40.20^{g}	812.71±21.72^{f}	392.41±10.58^{g}	48.56±1.37^{f}	1961.83±56.29^{h}	995.27±27.98^{f}	436.58±11.82^{f}
50	48.77±1.18^{e}	1856.87±44.70^{f}	1121.22±25.70^{e}	519.28±10.91^{f}	77.01±1.62^{e}	2684.62±50.99^{g}	1599.89±31.22^{e}	672.61±14.25^{e}
100	59.02±1.22^{c}	2257.81±45.88^{de}	1458.29±27.70^{b}	591.83±12.27^{cd}	94.95±1.50^{c}	3212.88±51.75^{de}	1898.92±24.01^{c}	791.21±12.38^{c}
AgNPs								
0	36.89±0.99^{f}	1512.64±43.05^{g}	815.67±22.89^{f}	389.59±9.69^{g}	48.61±1.34^{f}	1957.61±54.48^{h}	999.67±27.65^{f}	433.92±11.80^{f}
50	57.26±1.39^{cd}	2188.97±57.25^{e}	1289.66±29.04^{cd}	561.85±11.97^{de}	84.54±1.85^{d}	2967.82±63.74^{f}	1734.21±31.94^{d}	746.11±16.76^{d}
100	65.36±1.41^{b}	2479.36±57.26^{c}	1589.94±35.71^{a}	689.81±12.62^{b}	102.12±1.47^{b}	3615.28±55.10^{c}	2098.08±32.34^{b}	872.28±13.25^{b}
$ZnSO_4$								
0	36.26±1.00^{f}	1506.72±40.19^{g}	805.11±21.52^{f}	389.21±10.49^{g}	48.64±1.37^{f}	1956.43±56.14^{h}	998.81±28.08^{f}	441.18±11.95^{f}
50	53.88±1.30^{d}	2162.29±52.06^{e}	1239.12±28.40^{d}	543.84±11.43^{ef}	82.51±1.73^{d}	3149.72±59.83^{e}	1589.42±31.02^{e}	782.55±16.58^{cd}
100	68.89±1.43^{b}	2771.12±56.32^{b}	1584.97±30.11^{a}	658.13±13.64^{b}	101.24±1.60^{b}	3872.18±62.37^{b}	2081.68±26.32^{b}	878.18±13.74^{b}
ZnONPs								
0	36.22±0.99^{f}	1524.17±43.38^{g}	809.71±22.72^{f}	392.19±9.76^{g}	48.69±1.35^{f}	1959.15±54.52^{h}	1002.17±27.72^{f}	438.44±11.92^{f}
50	60.89±1.48^{c}	2382.18±62.30^{cd}	1346.46±30.32^{c}	614.48±13.09^{c}	92.14±2.02^{c}	3378.12±72.55^{d}	1829.41±33.69^{c}	841.91±18.91^{b}
100	74.61±1.59^{a}	3019.60±69.73^{a}	1658.43±37.25^{a}	729.71±13.36^{a}	109.95±1.58^{a}	4045.54±61.66^{a}	2244.78±34.60^{a}	971.58±14.75^{a}

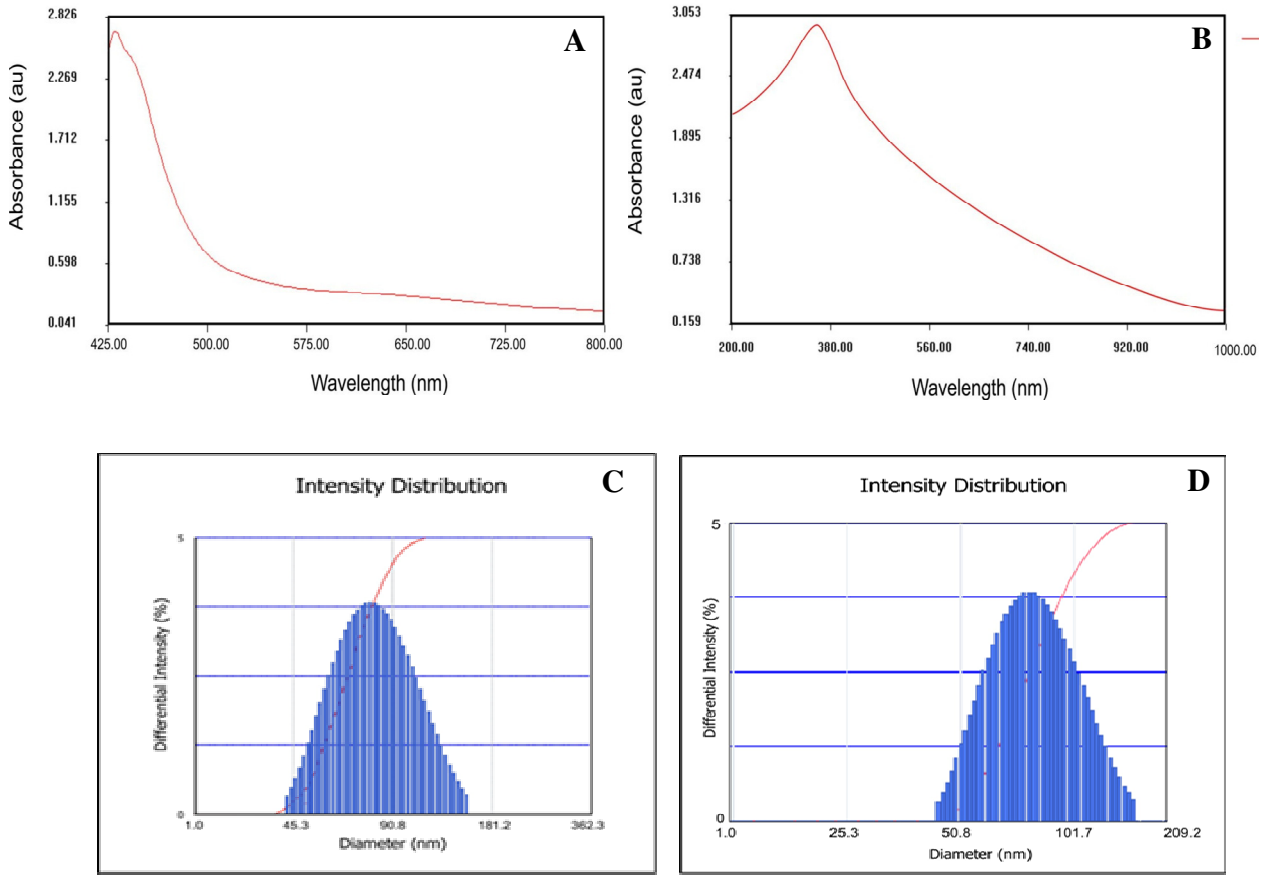

Fig. 1: UV-Vis spectrum of green synthesized AgNPs (A) and ZnO NPs (B) showing the absorption peak at 439 nm and 350 nm respectively.Dynamic light scattering of green synthesized Ag NPs (C) and ZnO NPs (D) showing the average particle size of 79.5 nm and 82.6 nm respectively.

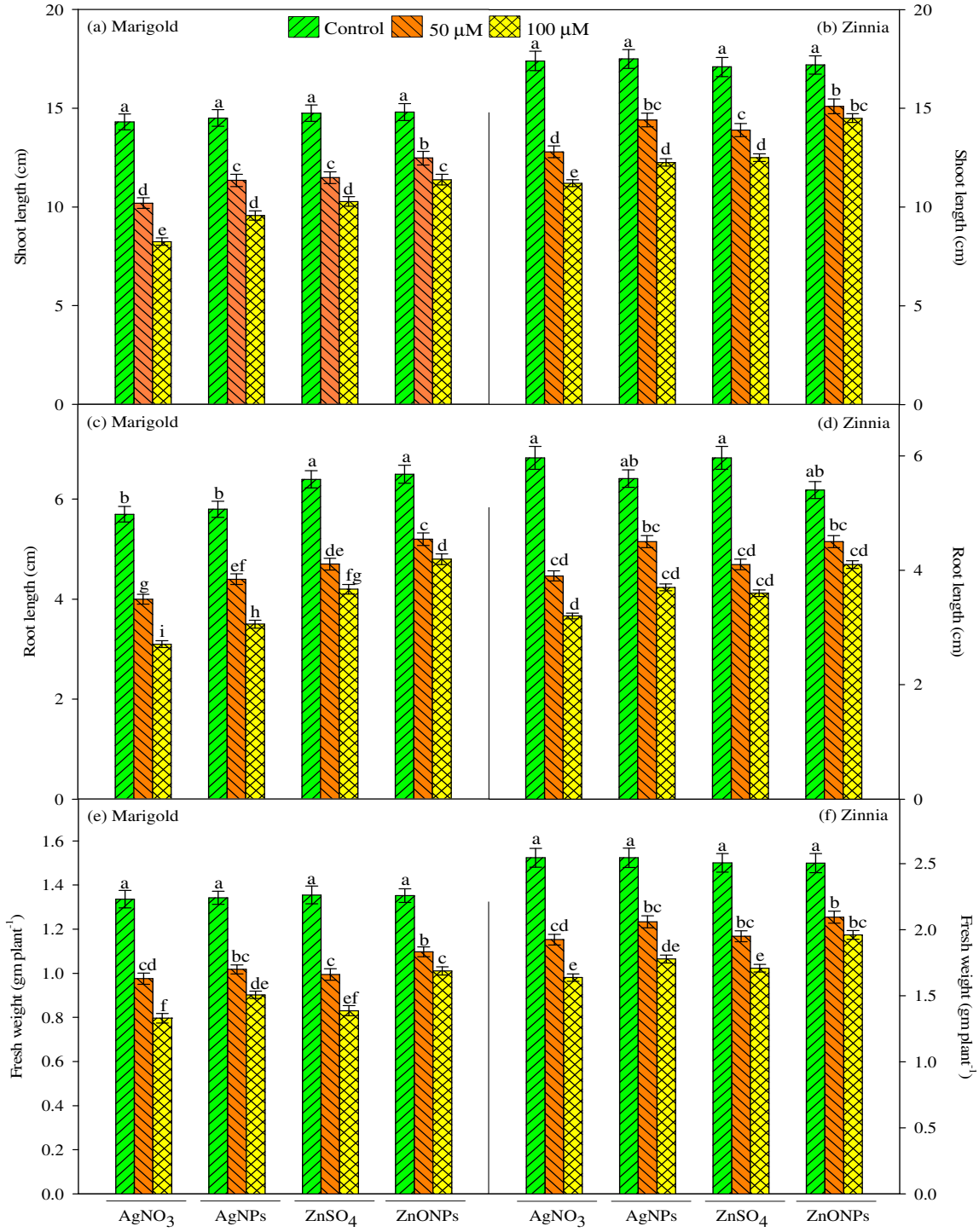

Fig.2: Impact of $AgNO_3$, AgNPs, $ZnSO_4$ and ZnO NPs treatments on root and shoot length, and fresh weight of marigold and zinnia. Data are mean ± standard error of three independent experiments with three replicates (n=3). Bars followed by differentletter(s) show significant difference at *P<0.05* significance level according to the Duncan'smultiple ranges test.

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