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Enhanced Activity of Anti-oxidant Enzymes by Foliar Spray of Nanoscale Zinc Oxide under Drought Stress Conditions in Peanut (*Arachis hypogaea* L.)

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ABSTRACT

Background: Zinc plays an important role in controlling both generations and also detoxifies free oxygen radicals that can damage membrane lipids and sulfhydryl groups. Zinc in particular has an action that prevents membrane damage induced by superoxide radicals that produce NADPH oxidase. The use of zinc oxide (ZnO) nanoparticles in agriculture had a significant impact on crop growth regulation, improved quality and improved stress tolerance. Hence, the current study aimed to study the foliar spray effect of nanoscale zinc oxide (25 nm mean particle size) at different concentrations and chelated bulk zinc suphate ($ZnSO_4$) and comparable control (unsprayed) in peanut to investigate the oxidative stress induction and enhanced antioxidant enzyme activity (SOD, POD, CAT) under conditions of water stress.

Methods: The experimental design was a randomized block design with two water regimes as main treatments *viz.*, well watered (WW) and water stress (WS) conditions, eight foliar sprays along with unsprayed treatment (control) as sub treatments and 3 replications. The eight foliar spray treatments, includes nanoscale ZnO concentrations @ 10, 20, 40, 50, 100, 300, 1000 ppm, chelated bulk $ZnSO_4$ (EDTA based) @ 0.1% (recommended dose). The antioxidant enzyme activity (SOD, POD, CAT) under conditions of both WW and WS was determined in the lab using UV spectrophotometer.

Result: The results of pot culture experiment revealed that nanoscale ZnO at a concentration of 50 ppm increased biomass and pod yield and promoted antioxidant enzyme activity under water stress and well watered conditions compared to chelated bulk $ZnSO_4$. Nanoscale ZnO showed increased activity under lower concentrations and inhibitory activity at higher concentrations that highlights the need for careful use of these particles in agriculture.

Key words: Antioxidant enzymes, Drought stress, Nanoscale Zinc oxide, Peanut.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an essential oil seed, confectionery, food and feed legume crop worldwide. Drought is one of the key environmental problems that hinder crop production and threatens global food security. Moisture stress causes many biochemical, molecular and physiological changes and reactions that affect various cellular and whole plant processes (Prasad *et al.*, 2008). In the current context of global warming and climate change, improving peanut for drought is crucial to ensure high productivity. At the cellular level, one of the effects of salt stress is to hinder the cellular function due to excess production of reactive oxygen species (ROS) (Hasanuzzaman *et al.,* 2012). To reduce ROS accumulation, plants produce antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), keeping ROS lower than toxic limit (Gill *et al.*, 2015).

Detoxification of excess ROS is achieved by antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione reductase and other related enzymes. Super oxide dismutase acts as the first line of defense and detoxifies the super oxide radicals to hydrogen peroxide, while Catalase suppresses hydrogen peroxide. Under limited water availability, the photosynthetic process in the plant is greatly reduced and the continuous accumulation of photo-reducing energy causes more electrochemical forces in the tissues, forming superoxide radicals and hydrogen peroxide (Hernandez *et al.*, 2001).

Zinc is one of the key nutrients in boosting the groundnut yields. Zn deficiency induce higher ROS levels in plants (Cakmak, 2000) and increases SOD, peroxidise (POD) and catalase (CAT) activities (Yu *et al.,* 1998). Yield losses in groundnut due to Zn deficiency have been reported to be 13.3% to 20% (Singh *et al.*, 2004).

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Nanomaterials control the plant growth and development at multiple levels (Kwak *et al.,* 2016). For example, the positive effect of nano-ZnO at low concentrations which is well documented in peanut (Prasad *et al.,* 2012), mung bean (Mahajan *et al.*, 2011) and tomato (Singh *et al.,* 2016), suggest that nanoparticles generate tolerance to different abiotic stresses, *viz.*, drought (Zaimenko *et al.,* 2014), salinity (Almutairi, 2016) and low temperature stress (Hawrylak-Nowak *et al.*, 2010). Mitigation of abiotic stress by nanoparticles was often associated with the increased activity of antioxidant enzymes (Sturikova *et al.*, 2018).

Nanoparticles of less than 100 nm in size fall into the transition zone, producing both positive and negative biological effects on living cells (Nel *et al.*, 2006). Studies related to the response of nanoscale zinc oxide particles on antioxidant enzyme activities in peanut due to drought stress are lacking. Therefore, the objective of the current study is to measure the levels of anti oxidant activity experienced by peanut crop under water deficit stress by the foliar application of nanoscale zinc oxide particles at different concentrations and to confirm the role of antioxidant enzymes in providing drought tolerance.

MATERIALS AND METHODS

The present study was carried at Department of Crop Physiology, Institute of Frontier Technology (IFT), Regional Agricultural Research Station (RARS), Acharya N G Ranga Agricultural University (ANGRAU), Tirupati andhra Pradesh (A.P.) during 2015-16. ZnO nano particles of size 15-30 nm from the Department of Nanotechnology, IFT, RARS, ANGRAU, Tirupati, A.P. were used in the study. Nano crystalline zinc oxide has been prepared using an oxalate decomposition process. The samples are characterized by transmission electron microscopy (HRTEM, JEOL 3010), scanning electron microscopy (SEM, FEI quanta 200) and energy dispersive analysis X-rays (EDA X FEI quanta 200) (Prasad *et al.,* 2012).

Peanut seeds of variety Narayani received from the Department of Genetics and Plant Breeding, RARS, ANGRAU, Tirupati, A.P. The pots (20 cm × 40 cm) were filled with equal quantity of soil and four seeds were sown per pot. Care was taken to use the same soil in all pots to reduce soil variability. The experimental design was a randomized block design with two water regimes as main treatments *viz.*, well watered (WW) and water stress (WS) conditions, eight foliar sprays along with unsprayed treatment (control) as sub treatments and 3 replications. Water stress (WS) regime is imposed by withholding irrigation for 40 days *i.e.*, from 40 to 80 days after sowing (DAS). The eight foliar spray treatments, includes nanoscale ZnO concentrations @ 10, 20, 40, 50, 100, 300, 1000 ppm, chelated bulk $ZnSO_4$ (EDTA based) @ 0.1% (recommended dose). The pots were maintained at field capacity (FC) until harvest under WW treatment, whereas under WS treatment, water stress was imposed @ 40% FC during flowering when 50% of the plants (at least two out of four plants) reached flowering. Foliar spray treatments were applied at 40 DAS and sampling was done after one week of foliar spray treatment.

SOD (EC 1.15.1.1) enzyme activity was estimated by its ability to inhibit photochemical reduction of Nitrobluete trazolium (NBT) method according to Madamanchi *et al.,* 1994). In this assay, 1 unit of SOD is defined as the amount required to inhibit the photoreduction of NBT by 50% and SOD activity was measured spectrophotometrically at 560 nm.

The activity of POD (EC 1.11.1.7) was measured by increased absorption of tetraguaiacol formation at 470 nm and the POD activity was measured as per extinction coefficient of its oxidation product, tetraguaiacol ε = 26.6 mM^{-1} cm^{-1} (Castillo *et al.,* 1984). Enzyme activity was estimated by monitoring the increase in absorption at 470 nm due to guaiacol oxidation.

CAT activity (EC 1.11.1.6) was determined by measuring the H_2O_2 disappearance (Aebi, 1984) in the reaction compound. The decrease in absorption at 240 nm on a UV spectrophotometer was observed for 1 min. Enzyme activity was measured by calculating the amount of H_2O_2 decomposed.

At harvest, shoot dry weight (SDW) and pod yields were recorded after drying of individual plants and expressed in g $plant^{-1}$. The statistical analysis was assessed by Two Way Analysis of Variance (ANOVA) using GENSTAT software and the critical difference values were calculated at 5% significance level to compare the mean values.

RESULTS AND DISCUSSION

Of the newest technological innovations, nanotechnology offers an important opportunity and occupies a prominent position improving the existing crop management strategies and food production practices (Nair *et al.*, 2010). The nano scale ZnO particles with mean diameter of 25 nm are crystalline as revealed by the high magnification image and the lattice of ZnO is clearly seen. Zhu *et al.,* 2009 also reported lattice spacing of 0.26 nm to 0.28 nm of WURTZITE ZnO.

Application of nano-ZnO promoted melatonin synthesis and increased the antioxidant enzyme system, which reduces drought-induced damage to mitochondria and chloroplast in corn (Luying Sun *et al.*, 2020). In the current study, water stress (WS) regime from 40 to 80 days after sowing (DAS) *i.e.*, from pegging to pod formation stage, reduced soil moisture content by 42 % at 0-5 cm soil depth and 40 % at 15-30 cm soil depth compared to well watered (WW) plot where moisture level was maintained optimum throughout the crop growth period. Irrespective of the foliar treatments, the mean activity of SOD, POD and CAT increased significantly by 31.2%, 12.0 and 28.0% respectively under WS compared to WW (Table 1). Kusvuran and Yildiz Dasgan (2017) in bean and Koushik Chakraborty *et al.* (2015) in peanut reported higher activity of antioxidant enzymes under moistures stress compared to control conditions. Under WS conditions, the activity of SOD, POD and CAT increased by 11%, 12% and 6.0% respectively in chelated $ZnSO_4$ @ 1000 ppm treatment compared to unsprayed control. SOD value increased

Table 1: Foliar treatment of nanoscale ZnO at different concentrations and chelated bulk $ZnSO_4$ on SOD, POD and CAT enzyme activity of peanut leaves under well water (WW) and water stress (WS) conditions.

Treatments	SOD activity (OD units min^{-1} g^{-1})		POD activity (OD units min^{-1} g^{-1})		CAT activity (OD units min^{-1} g^{-1})		Shoot biomass (g $plant^{-1}$)		Pod yield (g $plant^{-1}$)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
T1: Control (Unsprayed)	0.323	0.435	0.694	0.775	0.343	0.475	6.720	4.56	11.50	8.33
T2: Chelated bulk $ZnSO_4$ @ 1000 ppm	0.336	0.486	0.713	0.868	0.359	0.505	7.030	5.24	12.70	8.90
T3: Nanoscale ZnO@ 10 ppm	0.366	0.461	0.777	0.842	0.394	0.486	7.190	5.33	11.50	9.09
T4: Nanoscale ZnO@ 20 ppm	0.343	0.489	0.734	0.863	0.368	0.501	7.420	5.48	12.20	9.12
T5: Nanoscale ZnO@ 40 ppm	0.381	0.505	0.788	0.898	0.402	0.520	7.640	5.59	13.70	9.64
T6: Nanoscale ZnO@ 50 ppm	0.402	0.544	0.821	0.987	0.434	0.577	8.210	6.16	14.40	11.02
T7: Nanoscale ZnO@ 100 ppm	0.346	0.447	0.743	0.817	0.358	0.474	6.290	5.49	10.50	7.90
T8: Nanoscale ZnO@ 300 ppm	0.279	0.335	0.619	0.705	0.318	0.377	4.780	2.98	7.60	5.40
T9: Nanoscale ZnO@ 1000 ppm	0.164	0.155	0.522	0.402	0.205	0.174	4.050	2.58	5.40	3.14
Mean	0.327	0.429	0.712	0.795	0.353	0.454	6.592	4.82	11.06	8.06
	SEm	CD (5%)	CV (%)	SEm	CD (5%)	CV (%)	SEm	CD (5%)	CV (%)	SEm	CD (5%)	CV (%)	SEm	CD (5%)	CV (%)
Main treatments	0.005	0.012	3.6	0.004	0.012	4.6	0.002	0.005	3.2	0.14	0.40	6.5	0.28	0.85	7.5
Sub treatments	0.010	0.031		0.014	0.039		0.003	0.010		0.43	1.26		0.51	1.46	
Interaction	0.021	0.057		0.019	0.055		0.005	0.014		0.62	1.78		1.21	3.06	

Main Treatments: Water Regimes; Sub treatments: Foliar sprays.

significantly even with $ZnSO_4$ spraying denotes the stimulating action of Zn as it is one of the cofactors of SOD (Cu/Zn SOD). Zinc may act as a scavenger of oxygen free radical production for mitigating the injury on biomembranes under salt stress and hence, treatment with zinc could reduce the effects of salinity stress in soybean plants and adequate zinc also prevents uptake and accumulation of Na in shoot, by increasing membrane integrity of root cells (Weria Weisany *et al.,* 2012).

In the present study, among the different concentrations of nanoscale ZnO, significantly highest SOD, POD and CAT activity recorded in nanoscale ZnO @ 50 ppm treatment compared to unsprayed control and chelated bulk $ZnSO_4$ under WW conditions. Similarly, foliar spray of nanoscale ZnO @ 50 ppm significantly recorded highest SOD, POD and CAT activity and increased by 25.1, 27.4 and 25.5 % respectively compared to unsprayed control whereas SOD, POD and CAT activity increased by 11.9, 13.7 and 22.6 % respectively compared to chelated bulk $ZnSO_4$ respectively under WS conditions (Table 1; Fig 1). Drought results in accumulation of ROS, leading to lipid peroxidation of the cell membrane system, which is mainly due to the production of malondialdehyde. Luying *et al.* (2020), documented that, nano-ZnO significantly increased the activities of SOD, CAT and APX, which reduced the accumulation of H_2O_2 under drought and in agreement with this, the relative transcript abundance of Fe/Mn SOD, Cu/Zn SOD, APX and CAT in nano-ZnO plants was significantly up-regulated higher than that of non ZnO treatment plants under drought.

Biomass accumulation improved in the ZnO nanoparticle treated Chickpea seedlings and this response was associated with lower activity of prominent antioxidant enzymes, compared to control (Burman *et al.,* 2013). The study by García-López *et al.* (2018) on the effects of suspensions of zinc oxide nanoparticles on germination of *Capsicum chinense* seeds resulted in increased activities of peroxidase and catalase. *Vigna mungo* seeds treated with different concentrations of ZnO nano particles showed significant induction of the activities of Glutathione reductase, Guaiacol peroxidase and Catalase activity (Pavani *et al .,* 2020).

Sharifi *et al.* (2012) reported that the tolerant wheat lines revealed high activity of POD and CAT enzyme under drought conditions with higher yields, thus showing positive correlation between enzyme activity and yield in drought conditions. Prasad *et al.,* 2012 reported that, ZnO in the nanoscale form is absorbed by plants to a larger extent unlike bulk $ZnSO_4$ and these particles proved effective in enhancing plant growth, development and yield.

The inherent small size and the associated large surface area of nanoscale ZnO fertilizer may increase the uptake of Zn. All these factors may be responsible to give higher yields for nanoscale ZnO compared to chelated $ZnSO_4$.

The present study reveals that, shoot biomass and pod yields recorded highest under WW compared to WS conditions. Shoot biomass and pod yields decreased by 37% under WS compared to WW conditions. Chelated $ZnSO_4$ foliar treatment recorded 5.0% increase in shoot biomass under both WW and WS conditions compared to unsprayed control whereas Chelated $ZnSO_4$ foliar treatment recorded 10.0% and 7.0% enhanced pod yields under WW and WS conditions compared to unsprayed control (Table 1). Under various stressed conditions, optimum Zn concentrations enhanced the plant growth. The lower dry weight in the control treatments may be due to water limited stress as

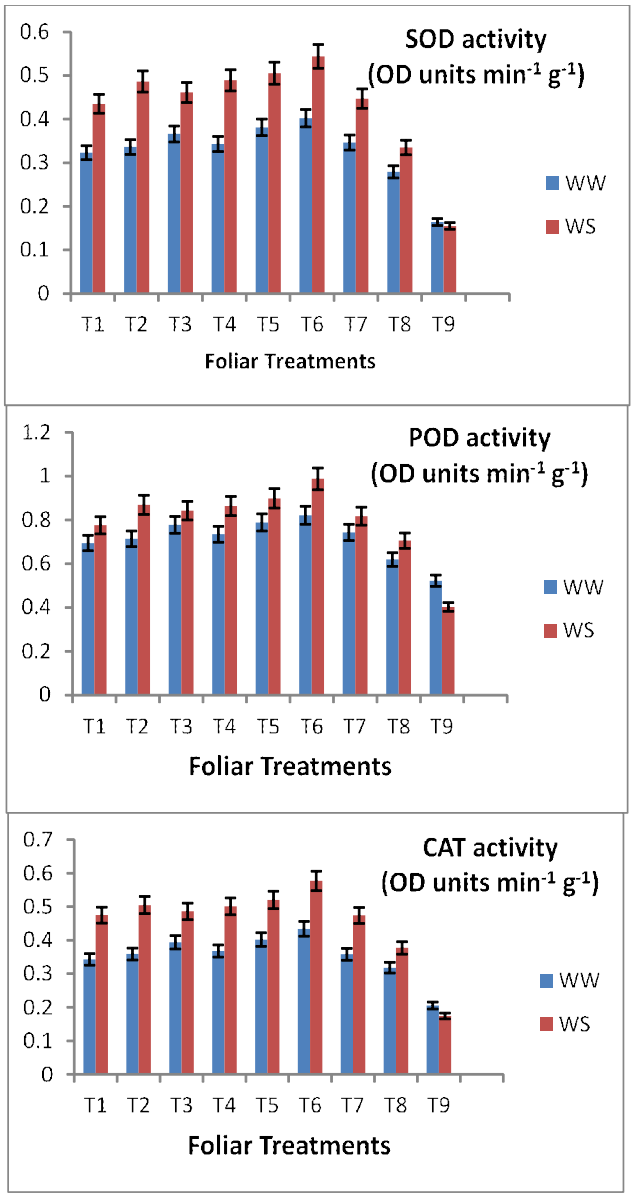

Fig 1: Anti oxidant enzyme activity by foliar spray of nanoscale zinc oxide under drought stress conditions in peanut.

T1: Unsprayed treatment (control), T2: chelated bulk $ZnSO_4$ (EDTA based) @ 0.1%, T3: Nanoscale ZnO @ 10 ppm, T4: Nanoscale ZnO@ 20 ppm, T5: Nanoscale ZnO@ 40 ppm, T6: Nanoscale ZnO, @ 50 ppm, T7: Nanoscale ZnO@ 100 ppm, T8: Nanoscale ZnO@ 300 ppm, T9: Nanoscale ZnO@ 1000 ppm.

water limited stress have been known to severely reduce the growth of plants (Adrees *et al.,* 2020). In the present study, significantly highest shoot biomass and pod yields were recorded at foliar spray treatment of nanoscale ZnO @ 50 ppm compared to unsprayed control and chelated bulk $ZnSO_4$ under WW conditions. Foliar spray of nanoscale ZnO @ 50 ppm significantly recorded highest shoot biomass and pod yields and increased by 35.0 and 32.3 % respectively compared to unsprayed control increased by 17.6 and 23.8 % respectively compared to chelated bulk $ZnSO_4$ under WS conditions (Table 1). Adrees *et al.* (2021) also reported that, the shoot, root and grain dry weights enhanced more under water deficit stress compared to normal water conditions where 100 mg l^{-1} ZnO NPs were applied, over their respective control treatments.

The study by Hesham Alharby *et al.* (2016) reveals that, lower concentration of nanoscale ZnO was beneficial compared to the higher concentration of nanoscale ZnO, at both salinity levels. The current study also observed that the nanoparticles at higher concentrations exhibit inhibitory effects on plants in terms of enzymatic activity and promontory activity at lower concentrations. At 1000 ppm, the antioxidant enzyme activity, shoot weight and pod yield significantly decreased compared to unsprayed control under both WW and WS conditions respectively in terms of plant growth showing phytotoxicity (Table 1).

CONCLUSION

Foliar application of chelated bulk $ZnSO_4$ increased antioxidant enzyme activity, shoot biomass and pod yield compared to unsprayed control both under WW and WS conditions. Among different concentrations of nanoscale ZnO, foliar treatment of nanoscale ZnO @ 50 ppm increased the activity of antioxidant enzymes, shoot biomass and pod yields both under WW and WS conditions compared to unsprayed control and chelated bulk $ZnSO_4$. Nanoscale ZnO at higher concentrations showed inhibitory activity of antioxidants, shoot biomass and pod yield both under WW and WS conditions. The research is continuing for further exploitation of nano ZnO effects on other antioxidant enzymes in peanut.

Conflict of interest: None.

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