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RESEARCH ARTICLE

Tissue Zn and P concentrations and certain biochemical constituents of *Ocimum sanctum* L. as influenced by varying Zn levels and P fertilization in alluvial soil (Entisol)

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Abstract

Experiment was conducted to study the effects of various levels of zinc (nil, 1, 2.5, 5 and 10 ppm) and a normal dose of phosphorus fertilizer (50 mg kg^{-1}) amendment in an alluvial soil (Entisol), omitted native soil (control) on some biochemical responses (pigments and protein contents, and activity of catalase and peroxidase) of *Ocimum sanctum* L. Tissue concentration of zinc and phosphorus was also determined. Soil properties were evaluated (texture sandy loam, pH 7.6, organic matter 3.8% , , N 85 and P 62 mg kg^{-1}, and extractable Zn 0.62, Cu 0.31 and Fe 5.5 ppm) before the transplantations of plants. Phosphorus fertilization could not influenced tissue-Zn and above biochemical constituents determined up to 2.5 ppm Zn amended soil, whereas its higher doses (5 and 10 ppm Zn) reduced tissue-P and biochemical constituents in *O. sanctum* leaves.

Keywords: Zinc fertilization, phosphorus, alluvial soil, *Ocimum sanctum*

Introduction

Most of the soils are low productive for the plants due to their nutrients-deficiency, a worldwide problem (Alloway 2009). About 159.7 million hectare land in India is used for agricultural practices, which are supporting socio-economic conditions of the 65.53% people. In India, about 91 million hectare land is degraded and 49% agricultural land is zinc (Zn) deficient (Arunachalam *et al.* 2013) and most prominently in alluvial soil (Agarwala *et al.* 1980). Zinc deficiency is more common in tropical and subtropical areas with low moisture, high temperature and coarse textured soil and such arable areas have been converted into waste land due to non-economically vible crop production under multi-nutritional disorder (Pandey 2017, Shukla *et al.* 2018). Zinc deficiency becomes more prominent in soils due to interactions with other nutrients (synergistic or antagonistic effects) such as phosphorus (Pandey 2018, Bhardwaj 2019). Also, the availability of Zn is highly influenced by soil properties, which ultimately influence the biochemical responses of plants (Marschner 2012, Brady and Weil 2001, Pandey 2020). In addition, abiotic stresses and other soil conditions highly influence the availability of nutrients to the plants (Tripathi and Pandey 2018), Several reports indicated that, a large area of India required zinc-deficiency management with respect to other nutrient(s) status for optimum crop production (Agarwala *et al.* 1980; Arunachalam *et al.* 2013, Shukla *et al.* 2018). Various management practices have also been reported for zinc deficiency management under the adequate status of macronutrients (N, P, S etc.) in soil for improvement in world's crop production (Cakmak 2008, Pandey *et al.* 2009). Zinc is significant micronutrient involves as a constituent of several enzymes in regulation of several cellular metabolic functions (Agarwala *et al.* 1980, Cakmak 2000) and in the biosynthesis of various molecules (Andreini *et al.* 2006). Zinc in adequate concentrations in plants supports growth; photosynthesis and reproductive yield (Sharma 2006, Pandey 2017). Zinc supports plant growth by protecting the alterations in chloroplast and

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Table 1: Physical and chemical properties of the soil (Lucknow University campus, Lucknow) used in the experiment

Texture	*pH*	*Bulk density (g/cc^3)*	*Organic matter (%)*	*$CaCO_3$ (%)*	*E.C (mS/cm)*	*N (mg Kg^{-1})*	*P (mg Kg^{-1})*	*DTPA Extractable (ppm)*		
								Zn	Cu	Fe
Sandy Loa	7.6	1.36	3.8	0.9	1.2	70	85	0.62	0.31	5.5

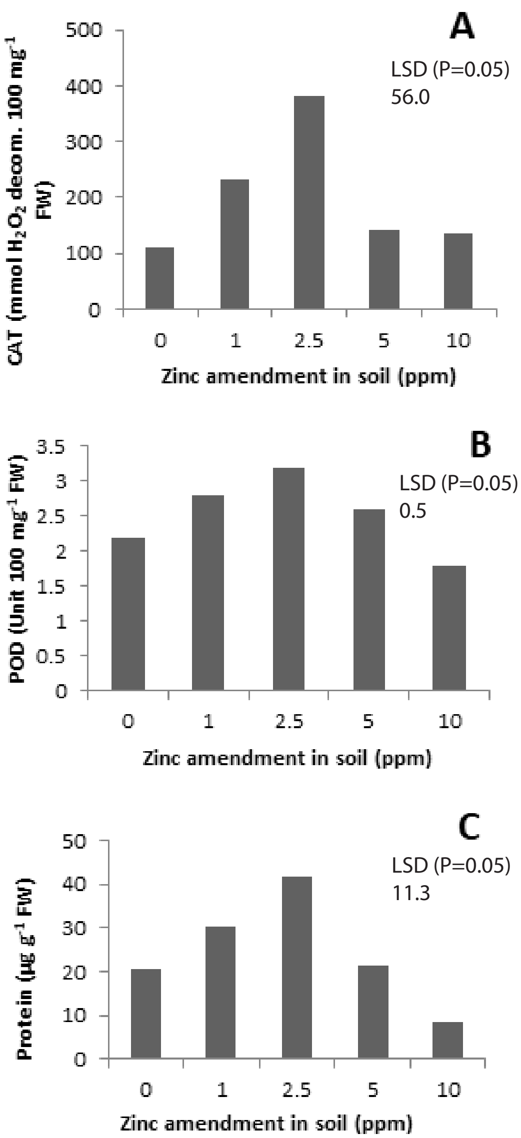

Figure 1: Effect of various doses of zinc amendment with phosphorus in soil on the activity of A. catalase, B. peroxidase and C- protein in *O. sanctum*.

cellular membrane system (Shrotri *et al.* 1978) and synthesis of auxin (Sharma 2006). It is a constituent of carbonic anhydrase which catalyzes the reaction of conversion of CO_2 to bicarbonate during the process of photosynthesis. Zinc is also constituent of antioxidant superoxide dismutase (Zn-SOD) which neutralize the reactive oxygen species (ROS) in plant cells produced during abiotic stresses (Cakmak 2000). The popular name holi 'Tulsi' (*Ocimum sanctum* L.) is commonly growing wild plant in subtropical region of India. Medicinal value of 'Tulsi' is due to its essential oil which has chemical compounds such as eugenol, methyl chavicol, camphor, and cinnamate (Bast 2014, Yamini *et al.* 2016). The world's market of medicinal aromatic plants will reach 5 trillion USD by 2050 (IFAD 2008). Least informations are available on the zinc-deficient soil management and Zn interaction with adequate phosphorus level in soil in relation to biochemical responses of medicinal aromatic plants 'Tulsi'. Therefore, study was conducted to observe the assessment of various zinc levels status with adequate phosphorus fertilazation in an alluvial North-Indian soil (Entisol), and their effect on some biochemical constituents including antioxidative responses of Tulsi (*Ocimum sanctum* L.) plants.

Materials and Methods

A bulk of composite soil sample was collected from the Lucknow University campus (U.P., India) (26° 51′ N and 80° 56′ E). This composite soil sample was analyzed for various physico-chemical properties following the standard methods as described by Piper (1942) for pH, organic matter content, electrical conductance, calcium carbonate, and available N and P contents. The available-N in soil was determined by Kjeldahl method and phosphorus by Olsen *et al.* (1954). Available (DTPA) extractable zinc, copper, and iron were estimated in the soil by the method of Lindsay and Norwell (1978). A clay pot (12 Kg size) experiment was conducted in triplicates. The 6 weeks of plants (*Ocimum sanctum* L.) of equal size and weight were transplanted in each pots amended with various levels of zinc viz. 0.0 (control), 1.0, 2.5, 5.0 and 10 ppm (as $ZnSO_4$). Also, in each pot of the treatment, 50 mg/kg P_2O_5 was mixed in the soil. Leaves of plants were harvested after 8 weeks of transplantation from each treatment for the estimation of some biochemical constituents (Total chlorophyll, chlorophyll a, chlorophyll b, carotenoids and protein contents) including the activity of some antioxidative enzymes catalase and peroxidase. Dried plants were digested in HNO_3, $HClO_4$ and H_2SO_4 in the ratio of 3:1:1, respectively for determination of tissue phosphorus. The concentration of phosphorus in shoot was determined with spectrophotometer by the phosphovanadate method as described by Hanson (1950). Dried leaves tissue (1g) was digested in nitric acid and perchloric acid (3:1 ratio v/v) for the determination of tissue zinc with the help of atomic absorption spectrophotometer (Parkin Elmer-250). The methods for determinations of pigments content by Lichtenthaler and Welburn (1983) and protein content by Lowry *et al.* (1951) in *O. sanctum* were followed. Enzymes catalase (Euler and Josephson !957) and peroxidase (Luck

Table 2: Some biochemical parameters in *Ocimum sanctum* L. grown at various levels of Zn and a normal dose of P fertilization (50 mg kg-1) in soil.

Parameters/ Treatments	*Nil (Control)*	*1 (ppm)*	*2.5 (ppm)*	*5 (ppm)*	*10 (ppm)*	*LSD (p=0.05)*
Total chlorophyll (mg^{-1} f.wt.)	0.53 (0.0)	0.92 (+73.6)	1.46 +175.5)	0.47 (-11.5)	0.43 (-19)	0.12
Chlorophyll 'a' (mg^{-1} f.wt.)	0.36 (0.0)	0.52 (+44.4)	0.64 (+77.8)	0.26 (-27.8)	0.20 (-44.4)	0.10
Chlorophyll 'b' (mg^{-1} f.wt.)	0.18 (0.0)	0.32 (+77.8)	0.51 (+183.3)	0.25 (+38.9)	0.12 (-33.3)	0.15
Carotenoids (mg^{-1} f.wt.)	0.47 (0.0)	0.62 (+31.9)	0.73 (+55.3)	0.52 (+10.6)	0.40 (-14.9)	0.21
Tissue Zinc (µg g^{-1} d.wt.)	16.5	25.6	38.5	140.0	186.0	52
Tissue phosphorus (mg g^{-1} d.wt.)	0.24	0.28	0.38	0.35	0.26	0.05

1963) activities were also determined. Data presented in the tables are mean values (n=3) and statistically analysed for their significance and homogenity for least significance difference one way ANOVA.

LSD=tn-2^2*MS within/n= tn-2*q

Where
MS= Mean square
q = Studentized range
n = Treatment

Results and Discussion

Soil collected from the Lucknow University Campus was alluvial in nature; a composite sample of this soil was assessed for some important physical and chemical properties presented in the Table 1. Sandy loam texture of this soil showed alkaline reaction (pH 7.6) and moderate compactness (bulk density <1.36 g/cc). Organic matter content was adequate (3.8%) calcium carbonate content was low (Brady and weill 2017). Available nitrogen content was near to the normal range, while phosphorus content was slightly deficient (Rupp *et al.* 2018). Among micronutrients Zn, Cu and Fe determined, only zinc was found in deficient range (<o.6 ppm). The normal range of DTPA extractable available Zn (0.8 ppm), Cu (0.6 ppm) and iron (5.6 ppm) have been reported (Agarwala & Sharma 1979, Pandey *et al.* 2009). Zinc element applied in the above soil (at the rate nil, 1, 2.5, 5 and 10 ppm), maximum increase in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content by 77.8, 183.3, 175.5 and 55.3% was observed in *Ocimum* leaves, respectively at 2.5 ppm Zn amendment in soil. The increase in pigments content could be attributed due to sufficient availability of tissue-zinc (Pandey 2020) protected disruption of chlorophyll synthesis (Hisamitsu *et al.* 2001) and as being a constituent activated proteins and enzymes involve in biosynthesis of pigments (Balashouri 1995). The higher Zn doses applied in soil (5 and 10 ppm) inhibited these pigments content. Maximum inhibition in chlorophyll a (-44.4%) was noticed, also reduction in chlorophyll b (-33%) and total chlorophyll content (-19%) was observed in *O. sanctum* grown at soil amended with 10 ppm Zn. The elevated Zn in soil might be interfere the absorption of Fe and other nutrients to plant roots by antagonistic effects inhibited pigments content in *Ocimum* (Rosen *et al.* 1977). The promotion in pigments content at lower doses of Zn (1 and 2.5 ppm) could be due to the Zn-assisted normal activity of antioxidants (Cakmak 2000, Pandey 2020). The enhanced biochemical responses such as pigments and protein contents determined (Table 2) can be correlated with the activity of the antioxidative enzymes catalase and peroxidase determined in *O. sanctum* which was maximum at 2.5 ppm Zn-amended soil. The elevated production of reactive oxygen species (ROS) at heavy metals or oter stresses damage the cellular membrane structures by rapid formation H_2O_2 (Sharma 2006, Pandey 2018). The activity of catalase and peroxidase can rapidly change the H_2O_2 to H_2O protect the normal cellular metabolism. The adequate tissue-Zn content in cell help to form a tetrahedral coordination bonds with some bimolecular structure including amino acids such as cysteine and histidine, Zn has more affinity to bind cysteine and histidine than the Fe (Berg and Shi 1996). Thus, block the formation of ROS via interactive reaction between cysteine, histidine and iron (Searle and Tomasi 1982, Girotti 1985). Variable doses of zinc (low and high) with a normal phosphorus dose application (50 mg kg^{-1}) in soil influenced their tissue accumulation (Table 2). Zinc accumulation in tissue was dose dependent, maximum 186 µg Zn g^{-1} dry weight was determined at 10 ppm of zinc amendment in soil inhibited above determined biochemical constituents. The test plants grown at native soil (without Zn and P application), showed 16.5 µg Zn g^{-1} dry weight and 0.14 mg kg^{-1} dry weight of phosphorus contents, both was in a critical deficient range as reported earlier (Marschner 2012, Cakmak 2018). Adequate phosphorus dose

in soil slightly suppressed the tissue-Zn at lower Zn status in soil (1 and 2.5 ppm), whereas a trend of suppression in tissue-P was observed at higher doses of Zn application in soil (5 and 10 ppm). These results showed antagonistic effect of Zn and P application in soil. A high status of P in soil causes Zn deficiency in plants but its normal status is not effective as reported earlier (Bhardwaj 2019). The maximum tissue-P was at 2.5 ppm supply of Zn in soil, thereafter decreased with increasing in doses of soil-Zn. Thus, high Zn levels in soil suppressed the tissue-P could be due to their antagonistic effects for the absorption in roots (Alloway 2009).

Conclusion

Therefore, study concluded that low Zn status in soil reduced pigments and protein content in *Ocimum*. Adequate phosphorus fertilization supported enhanced biochemical constituents (pigments and protein contents) including the activity of antioxidative enzymes catalase and peroxidase maximum at 2.5 ppm Zn amendment in alluvial soil (Entisol). These estimated biochemical constituents showed a prominent inhibition by higher doses of Zn (5 and 10 ppm) and an adequate P (50 mg/kg) level fertilization in soil, Also higher levels of Zn showed antagonistic effects with P as decreased tissue-P in *O. sanctum* L.

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