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Response of Different Doses of Zinc Oxide Nanoparticles in Early Growth of Mung Bean Seedlings to Seed Priming under Salinity Stress Condition

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ABSTRACT

Background: Salinity impacts physiological processes, including germination, seedling development, ionic balance and water relations, leading to growth inhibition. Mung bean's early stage is susceptible to salt stress. Our study aimed to mitigate salt stress at early stage using zinc oxide nanoparticles (ZnO-NPs) to enhance mung bean tolerance.

Methods: Pot experiment was carried out to incorporate ZnO-NPs into mung bean seedlings. Two Mung bean genotypes, TMB-37 (tolerant) and MH-1314 (sensitive), were chosen. Seeds were primed with ZnO-NPs at various concentrations (0.00 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm) and shown in saline soil.

Result: ZnO-NP priming notably increased germination percentage, shoot length and shoot dry weight in both genotypes. In 25-days-old seedlings, ZnO-NPs elevated antioxidant enzyme activity, proline content, especially superoxide dismutase (SOD) and peroxidase (POX) activity, while reducing lipid peroxidation and membrane injury. 1000 ppm ZnO-NPs had the negative impact on the root trait of sensitive genotype. Lower doses of ZnO-NP (50 ppm) concentrations was very effective in mitigating the adverse effect of salinity stress in both the genotypes offering a key approach for Mung bean's salt stress mitigation.

Key words: Lipid peroxidation, Mung bean, Salinity, Superoxide dismutase, ZnO-nanoparticle.

INTRODUCTION

Salinity significantly affects plant growth, causing osmotic stress due to ion toxicity in roots, disrupting water/nutrient balance and impacting growth and photosynthesis (Ijaz *et al*., 2019). This stress induces Reactive Oxygen Species (ROS), damaging membranes, integrity, signalling and compartmentalization process (Hasanuzzaman *et al*., 2020). Salt's impact and its secondary stressors impede progress in breeding salt-tolerant crops (Arif *et al*., 2020). Salinization from natural/human factors limits crop output. Mung bean germination, fresh and dry weight, seedling length, antioxidant enzyme such as catalase, peroxidase and superoxide dismutase activity, protein synthesis and photosynthesis as well as yield qualities are also affected by salinity stress (Singh and Singh, 2011).

Zinc (Zn), a vital micronutrient, plays a role in enzymes activity, auxin synthesis and carbohydrate metabolism (Das and Das, 2019). It influences enzyme, cell, pigment processes, membrane integrity and phospholipid accumulation (Rizwan *et al*., 2019; Czyżowska and Barbasz, 2022). Overall Zn boosts production of growth regulator like IAA, encouraging growth, Scavange ROS, enhancing abiotic stress tolerance (Zhang *et al*., 2021; Ahmad and Akhtar, 2019). Zn limited solubility in saline soil restricts access in condition of salinity, so directly priming of seed with ZnO-NPs could serve as important agronomic approach and additional strategy to complex breeding process to enhance tolerance of mungbean crop against salinity. However, the potential of ZnO-NPs to mitigate salinity's adverse effects remains largely unexplored (Keller *et al*., 2013). The influence of various doses of ZnO-NPs at early stage of mung bean crop, as well as dosage optimization and its impact on associated physiological and biochemical parameters, were the main focuses of our investigation.

Therefore, our study proposed to explore the inclusion of nanoparticles in plant system in regard to mitigate the toxic effect of salinity stress in which a limited study has been performed especially in legume crops.

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MATERIALS AND METHODS

The research conducted at, department of Botany, Plant Physiology and Biochemistry, Dr. Rajendra Prasad Central Agricultural University in Samastipur, Bihar, 2019. Mung bean genotypes TMB-37 and MH-1314 were collected from genetics and plant breeding department. Control soil were collected from the Pusa farm and saline soil from Motihari village, Tazpur district, Bihar. Detail of soil physical and chemical parameters and meteorological data are mentioned in Table 1. ZnO-NPS formed by dissolving reagent-grade zinc chloride ($ZnCl_2$) in distilled water for 0.25M solutions. A mix of 20 ml citric acid solution (pH>6) and 10% diluted citric acid heated led to haziness and deposition. Incubation and separation yielded 20-35 nm particles. For priming healthy seeds of mung bean genotype were used and priming were done with different concentrations of ZnO-NPS solutions (0.00, 50.0, 100, 500 and 1000 ppm) for 3 hours then air-dried and planted in plastic pots in separate treatments. Manual watering was maintained. Germination (%) calculated as:

$$\text{Germination \%} = \frac{\text{Emerged seedlings}}{\text{Total seeds}} \times 100$$

Seedling length in cm, dry weight in mg were assessed and membrane stability index (MSI) as per Sairam, (1994) Fresh leaves (0.1g) in tubes with distilled water underwent electrical conductivity tests at 40°C (30 mins) and 100°C (10 mins). The MSI (%) is determined using following formula.

$$\text{MSI} = \left[1 - \left(\frac{C1}{C2}\right)\right] \times 100$$

Where,

C_1 = Conductivity at 40°C.

C_2 = Conductivity at 100°C.

Lipid peroxidation were measured as per TBA technique (Assaha *et al*., 2015). Fresh leaves homogenized in extraction buffer (10 mM HEPES, pH 7.0, 15% tricarboxylic acid, 0.375% TBA (thiobarbituric acid), 0.25 N HCl, 0.04% BHT (butylated hydroxyl toluene), 2% ethanol, heated at 95°C, then centrifuged. Supernatant wavelength measured at 532-600 nm. MDA (melondialdehyde) concentration determined via extinction coefficient (155 mM^{-1} cm^{-1}), ignoring absorbance at 600 nm (Heath and Packer, 1968).

Antioxidant estimation (enzymatic and non-enzymatic)

Leaf sample (1 g) grinded in 10 ml extraction solution (1 M phosphate buffer, pH 7.5, 0.5 mM EDTA). Centrifugation at 15,000 rpm for 20 minutes determined enzyme content in supernatant. Superoxide dismutase (SOD) activity calculated per Dhindsa *et al*. (1981) 3 ml of the reaction mixture containing 0.2 ml; 200 mM nitro blue tetrazolium chloride (NBT); 0.1 ml; 2.25 mM, sodium carbonate 0.1 ml; 1.5 M, enzyme 0.1 ml; 1.5 M and distilled water. Test tubes were placed under two 15W fluorescent lamps for 15 minutes to start the reaction after being filled with 0.1 ml of 2 M riboflavin followed by placing the tubes in a dark condition. 560 nm was used to measure the absorbency. Activity expressed as mg^{-1} protein $minute^{-1}$. Peroxidase (POX) activity estimated as per Castillo *et al*. (1984) method. Activity calculated using tetra-guaiacol oxidation product's extinction coefficient, absorbance at 470 nm. Activity expressed as micromole tetra-guaiacol formed min^{-1} mg^{-1} protein. Proline Content Determination was done as per Bates *et al*., (1973) Fresh leaves (0.5g) extracted with sulfosalicylic acid, Ninhydrin and glacial acetic acid Sample heated to 100°C with added toluene. Absorbance measured at 530 nm, using toluene as blank. Proline expressed as mg/g fresh weight. Statistical analysis were done using Genstat 14th edition data analysis software, in which three factor ANOVA was applied to data collected from various observations for various treatments comprising three replication under (complete randomised design) CRD. Post to ANOVA, Duncan's multiple range test used to ascertain significant differences in treatment means at 5% level of significance.

RESULTS AND DISCUSSION

Germination percentage

Salinity stress significantly reduced germination percentage in both genotypes. The susceptible MH-1314 showed higher reduction (30.14%) than the tolerant TMB-37 (24.99%). Both genotypes exhibited increased germination percent in control as well as saline condition when primed with ZnO-NPS. However, 50 ppm treatment was found highly effective in increasing the germination percentage in TMB-37 (7.14% and 23.81%) and MH-1314 (7.40% and 26.84%) under control and saline condition respectively (Table 2).

Sensitive genotype MH-1314 had higher reduction percent than tolerant TMB-37, indicating better germination and salt resistance. Similar is documented in *Brassica napus* (Ali *et al*. 2021), salinity's impact observed may be attributed to water deficit due to osmotic stress and hindered water intake (Ahemed *et al*., 2017; Sinha *et al*., 2020). Prior studies on crops like wheat showed improved germination with zinc nanoparticles (Rai-Kalal and Jajoo 2021). Zn's multifaceted roles in plants, including enzyme co-factor, protein, carbohydrate metabolism (Gardea-Torresdey, 2014) and antioxidant enzymes and ROS scavenging (Jiang *et al*., 2014), possibly enhance germination vital processes.

Seedling length and dry weight

Salinity stress significantly reduces shoot length (SL) and shoot dry weight (SW) in both genotypes compared to controls. In sensitive genotype MH-1314 percent decrease was higher in SL (57.69%) and SW (17.54%) than tolerant genotype TMB-37 in SL (56.84 %) and SW (15.96%). Similarly, susceptible genotype (MH-1314) shows higher decrease percent in root length (RL) (54.05%) and root dry weight (RW) (17.25%), while TMB-37 displays non-significant increase in RL and significant decrease in RW (15.57%) against its respective control.

Zinc nanoparticle doses (50, 100, 500, 1000 ppm) significantly enhance SL and SW in both genotypes in both control and saline condition. However, 50 ppm treatment stand out i.e. by increasing following percent increase such as in TMB-37 SL: 11.69%, MH-1314 SL: 24.38%; TMB-37 SW: 21.81%, MH-1314 SW: 27.48% under control condition and TMB-37 SL: 36.23%; MH-1314 SL: 49.63%; TMB-37 SW: 18.98%; MH-1314 SW: 16.31% under saline condition. Similar trends occur in RL and RW, increased in percent change such as in TMB-37 RL: 37.82%, MH-1314 RL: 27.02%; TMB-37 RW: 21.94%, MH-1314 RW: 27.64% under control condition and TMB-37 RL: 34.32%, MH-1314 RL: 19.81%; TMB-37 RW: 18.52%, MH-1314 RW: 16.70% under saline condition (Table 3, Fig 1).

Research shows lower doses enhance seedling traits such as 50 ppm for broad beans, even lower concentration for crops like rapeseed (Kahlel *et al*., 2020; Hezaveh *et al*., 2019). Zinc's roles include water uptake, auxin biosynthesis, cell division and enlargement correlating with improved shoot and root traits (Kahlel *et al*., 2020). Results of reduced root length and dry mass in wheat with higher doses such as 1100 ppm ZnO nanoparticles aligns with our result (Du W *et al.* 2019) in which susceptible genotype's MH-1314 root traits decreases with 1000 ppm i.*e*. RL (13.22%) and RW (8.96%) in control condition and RL (17.33%) and RW (11.43%) under saline condition. Roots are foremost to get affected by ion toxicity derived from salt stress showing susceptibility to morphological and dry mass changes (Dimkpa *et al*., 2012). And also the fact that Zn is

Table 1: Details of soils and meteorological data.

Properties of soil	Control	Saline soil
Soil electrical conductivity (dSm^{-1}) at ambient temperature (25°C)	0.34	4.10
At ambient temperature (25°C), the electrical conductivity of saturation extract (dSm^{-1})	1.95	7.80
Soil pH	7.20	8.27
Sand (%)	18.09	48.09
Silt (%)	41.71	19.00
Clay (%)	38.53	32.71
Organic carbon (%)	1.18	0.25
Available nitrogen (kg ha^{-1})	225	95
Available P_2O_5 (kg ha^{-1})	63.98	31.58
Available K_2O (kg ha^{-1})	226	305
Zn (ppm)	0.98	0.60
Cu (ppm)	0.80	0.81
Fe (ppm)	2.72	4.49
Mn (ppm)	3.58	2.77
B (ppm)	0.31	0.31
S (ppm)	72.02	70.02
Meteorological parameters		
Average maximum temperature (°C)	32±5	
Average minimum temperature (°C)	22±5	
Rainfall (mm)	10.4	
Relative humidity (%) morning	85±5	
Relative humidity (%) evening	60±5	
Wind speed (km/hr)	11.68	
Evaporation (mm)	3.87	

Table 2: Effect of seed priming with ZnO nanoparticles on germination percentage (%) of mungbean (*Vigna radiata*) genotypes under salinity stress condition.

Genotypes	Condition	ZnO-NPs (ppm)				
		0 .00	50.00	100.0	500.0	1000
TMB-37	Control	93.33±0.33^{c}	100.0±0.10^{a}	100.0±0.33^{a}	93.33±0.33^{c}	93.33±0.33^{c}
	Saline	70.00±0.33^{i}	86.67±0.33e	76.67±0.33^{g}	80.00±0.10^{f}	76.67±0.33^{g}
MH-1314	Control	90.00±0.33^{d}	96.67±0.33^{b}	96.67±0.33^{b}	90.00±0.33^{d}	90.00±0.10^{d}
	Saline	63.33±0.33^{k}	80.67±0.33^{f}	73.33±0.33^{h}	70.00±0.14^{i}	66.67±0.33^{j}

Results represented are the averages of three replicates *(n=3)* and distinct superscript letters for each treatment are used to indicate statistically significant variations (pd"0.05) between treatments.

Table 3: Effect of seed priming with ZnO nanoparticles on 10-days old Mung bean (*Vigna radiata*) seedlings under salinity stress condition (±SEM) on Shoot length (cm), Shoot dry weight (mg), Root length (cm), Root dry weight (mg).

Genotypes	Condition	Treatments (ZnO-NPs)	Shoot length (cm)	Shoot dry weight (mg)	Root length (cm)	Root dry weight (mg)
TMB-37	Control	0 PPM	16.50±0.50^{b}	18.8±0.26^{de}	4.23±0.50^{hi}	13.17±0.29^{de}
		50 ppm	18.43±0.30^{a}	22.9±0.64^{a}	5.83±0.17^{de}	16.06±0.59^{a}
		100 ppm	18.21±0.20^{a}	21.7±0.76^{b}	5.07±0.57^{efg}	15.24±0.45^{b}
		500 ppm	18.34±0.31^{a}	19.4±0.26^{d}	4.83±0.44^{fgh}	13.59±0.31^{d}
		1000 ppm	18.20±0.30^{a}	21.0±0.30^{bc}	4.83±0.34^{fgh}	13.59±0.32^{d}
	Saline	0 PPM	07.12±0.58^{f}	15.8±0.54^{gh}	4.37±0.19^{ghi}	11.12±0.55^{g}
		50 ppm	09.70±0.47^{d}	18.8±0.29^{de}	5.87±0.24^{d}	13.18±0.16^{de}
		100 ppm	09.10±0.58^{de}	17.0±0.59^{f}	5.43±0.33^{def}	11.94±0.58^{f}
		500 ppm	08.90±0.38^{de}	16.4±0.19^{fg}	5.60±0.15^{de}	11.53±0.65^{fg}
		1000 ppm	08.84±0.32^{de}	16.4±0.29^{fg}	5.43±0.15^{def}	11.54±0.63^{fg}
MH-1314	Control	0 PPM	13.00±0.50^{c}	17.1±0.60^{f}	7.03±0.03^{c}	11.94±0.59^{f}
		50 ppm	16.17±0.59^{b}	21.8±0.48^{b}	8.93±0.30^{a}	15.24±0.44^{b}
		100 ppm	15.37±0.27^{b}	19.4±0.36^{d}	8.30±0.44^{ab}	13.59±0.31^{d}
		500 ppm	15.40±0.29^{b}	18.8±0.57^{de}	7.70±0.44^{bc}	13.18±0.52^{de}
		1000 ppm	15.90±0.47^{b}	18.8±0.56^{de}	6.10±0.22^{d}	10.87±0.49^{g}
	Saline	0 PPM	05.50±0.29^{g}	14.1±0.32^{i}	3.23±0.15^{jk}	09.88±0.39^{h}
		50 ppm	08.23±0.50^{ef}	16.4±0.44^{fg}	3.87±0.38^{ij}	11.53±0.33^{fg}
		100 ppm	07.17±0.73^{f}	15.8±0.15^{gh}	3.90±0.56^{ij}	11.12±0.56^{g}
		500 ppm	07.10±0.73^{f}	15.9±0.31^{gh}	3.80±07.76^{ij}	11.12±0.04^{g}
		1000 ppm	07.20±0.55^{f}	15.3±0.45^{h}	2.67±0.35^{k}	08.75±0.38^{i}

Result represented are the averages of three replicates *(n=3)* and distinct superscript letters for each treatment are used to indicate statistically significant variations (p≤0.05) between treatments.

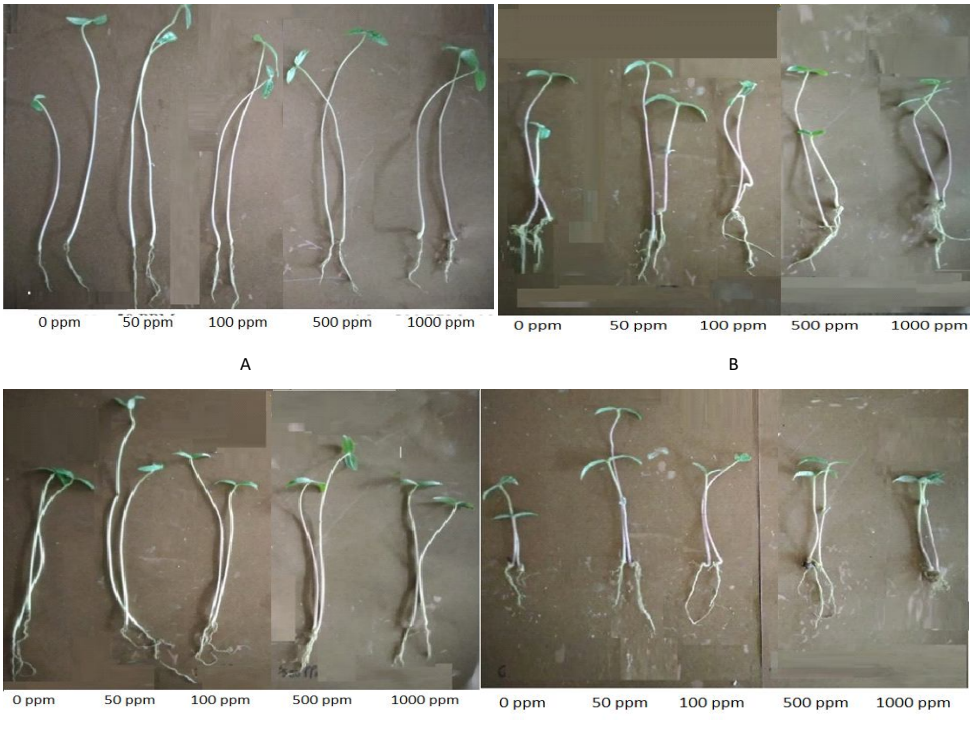

Figure shows seedling length of: TMB-37 under control condition (A); TMB-37 under saline condition (B); MH-1314 under control condition (C); MH-1314 under saline condition (D).

Fig 1: Effect of seed priming with various ZnO nanoparticle concentrations on the length of seedlings in 10-day-old Mung bean genotypes.

Table 4: Effect of seed priming with ZnO nanoparticles on proline content (µg g $^{-1}$ FW), (MSI) membrane stability index (%), (MDA content) lipid peroxidation (nmol TBARS g^{-1} FW), (SOD) superoxide dismutase activity (Units mg $^{-1}$ protein min^{-1}) and (POX) peroxidase activity (µg mol TG mg $^{-1}$ protein min^{-1}) (±SEM) of 25-days old Mung bean (*Vigna radiata*) seedlings under salinity stress condition.

Genotypes	Condion	ZnO-NPs	Proline	MSI	MDA content	SOD	POX
TMB-37	Control	0 PPM	44.87±0.37^{g}	67.56±0.30^{c}	36.03±0.61^{c}	16.74±0.16^{e}	3.72±0.15^{d}
		50 PPM	47.74±0.32^{e}	71.43±0.14^{a}	31.39±0.51^{d}	17.29±0.29^{de}	4.04±0.06^{cd}
	Saline	0 PPM	56.64±0.29^{c}	63.29±0.23^{e}	40.76±0.15^{b}	20.11±0.11^{b}	4.13±0.08^{bc}
		50 PPM	63.64±0.82^{a}	67.49±0.24^{cd}	35.38±0.32^{c}	21.54±0.29^{a}	4.84±0.31^{a}
MH-1314	Control	0 PPM	42.08±0.76^{h}	66.65±0.28^{d}	30.94±0.37^{d}	15.91±0.10^{f}	3.66±0.13^{d}
		50 PPM	45.12±0.78^{f}	69.21±0.31^{b}	28.70±0.87^{e}	16.92±0.16^{e}	3.98±0.08^{cd}
	Saline	0 PPM	52.80±0.35^{d}	58.33±0.16^{g}	46.04±0.26^{a}	17.77±0.14^{d}	4.11±0.08^{cd}
		50 PPM	58.19±0.42^{b}	61.39±0.29^{f}	44.89±0.41^{b}	18.79±0.18^{c}	4.63±0.20^{ab}

Results represented are the averages of three replicates *(n=3)* and distinct superscript letters for each treatment are used to indicate statistically significant variations ($p \leq 0.05$) between treatments.

micronutrient, when applied in higher concentration might have led to its adverse effect, *e.g.*, 1000 ppm ZnO-NPs.

Proline accumulation

Compared to controls, salinity stress significantly elevates proline with a higher percent increase in TMB-37 (26.23%) vs. MH-1314 (25.51%) (Table 4). Enhanced proline content under salt stress is widely documented (Soleimani *et al*. 2017). Proline, crucial for cell osmoregulation under salt stress aids in countering osmotic stress through improved protein synthesis (Ahmad *et al*., 2019; Pradhan *et al*., 2023). Seed priming with 50 ppm enhances proline content in TMB-37 (4.88%) and MH-1314 (7.25%) under control condition and TMB-37 (12.35%) and MH-1314 (10.198%) under saline condition. Similar findings was reported in rapeseed (Hezaveh *et al*. 2019). Proline, a non-enzymatic antioxidant, combats free radicals and singlet oxygen under stress, possibly induced by Zn's interaction with the non-enzymatic antioxidant system and protein synthesis (Szabados *et al*. 2010). Conversely, Zn treatments might generate modest ROS levels for tolerance against toxicity (Du W *et al*. 2019).

Membrane stability and lipid peroxidation

Salinity stress reduces MSI significantly higher in MH-1314 (12.48%) vs. TMB-37 (6.32%), while increase lipid peroxidation in MH-1314 (48.80%) vs. TMB-37 (13.11%) against its respective control (Table 4). Similar findings in prior research report higher lipid peroxidation and MDA concentration under salt stress in maize leaves (Nahar *et al*., 2016). Osmotic stress from salinity affects water, nutrient intake, potentially reducing phospholipid accumulation, harming membrane integrity (Mansour, 2013; Mandhania, 2006). ROS further damage membranes and elevate MDA (a marker for oxidative damage) (Zheng Jia-Lang, 2016). Seed priming with 50 ppm improves MSI in TMB-37 (5.73%) and MH-1314 (3.38%) under control condition and in TMB-37 (6.63%) and MH-1314 (5.25%) under saline condition. It also reduces lipid peroxidation in TMB-37 (12.89%) and MH-1314 (7.22%) under control condition and in TMB-37 (13.20%) and MH-1314 (11.18%) under saline condition (Table 4). Zn nanoparticles enhance rice resistance to moisture stress by maintaining MSI (Rameshraddy *et al*., 2017). Zn's role in nutrient transport may boost phospholipid buildup, maintain biomembrane structural integrity. Similarly reported, ZnO nanoparticle treatment reduces lipid peroxidation in Cilantro and Lupine plants (Reddy Pullagurala *et al*., 2018; Latef *et al*., 2016), likely due to changes in membrane permeability along with Zn aided protection from oxidative stress (Burman *et al*., 2013).

Antioxidant enzyme activity

Salinity stress raises SOD and POX activity significantly higher in TMB-37 i.e. SOD (20.10%) and POX (11.02 %) vs. MH-1314 SOD (11.68%) and POX (12.34 %) against its respective control (Table 4). Similar findings in other crops (Unal *et al*. 2014) suggest salt stress boosts ROS production, antioxidant enzyme activity (Das and Das 2019). Seed priming with 50 ppm raises SOD in TMB-37 (3.25%) and MH-1314 (6.38%) under control condition and TMB-37 (7.09%) and MH-1314 (5.77%) under saline soil. Similarly, it increases POX non-significantly (NS) in both genotype (C.D. = 0.199 among treatments) under control condition and significantly in TMB-37 (17.26%) and MH-1314 (12.52%) under saline condition. It is also documented that 50 ppm ZnO nanoparticles increases antioxidant enzymes in wheat (Du W *et al*., 2019), combined with salinity stress improves enzyme activity also (Latef *et al*., 2016). Elevated SOD, POX activity linked to Zn's role in enzyme, non-enzymatic antioxidant production and Initial ROS generation from Zn treatment could signal chain reaction, increasing enzyme activity may be attributed for the effect (Rezaei and Abbasi 2014; Soliman *et al*., 2015).

CONCLUSION

TMB-37 displays stronger root development, higher antioxidant enzyme activities and more proline content than MH-1314 in saline conditions, resulting in better adaptation. An optimum dose of ZnO-NPs (50 ppm) significantly boosts dry biomass by mitigating the adverse effect of salinity

stress by enhancing antioxidant mechanism in both genotypes. In this experiment identified dose i.e. priming with 50 ppm of ZnO-NPs alleviated salt stress impact on mung bean seedlings that was reflected in terms of increased seedling dry weight. This technique of nano priming could also mitigate stress effects and improve stress tolerance as well as enhance growth under normal condition in mung bean. More, real world field experiment should be conducted, also the toxic effect of higher doses of nanoparticles on crop and environment should be studied.

Conflict of interest

All authors declare that they have no conflicts of interest.

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