

Effect of Zinc on Lentil (*Lens culinaris L.*) Metabolites and Antioxidant Enzyme Activities.

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ABSTRACT

In the given study, the toxic effect of zinc (Zn) was studied with special attention being given to the biochemical response of *Lens culinaris L.*, exposed to zinc toxicity at different concentration levels. The research carried out in a randomized complete block design with 3 replications of each treatment. The major change was observed in the production and accumulation of primary metabolites (i.e. protein and protease) and activity of antioxidant enzymes i.e. catalase (CAT) and acid phosphatase (APS). The data showed that the low concentration of Zn addition support soluble protein accumulation in lentil leaves, although protease activity greatly arrested the protein content and appeared as a negative factor with increasing Zn levels. The response of antioxidant enzymes was also dose dependant, it increases their activity to suppress oxidative stress produced by heavy metal but at high dose they become ineffective due to increase in oxidation process controlled by metal application. The peak of activity appeared at 4ppm to 8ppm Zn.a

Keywords: *Antioxidant enzymes, acid phosphatase (APS), catalase (CAT), Zinc toxicity.*

INTRODUCTION

Zinc is an essential micronutrient required for optimum crop growth. Numerous researches were conducted on Zinc. Nand & Ram (1996) and Selvi & Ramaswami (1995) observed that, at higher concentrations Zn leads to physiological and morphological disturbances and, eventually to decreased crop yield. Higher concentration of Zn in the plant tissue seriously affects activity of several enzymes and other fundamental metabolic processes. An excess of Zn also reduced photosynthetic rate as a part of enzymes concerned in the photosynthesis. A toxic concentration of Zn in plant tissue seriously affects activity of several enzymes and other fundamental metabolic processes. Ali et al., (2000) carried out a broad study and affirmed that an excess of Zn also reduced photosynthetic rate as a part of enzymes concerned in the photosynthesis. Nitrogen metabolism is also affected in diverse ways by an

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excess of Zn. The protein content is found to be reduced; nitrogen-fixation and nitrate reductase activity was also concealed by Zn toxicity (Phalson, 1989). Excessive Zn in plants can profoundly affect normal ionic homeostatic systems by interfering with the uptake, transport, osmotic and regulation of essential ions and results in the disruption of metabolic processes such as transpiration, photosynthesis and enzyme activities related to metabolism (Broadley et al., 2007; Abbas et al., 2009). Zn phytotoxicity also induces oxidative stress by generating free radicals and reactive oxygen species (ROS) (Weckx & Clijsters, 1997). These ROS are highly reactive and cause the death of plants by damaging membrane lipids, proteins, pigments and nucleic acids. To cope up with the damages caused by the ROS, cells possess their own comprehensive and integrated endogenous antioxidant defense system composed of both enzymatic as well as non-enzymatic components (Miller et al., 2008). Superoxide dismutase (SOD),

peroxidase (POD) and catalase (CAT) represent the endogenous defense of plant cells. These enzymes are present in different isoforms in several cell compartments and their expression is genetically controlled and regulated both by developmental environmental stimuli, according to the necessity to remove ROS produced in cells (Mittler et al., 2004). It is also well documented that flavonoids and polyphenols are natural antioxidants. Flavonoids can directly react with superoxide anions and lipid peroxy radical and consequently inhibit or break the chain of lipid peroxidation. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols or flavonoids, thus contributing to their electron/hydrogen donating ability. Plant phenolic compounds also participated in the defence system of plant against oxidative stress. The antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, with ability to bind particularly Fe⁺² and Cu⁺² (Jung et al., 2003). The roots of many plants exposed to heavy metals exude high levels of phenolics (Wink El-Shirl, 2002). They may inactivate iron ions by chelating and additionally suppressing the superoxide-driven fenton reaction, which is believed to be the most important source of ROS (Arora et al., 1998). This general chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than to specific chelating groups within the molecule (Morgan et al., 1997). An enhancement of phenylpropanoid metabolism and the amount of phenolic compounds can be observed under different environmental factors and stress conditions (Sakihama et al., 2002). The induction of phenolic compound biosynthesis was observed in wheat in response to Ni⁺² toxicity (Díaz et al., 2001) and in maize in response to Al⁺² (Wink El-Shirl, 2002). *Phaseolus vulgaris* exposed to Cd⁺² accumulate soluble and insoluble phenolics. Similarly, An increase in soluble phenolics, such as intermediates in lignin biosynthesis can reflect the typical anatomical change induced by stressors: increase in cell wall endurance and the creation of physical barriers preventing response against harmful

action of heavy metals (Díaz et al., 2001).

MATERIALS AND METHODS

The present research was to investigate the alleviating effect of different Zn concentrations on growth inhibition and oxidative stress in *Lens culinaris* L. (Lentil). The experiments were carried out using plastic pots with holes at the bottom to facilitate water percolation. The Zn was added to the soil at concentrations of 0 (Control), 2 mg/kg (T1), 4mg/kg (T2), 6mg/kg (T3), 8mg/kg (T4), and 10mg/kg (T5). Each treatment was replicated three times. Ten seeds of Lentil (*Lens culinaris*) were sown per pot separately. The experiment was carried out in a greenhouse with a 14 h (26°C)/10 h (13°C) day/night cycle. Soil water content was adjusted regularly. The plants were harvested after 21 days of treatment and subjected to the analysis of physiological and biochemical test to check the toxic effect of Zn on primary, secondary metabolites and antioxidant contents of plant. Total protein was estimated by Bradford Method (1976) respectively. Protease activity was determined by the method of McDonald and Chen (1965). Some important antioxidant enzymes assay including CAT and APS, and secondary metabolites were also performed to investigate the oxidative stress produced in plants under Zn alleviated concentration stress. CAT activity was assayed by using method of Maehly & Chance (1959). Activity of Acid Phosphatase (AP) was determined according to McDonald and Chen (1965). While, total phenolics estimation was done by methods used by Ainsworth & Gillespie (2007). Flavanoid estimation was done by adapting methods from Woisky, R. And Salatino, A. (1998).

RESULTS AND DISCUSSION

Zinc effect on total protein and protease activity:

Table -1 showed that, with an elevated Zn concentration, there was an increased in protease activity and decreased protein content was found in lentil crops (Fig-1). Similar results are reported by

Barcelo et al., (1985). Decrease in protein related with the heavy metal effect on protein accumulation and production. Kastori et al., (1992) also reported in *Helianthus annuus* that content of soluble proteins decreases with high concentration of heavy metals. Protein content under heavy metal influence may be affected due to Enhanced protein hydrolysis (proteolysis) resulting in decreased concentration of soluble proteins (Melnichuk et al., 1982).

Table-I: Effect of Zn on Primary Metabolite of Lentil (*Lens culinaris*)

TREATMENT	PRIMARY METABOLITES	
	Total Protein	Protease
	(uMole/gm fr.wt.)	mg ⁻¹ protein mg ⁻¹ fr.wt
T0 CONTROL	3.197 a (0)	16.69 f (0)
T1 2ppm Zn	2.388 b (-25.30)	17.24 e (+3.29)
T2 4ppm Zn	2.354 c (-26.36)	19.9 d (+19.23)
T3 6ppm Zn	2.312 d (-27.68)	21.55 c (+29.11)
T4 8ppm Zn	2.215 e (-30.71)	31.23 b (+87.11)
T5 10ppm Zn	1.627 f (49.10)	37.24 a (+123.12)

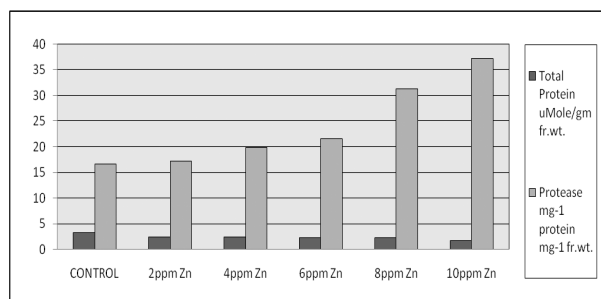


Figure 1: Effect of Zn on Primary Metabolites of Masoor (*Lens culinaris*)

Zinc effect on antioxidant enzymes activity: In the present research work, activity of antioxidant enzymes increased with Zn supply up to certain level i.e. 4-6ppm, and also excess of Zn produced oxidative stress in plants. Antioxidant enzymes may alter the H₂O₂ to the H₂O in the plant cells and counteract the toxicity effect of H₂O₂ (Rezai and Farboodnia, 2008). Hence to shield cells against oxidative stress, antioxidant enzymes augmented proportionally, which is also consistent with our results (Fig.2). Rate of POD was highest among three antioxidant enzymes, followed by CAT and APS at all treatments. The given data revealed the decreased activities of antioxidative enzymes in studied crop under high Zn stress. Maximum increase in lipid peroxidation was observed in plants treated with 6ppm Zn as

compared to non treated seedlings. Similarly, the activity of CAT was also increased by the treatment with 8ppm Zn.

Table-II: Effect of Zn on Antioxidant enzymes activity of Lentil (*Lens culinaris*)

TREATMENT	ANTIOXIDANT EZMYME ACTIVITY	
	Catalase (CAT)	Acid Phosphatase (APs)
	uMH ₂ O ₂ min ⁻¹ mg ⁻¹ protein gm ⁻¹ fr.wt	uMH ₂ O ₂ min ⁻¹ mg ⁻¹ protei gm ⁻¹ fr.wt
T0 CONTROL	0.46 e (0)	0.539 d (0)
T1 2ppm Zn	0.599 d (+30.21)	0.644 c (+19.48)
T2 4ppm Zn	0.802 c (+74.34)	0.652 b (+20.96)
T3 6ppm Zn	1.897 b (+312.39)	0.703 a (+30.42)
T4 8ppm Zn	2.56 a (+456.52)	0.503 e (-6.67)
T5 10ppm Zn	0.423 f (-8.043)	0.489 f (-9.27)

Value in parenthesis indicate percent increase (+) or decrease (-) over control.

Means followed by different letter shows significant result at the level of Standard deviation

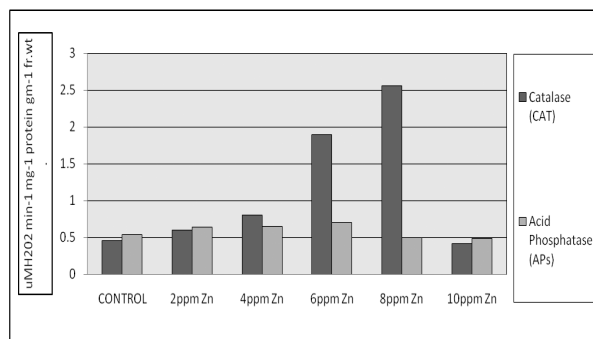


Figure 2: Effect of Zn on Antioxidant enzymes of Masoor (*Lens culinaris*)

Catalase activity: CAT is one of the major antioxidant enzymes that play a very important role in the protection against oxidative damage by breaking down hydrogen peroxide and plays an important role in plant defense, aging and senescence (Mittler et al., 2004). The CAT activities in shoot significantly increased with the zinc concentrations. The induction of this enzyme under zinc stress indicated that it helps in inhibiting the oxygen radical accumulation. Similar to the present study, an increase in CAT activity has been reported in other plant species exposed to zinc stress (Prasad et al., 1999; McGeer et al., 2000). Our findings provide the evidence that CAT may provide an additional protection against the oxidative damage induced by zinc stress. A gradual decline in CAT activity in lentil plants grown under excess Zn stress i.e. above 8ppm was observed. Maximum CAT activity (2.56 mM H₂O₂ min⁻¹ mg-

1 protein) was observed in plant treated with 8ppm Zn as compared to control plants (0.46 mM H₂O₂ min⁻¹ mg⁻¹ protein). This finding matches with the work of Andrade et al., (2009). The decrease in CAT activity observed in plant supplemented with excess Zn might be due to inhibition of enzyme synthesis or a change in the assembly of enzyme subunits (Radic et al., 2010).

Acid phosphatase (APS) activity: APS is the most important peroxidase in H₂O₂ detoxification operating both in cytosol and chloroplasts (Mittova et al., 2000). Initially it increase at 6ppm Zn (p<0.005) in lens plant and then gradually decrease with the elevated concentration of the Zn. Increases in the acid phosphatase activity with high Zn treatments might be due to the decline of phosphate (P) level in the cell of P starvation and that intracellular and extracellular acid phosphatases are integral components of plants response to P deficiency (Duff et al., 1993).

CONCLUSION

In conclusion, even though Zn is an essential micronutrient for plants, higher Zn concentrations (above 6mg/kg) reduced plant growth and induced oxidative damage. Moderate Zn supplementation (2mg/kg to 4mg/kg) played an important role in protecting plants from oxidative stress induced by excess Zn exposure. Although these antioxidant enzymes showed different patterns of activities exposed to zinc toxicity, the total activity of these enzymes were significantly enhanced, which reflect an increased degree of oxidative stress. Our findings suggested that the defensive system of plant regulated the changes of enzyme activities in order to enhance the defensive function against excessive zinc.

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