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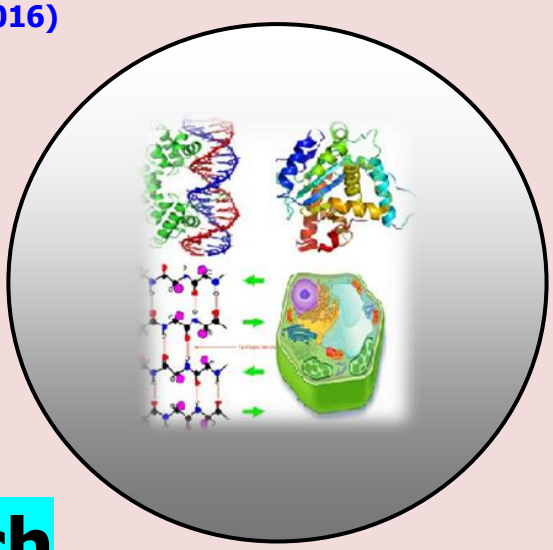

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RESEARCH PAPER

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Acquisition of Zinc from Soil and Foliar Application in Oat Plant: Physiological Approach

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ABSTRACT

The present study aims to investigate the acquisition of zinc through soil and foliar application in oat (Avena sativa c.v. shalimar), from the soil which is beneficial for human health. The combined effect of both soil and foliar applications with 0.5% Zn at different growth stages (pre-flowering and post-flowering growth stage) was studied to enhance the Zn uptake in oat. Treatments consisted of five levels of Zn- control (T1), 15 mg kg^{-1} soil + pre-flowering foliar spray (T2), 15 mg kg^{-1} soil + post-flowering foliar spray (T3), 30 mg kg^{-1} soil + pre-flowering foliar spray (T4), 30 mg kg^{-1} soil + post-flowering foliar spray (T5). The results of this experiments showed that at T2 Zn level increased plant growth, biomass, photosynthetic activity but enzyme- catalase and peroxidase activity were decreased. Enzyme- carbonic anhydrase activity was maximum at T4 Zn level. The accretion of zinc can improve physiological characteristics, yield and biomass of oat as compared to control.

Key words: Avena sativa, Carbonic Anhydrase, Catalase, Foliar Application and Peroxidase.

INTRODUCTION

The main purpose of sustainable agriculture is to provide food and nutritional security by enhancing crop productivity without impairing soil health. Micronutrients are as important as macronutrients for adequate plant growth and occupy an important position by virtue of their essentiality in plant metabolism. In fact, their essential role in plant nutrition and increasing soil productivity makes their importance even greater (Zain *et al*., 2015). Deficiency of just one nutrient can greatly reduce growth and yield. Adequate plant nutrition with macro and micronutrients depends on many factors which include the ability of soil to supply these nutrients and rate of absorption of nutrients to functional sites, and nutrients' mobility within the plants.

In different states of India, micronutrients such as zinc, boron, iron, manganese, copper and molybdenum have been reported to be deficient in soil. In most of the soils of Uttar Pradesh, the deficiency of zinc is very common among all the micronutrients, which results in major loss of crop productivity (Shukla *et al*., 2016). He analysed 8072 soil samples from U.P. state and observed that deficiency of Zn was found in 32.4% soils. Zinc deficiency is generally noticed in Barabanki, Lucknow, Unnao, Kanpur, Rampur, Bareilly, Badaun, Bahraich, Gonda, Mainpuri, Mathura and Saharanpur area. Growing plants on micronutrient-deficient soils further reduces their concentration in crop plants (Cakmak *et al.*, 2010; Shukla *et al.*, 2014). Indian diets mainly consisting of cereals are inherently low in micronutrients.

Zinc is an essential nutrient in plants, animals and humans, and plays a fundamental role in several critical functions as in protein metabolism, gene expression, structural and functional integrity of bio-membranes and photosynthetic carbon metabolism of plant as a cofactor for more than 300 enzymes

(Poblaciones and Rengel, 2016). In humans, Zn deficiency is associated with severe health complications, including impairments of physical growth, learning ability, an immune system, and increased risk of infections and DNA damage and cancer development (Levenson and Morris, 2011). Deficiency of Zn is reported to be 5th highest among various health risk factors in developing countries, and they have high mortality rates (Clemens S., 2014; Shahzad *et al*., 2014). Normally cereals are reported to contain low Zn (15-30 mg Zn kg^{-1}) content against the required adequate concentration of 40-60 mg Zn kg^{-1} for better nutrition (Cakmak, 2004).

The main reason of Zn deficiency is minimum diversification in food items and less amount of its bio-available forms. The challenge to agriculture is not only to feed the masses but also to provide the nutrient rich food to the human being. For this purpose we have to design the agriculture that focuses on the health of masses too (Saleem *et al*., 2016). One such interesting technique is foliar feeding in plants applying liquid nutrient or fertilizer directly to the leaves. The plants absorb essential elements through their leaves via stomata. Foliar nutrient uptake is a mean of rapid nutrient supply, especially when soil nutrient availability or root activity is reduced or mobility of particular nutrient in plant system is low. Oat (*Avena sativa*) is one of the sixth main cereal crops in world including wheat, maize, rice, barley and sorghum. Oat bran and whole oats are beneficial for preventing heart disease, gallstones, controlling high blood pressure; high cholesterol; diabetes; colon and stomach cancer, and digestion problems including inflammatory bowel disease, diarrhoea and constipation. There is a need to enhance the contents of Zn in the oat crop not only for better growth and yield of plants but even increase the Zn availability in grain for human. This study was designed to increase the Zn content in oat crop eventually to minimize the threat of human Zn deficiency as the human especially with vegetarian diet exclusively depend on plant produce for the fulfilment of their nutrient and vitamin needs.

MATERIAL AND METHODS

Experimental design

The pot experiment was conducted under controlled conditions in wire house in Botany Department, University of Lucknow (26^{0}55'N Latitude, 80^{0}59'E Longitude). Five treatments, with three replications of each treatment were arranged in a completely randomized design. Treatments consisted of five levels of Zn- control (T1), 15 mg kg^{-1} soil + pre-flowering foliar spray (T2), 15 mg kg^{-1} soil + post-flowering foliar spray (T3), 30 mg kg^1 soil + pre-flowering foliar spray (T4), 30 mg kg^{-1} soil + post-flowering foliar spray (T5). A foliar application was given as 0.5% Zn as $ZnSO_4.7H_2O$ (early morning, 25-30^{0}C temp) at pre-flowering and post-flowering growth stages. According to the climate data obtained from a weather station, temperature and relative humidity during the experiment were 24 ^{0}C (sunny) and 34-45%, respectively. Ten kilograms of soil (soil: FYM - 5:2) was filled in each earthen pot (10 inch). The viable seeds of oat (*Avena sativa* c.v. Shalimar variety) with viability percentage more than 80 were collected and used for the sowing purpose. Seeds were planted about 2 cm deep in soil and thinned to four uniform stands 2 week after emergence. The second and third youngest fully opened leaves of one pot of each treatment were cut for analysis of enzymes (carbonic anhydrase, catalase and peroxidase) and photosynthetic activities.

Analysis of soil

Electric conductivity (EC) and pH of soil was measured by method of Jackson (1973).

Analysis of growth (External morphology)

The plants were sampled for determination of plant height, fresh and dry weight of stem and leaf at three physiological growth stages i.e. vegetative, pre-flowering and post-flowering growth stages at 45, 65 and 85 DAS (Days after sowing). The plant samples were thoroughly washed with running tap water then distilled water to remove surface contamination and gently blotted to wipe out. Then plant was separated into different plant parts (stem and leaf) and weighed quickly on balance to avoid excessive loss of water by evaporation. The dry weight was determined after drying these fresh samples in pre heated oven at 70 ^{0}C for 48 hours. The mean plant height was expressed in centimeter (cm) and weight in gram (g).

Analysis of enzymes

Carbonic anhydrase (EC 4.2.1.1; CA) enzyme

The electrometric method of Wilbur and Anderson (1948) was adopted in which the time required (in seconds) for a saturated CO_2 solution to lower the pH of 0.012M Tris HCl buffer from 8.3 to 6.3 at 0^{o}C is determined. The time without enzyme is recorded at T_0 with enzyme T.

A Unit of activity = $\frac{2\times(To-T)}{T}$

Unit/mg = $\frac{2\times(To-T)}{(T\times mg\ enzyme\ \ in\ reaction\ \ mixture\ )}$

Which: T_o = reading of blank; T = reading of sample

The unit of enzyme was expressed in EU mg^{-1} protein.

Catalase (EC 1.11.1.6; CAT) enzyme

Activity of enzyme was determined by Euler and Josephson (1927). The enzyme activity was expressed as µM H_2O_2 degraded mg^{-1} protein.

Peroxidase (EC 1.11.1.7; POD) enzyme

Peroxidase activity was assayed by the method of Luck (1963). Absorbance was measured by spectrophotometer (Toshniwal Visible spectrophotometer-TSUV 75). The enzyme activity was expressed as change in optical density between the sample and the blank (or EU) ΔO.D. mg^{-1} protein.

Photosynthetic efficiency

The pigment content (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) was estimated by the method of Arnon (1949), and their quantity was expressed in mg g^{-1} fresh weight of tissue. Carotenoid content were calculated on leaf fresh weight basis according to formula given by Duxbury and Yentsch (1956).

Total protein

Total protein was measured according to Lowry *et al*. (1951). It was expressed in mg mg^{-1} fresh tissue.

Statistical analysis

The experiment was conducted in completely randomized design (CRD) with 3 replications. The data were analysed by One Way ANOVA using software program Sigma stats 4.0. It was followed by comparison of mean values using Holm Sidak method at $p \leq 0.05$.

RESULTS

Soil analysis

The soil used in the study was sandy loam in texture with pH of 6.5; electrical conductivity (EC) of 1.09 dSm^{-1} (Table 1).

Table 1. Physical properties of soil used in experiment. The values are mean of 3 replicates ± S.E.

Soil Property	Value	Method used
Texture	Sandy loam	Jackson, 1973
pH	6.5± 0.05	
EC	1.09 ± 0.002 dSm^{-1}	

Growth of oat in presence of Zn

Results of the present study showed that the effect of Zn on plant growth in terms of plant height, leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW) and stem dry weight (SDW) at 45, 65 and 85 DAS are given in fig 1(A-E). It was calculated in terms of percentage increase or decrease over the control. Growth of plant was significantly ($p<0.05$) increased in a time-point and zinc concentration dependent manner. The most luxuriant growth of plants was observed at 15 mg kg^{-1} Zn concentration both stages (pre-flowering and post-flowering growth stages) during 45, 65 and 85 DAS as compared to control (without Zn). The maximum plant height, LFW, LDW, SFW and SDW was observed at T2 Zn level (15 mg kg^{-1} soil+ pre flowering foliar spray) with the increase by 33.44, 35.53, 43.53, 58.76, 58.40% (45 DAS); 22.02, 35.2, 47.98, 60.16, 65.9% (65 DAS) and 22.27, 34.5, 73.96, 79.36, 81.46% (85 DAS) respectively, when compared with the control.

Enzyme activity in leaves of oat

Carbonic anhydrase (CA)

The activity of carbonic anhydrase (CA) was observed as EU mg^{-1} protein as shown in fig 2 but for the comparative expression variation from the control, it was calculated in terms of percentage increase or decrease over the control. Enzymatic activity of CA in leaf was significantly ($p<0.05$) increased with increase in Zn supply at all growth stages as compared to control. The maximum enzyme activity was observed at T4 which was found 140.16, 130.36 and 71.90% at 45, 65 and 85 DAS respectively over the control. CA activity was observed 107.95, 90.39, 140.16, 97.27% (45 DAS); 89.69, 53.53, 130.36, 119.84% (65 DAS) and 51.79, 19.59, 71.90, 13.70% (85 DAS) respectively, with increasing concentration of Zn as compared to control.

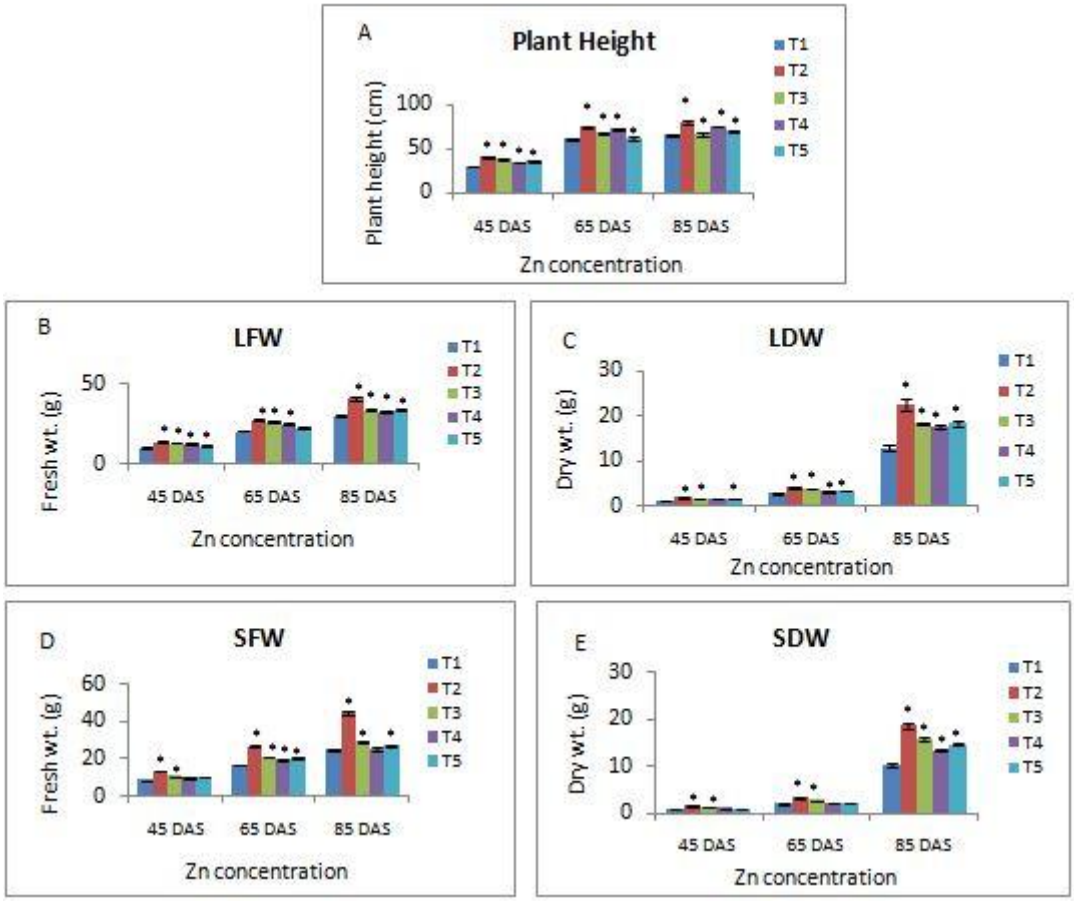

Figure 1. (A, B, C, D and E): Graphical illustration of the effect of Zn on growth and biomass of Oat at different physiological growth stages. [T1= control, T2= Zn (15 mg kg^{-1} soil + pre flowering foliar spray), T3= Zn (15 mg kg^{-1} soil+ post flowering foliar spray), T4= Zn (30 mg kg^{-1} soil + pre flowering foliar spray), T5= Zn (30 mg kg^{-1} soil + post flowering foliar spray), plant height (A), LFW= leaf fresh weight (B), LDW= leaf dry weight (C), SFW= stem fresh weight (D), SDW= stem dry weight (E)] The values are mean of 3 replicates ± S.E. *Data significant at p<0.05. Multiple comparisons Vs control group (Holm Sidak method). Overall significant level=0.05.

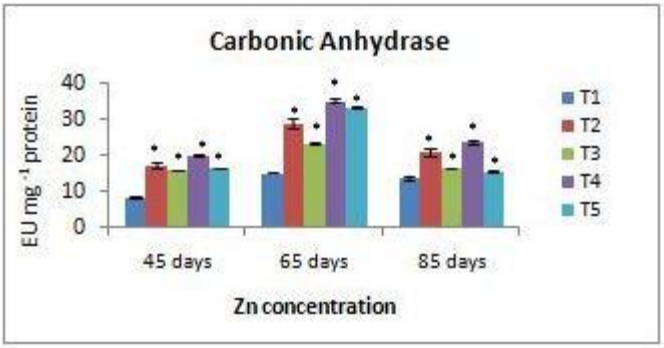

Figure 2. Graphical illustration for the effect of Zn on enzyme carbonic anhydrase in leaves of Oat at different physiological growth stages. [Carbonic Anhydrase (EU mg^{-1} protein); T1= control, T2= Zn (15 mg kg^{-1} soil + pre flowering foliar spray), T3= Zn (15 mg kg^{-1} soil+ post flowering foliar spray), T4= Zn (30 mg kg^{-1} soil + pre flowering foliar spray), T5= Zn (30 mg kg^{-1} soil + post flowering foliar spray)]. The values are mean of 3 replicates ± S.E. *Data significant at p<0.05. Multiple comparisons Vs control group (Holm Sidak method). Overall significant level=0.05.

Catalase (CAT)

The effect of Zn on enzyme CAT activity was observed as µM H_2O_2 degraded mg^{-1} protein as shown in fig 3 but for the comparative expression variation from the control. It was calculated in terms of percentage increase or decrease over the control. The activity of CAT on three time points 45, 65 and 85 DAS was observed. The CAT activity was first reduced and then increased significantly ($p<0.05$) with increasing the concentration of Zn when compared with control. The maximum enzyme activity was 35.62, 6.43 and 5.66% at T5 Zn level 45, 65 and 85 DAS with respect to control, respectively. The lowest activity of enzyme was observed at T2 Zn level; in contrast, there was further increase with increase in Zn concentration. The decreased CAT activity was 19.92, 6.45 and 17.89% at 45, 65, 85 DAS respectively at the same treatment (T2).

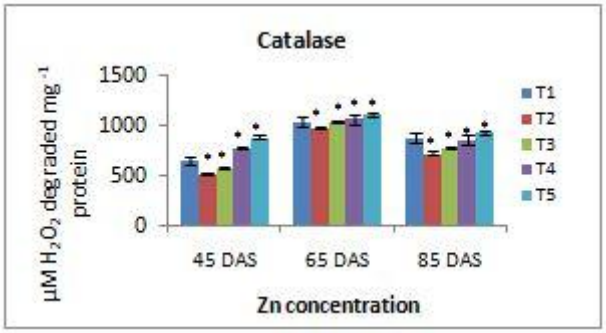

Figure 3. Graphical illustration for the effect of Zn on enzyme catalase of Oat at different physiological growth stages. [Catalase (EU µM H_2O_2 degraded mg^{-1} protein); T1= control, T2= Zn (15 mg kg^{-1} soil + pre flowering foliar spray), T3= Zn (15 mg kg^{-1} soil+ post flowering foliar spray), T4= Zn (30 mg kg^{-1} soil + pre flowering foliar spray), T5= Zn (30 mg kg^{-1} soil + post flowering foliar spray)]. The values are mean of 3 replicates ± S.E. *Data significant at $p<0.05$. Multiple comparisons Vs control group (Holm Sidak method). Overall significant level=0.05.

Peroxidase (POD)

The effect of Zn concentration on enzyme peroxidase (POD) activity was observed as ΔO.D. mg^{-1} protein as shown in fig-4 but for the comparative expression variation from the control. It was calculated in terms of percentage increase or decrease over the control is shown in fig 4. The enzyme activity was significantly ($p<0.05$) decreased on increasing the Zn concentration. The enzyme activity of POD was decreased which was found 51.3, 40.41, 40, 28.3% at 45 DAS; 33.08, 22.32, 18.98, 13.3% at 65 DAS and 47.64, 45.04, 22.32, 11.28% 85 DAS respectively, over the control. The lowest enzyme activity was observed in T2 Zn level at 45, 65, 85 DAS (51.3, 33.08 and 47.64% respectively).

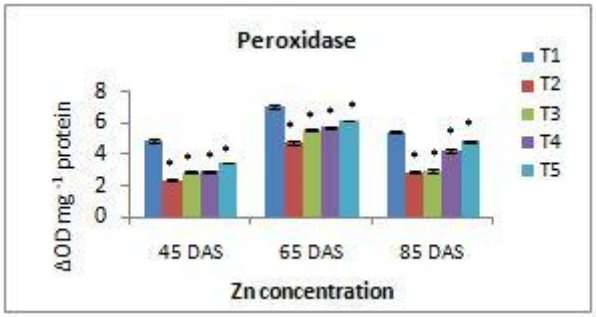

Figure 4. Graphical illustration for the effect of Zn on enzyme peroxidase of Oat at different physiological growth stages. [Peroxidase (EU ΔO.D. mg^{-1} protein); T1= control, T2= Zn (15 mg kg^{-1} soil + pre flowering foliar spray), T3= Zn (15 mg kg^{-1} soil + post flowering foliar spray), T4= Zn (30 mg kg^{-1} soil + pre flowering foliar spray), T5= Zn (30 mg kg^{-1} soil + post flowering foliar spray)]. The values are mean of 3 replicates ± S.E. *Data significant at $p<0.05$. Multiple comparisons Vs control group (Holm Sidak method). Overall significant level=0.05.

Photosynthetic efficiency in leaves of oat

The effect of Zn concentration on photosynthetic efficiency activity was observed as mg g^{-1} fresh weight as shown in fig 5 (A, B) but for the comparative expression variation from the control. It was calculated in terms of percentage increase or decrease over the control. A significant ($p<0.05$) difference was observed in photosynthetic activity. The maximum photosynthetic activity (chlorophyll a, b and total) was observed at T2 which was found 55.17, 43.08 and 60.32% at 85 DAS respectively, over the control. The minimum photosynthetic activity (chlorophyll a, b and total) was observed at T4 and it was found i.e. 2.68, 1.24 and 2.53 % (minus) at 45 DAS respectively, when compared with control.

As shown in fig 5 (B), a significant ($p<0.05$) effect of different Zn concentration was found in carotenoid activity. The maximum carotenoid activity was observed at T2 which was found 107.95, 89.69 and 51.79% at 45, 65 and 85 DAS respectively, over the control.

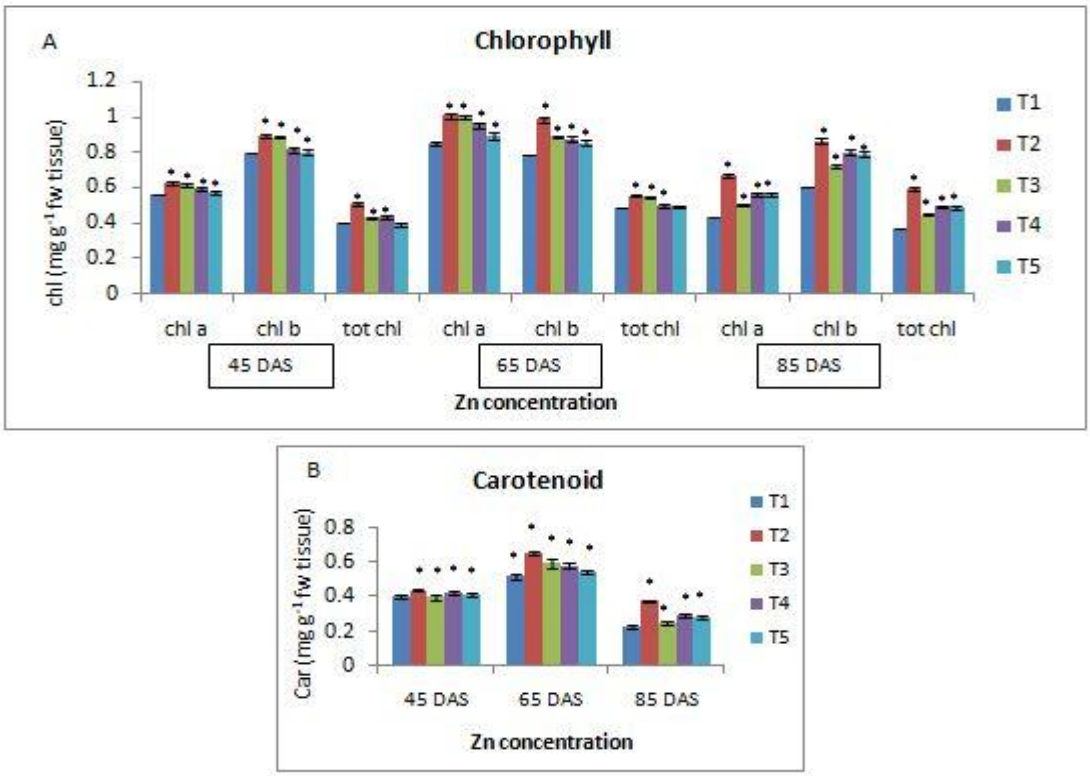

Figure 5. (A, B). Graphical illustration for the effect of Zn on photosynthetic efficiency of Oat at different physiological growth stages. [A= chlorophyll (mg g^{-1} fresh weight), B= Carotenoid; Chl a= chlorophyll a (mg g^{-1} fresh weight); Chl b= chlorophyll b (mg g^{-1} fresh weight); Tot Chl = total chlorophyll (mg g^{-1} fresh weight), car= carotenoid; T1= control, T2= Zn (15 mg kg^{-1} soil + pre flowering foliar spray), T3= Zn (15 mg kg^{-1} soil+ post flowering foliar spray), T4= Zn (30 mg kg^{-1} soil + pre flowering foliar spray), T5= Zn (30 mg kg^{-1} soil + post flowering foliar spray)]. The values are mean of 3 replicates ± S.E. *Data significant at $p<0.05$. Multiple comparisons Vs control group (Holm Sidak method). Overall significant level=0.05.

DISCUSSION

Zinc is an essential element for plant growth which plays fundamental role in several critical functions. Plant growth is an important morphological attribute; it is function of combined effects of genetic makeup of plant, soil nutrient status, foliar application and environmental conditions under which it is grown. Zn is an essential component or activator of many enzymes. In IAA biosynthesis, it has an important role in plant growth (Graham *et al*., 1992). The most luxuriant growth (plant height, LFW, LDW, SFW and SDW) of plants was observed at 15 mg kg^{-1} Zn concentration during 45, 65 and 85 DAS with respect to control (fig 1: A-E). This result is consistent with the results of Keram et al. (2012) and Rehman et al. (2012). Singh et al. (2012) and Cakmak et al. (2010) also reported that increase in levels of zinc increased wheat yield. The essentiality of Zn for growth of crop plant has been reported by several workers (Alloway, 2004; Naz *et al*. 2015).

CA is Zn dependent metalloenzyme i.e. directly correlated with Zn which was described previously by Gangwar *et al*. (1989) in rice, Rengel (1955) in wheat. Soltangheisi *et al*. (2014) suggested that Zn dependent CA facilitates diffusion of the CO_2 assimilated in photosynthesis on its path through the liquid phase of chloroplast. Enzyme located in cytosol, root plastids and chloroplasts (Soltangheisi *et al*., 2014). Activity of CA in leaves increased with increasing concentration of Zn. The maximum enzyme activity was observed at T4 Zn level when compared with control. Similar results were obtained by Soltangheisi *et al*. (2014) and Pandey *et al*. (2013), who observed maximum activity of enzyme in sweet corn and black gram with increasing concentration of Zn respectively.

The activity of CAT was reduced significantly at lower concentration of Zn, then after increased with increasing Zn concentration. These results are somewhat similar to Xu *et al*. (2005), Jiang *et al*. (2009), Zhou *et al.* (2017). However increase in CAT activity, as observed in the present study, indicates that this enzyme is involved in providing protection. POD activity was decreased as compared to control. Maximum decreased was found in T2 level. Activity is lower at low level of Zn due to normal growth of plants; in contrast the high Zn increased activity of enzyme. Somewhat similar result obtained by Xu *et al*. (2005), Jiang *et al*. (2009), Zhou *et al*. (2017).

Photosynthetic molecule such as Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were increased by increasing Zn concentration as compare to control. Maximum photosynthetic activity was observed in T4 Zn level. According to Jaffe (2004), Zn was essential for production and function of δ amino levulinic acid dehydratase, which catalysis the condensation of two molecule of δ amino levulinic acid to form a molecule of porphobilinogen. It is precursor of chlorophyll synthesis (Komai *et al*., 1968; Jaffe, 1993). Baker *et al*. (1982) suggested that Zn acts at 2 sites, site I is on oxidizing site of photosystem II and site II is between photosystem I and II and this electron flow can be positively or negatively affected by Zn depending on light intensity. Carotenoids are organic pigments that are found in the chloroplasts and chromoplasts of plants. These are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). Maximum carotenoid activity was observed in T4 Zn level. Similar result was reported by Paivoke (1983).

CONCLUSION

It was deduced from the present study that Zn concentration can be increased in cereals if Zn is applied in soil as well as through foliar application in appropriate concentration. The best Zn supply level for having more growth and enzyme activity in oat plant was observed at T2 Zn level. As the increasing Zn supply level, activity of CA revealed an increasing trend. Pre-flowering stage is suitable for foliar spray because at that time Zn directly reached to the grain and increases the Zn concentration in grains. Foliar application of the correct nutrients in relatively low concentrations at critical stages in crop development contributes significantly to higher yields and improved quality.

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