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Iron impact assessment in maize: Growth, biomass, pigments and related enzymes

Abstract

Health of human being continues to be affected adversely due to lack of micronutrients in India. Iron (Fe) deficiency also occurs in crops and human on a world scale and is now regarded as next to zinc. The present study aims to investigate the growth, biomass, pigments and related enzymes of maize (*Zea mays* c.v. HY 7074) by soil and foliar application of iron. In pot experiment, performance of maize was studied under five different concentration of iron-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). Growth (plant height), biomass (Fresh and dry weight of leaf, stem and root) and biochemical parameters (enzyme activity- acid phosphatase, catalase, peroxidase, lipid peroxidation and photosynthesis pigments) were studied. The study concluded that growth, biomass, pigment and related enzymes were increased significantly with increasing Fe concentration as compared to control. The soil and foliar application of iron was most beneficial, and gave higher enzymatic activity and photosynthesis pigments as compare to control (without Iron).

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Keywords

Acid phosphatase, catalase, peroxidase, photosynthesis pigments, *Zea mays*

Introduction

Hidden hunger or micronutrient deficiency retards the growth and development of both crops and humans. Micronutrient deficiency in soil limits the crop productivity and nutritional quality of foods, which together affect nutrition and human health (Valenca *et al.*,2017). Micronutrients follow a path from the soil through the crop and food into the human body. Diets in India (especially poor and vegetarian) are often low in diversity and dominated by staple crops such as maize, rice, oat, sorghum, wheat, millet and barley. Such diets are poor in micronutrients (minerals and vitamins). The lack of micronutrients can severe but often invisible health problems, especially among women and young children (Black *et al.*, 2013). Worldwide over 2 billion people suffer from iron (Fe), zinc (Zn) and other micronutrient deficiencies (WHO, 2016). Iron (Fe) deficiency is the most common micronutrient deficiency worldwide and disproportionately affects the poorest and most vulnerable populations in India (Finkelstein *et al.*, 2017). Iron is an essential micronutrient in biological system. According to a report of the World Health Organization in 2002, Fe deficiency affects over 3 billion people in the world, especially in developing countries. Iron deficiency causes impairments in mental and psychomotor development in children and diminished productivity in adults and represents the most common cause of anemia (Neumann *et al.,* 2002).

Biofortification is the process of increasing the content and bioavailability of essential vitamins and minerals in staple crops, through plant breeding or agronomic practices, to improve nutritional status (Bouis *et al.,* 2011). Biofortification is a promising and sustainable agriculture-based strategy to target iron deficiency, particularly in high-risk populations in resource-limited settings (Garcia *et al.*, 2016; Vasconcelos *et al.*, 2016; Petry *et al.*, 2016). The approach not only seeks to reduce the prevalence of micronutrient deficiencies but also provides a means of improving the nutritional status of people. As compared to supplementation and fortification, biofortification provides a feasible means of reaching malnourished population who are mostly found in the rural areas and who have a limited access to commercially marketed fortified foods and supplements which mainly target urban populations that consume processed foods (Ronoh *et al.,* 2017). Agronomic biofortification is achieved through micronutrient fertilizer application to the soil and/or foliar application directly to the leaves of the crop to increase micronutrient contents of the edible part of food crops. Biofortification is mainly focused on starchy staple crops (rice, wheat, maize, sorghum, millet, sweet potato and legumes) delete because they dominate diets worldwide especially among groups vulnerable to micronutrient deficiencies and provide a feasible means of reaching malnourished populations with limited access to diverse diets, supplements, and commercially fortified foods (Saltzman *et al.*, 2013; Basavesha *et al.*, 2016; Devi *et al.*, 2016;).

Maize (*Zea mays* L.) is one of the world's major crops, ranking third in importance after wheat and rice. Maize is a part of the basic diet in human nutrition, as it is a good source of starch, proteins, polyphenols, carotenoids, vitamins and dietary fiber (Tejada *et al.*, 2018). Consequently, studying the response of this crop to foliar application of iron could be of great interest to the farmer.

In the present study, an approach was made for soil and foliar application of iron to enhance crop production in maize (*Zea mays* L.) cv. HY 7074. The study was conducted in order to assess the physiological and biochemical activity of maize under varying levels of Fe application and to find out appropriate physiological growth stage of Fe application.

Materials and Methods

Experimental design: The soil pot experiment was conducted under controlled conditions in wirehouse 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 Fe- 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% Fe as $FeSO_4.7H_2O$ (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, the maximum and minimum temperatures were 37 and 23 ^{0}C during crop growth period. The relative humidity during the experiment was 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 maize (*Zea mays* L.) c.v. HY 7074 with viability percentage more than 80 were collected and used for the sowing purpose. Seeds were sown at 3 cm deep in soil and were grown under rainfed condition during kharif season.

The second and third youngest fully opened leaves of one pot of each treatment were cut for analysis of enzymes (acid phosphatase, starch phosphatase, catalase, peroxidase and lipid peroxidation) and photosynthetic activities.

Analysis of soil: Electric conductivity (EC) and pH of soil were measured by the method of Jackson (1973).

Analysis of growth and biomass: The plants were sampled for determination of plant height, fresh and dry weight of stem and leaf. 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 (Mx-Rady: max=250g, d=0.01g) was determined after drying these fresh samples in preheated oven (Ambassador- Oven) at 70 ^{0}C for 48 hours. The mean plant height was expressed in centimeter (cm) and weight in gram (g).

Biochemical analysis:

Acid Phosphatase (EC 3.1.3.2): Acid phosphatase activity was assayed by the method of Schmidt (1955). Absorbance was measured by spectrophotometer (Toshniwal Visible spectrophotometer- TSUV 75) on 640nm. The unit of enzyme activity was expressed in EU mg^{-1} protein.

Catalase (EC 1.11.1.6; CAT): Activity of catalase 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): Peroxidase activity was assayed by the method of Luck (1963). Absorbance was measured by the same spectrophotometer on 485nm. The enzyme activity was expressed as change in optical density between the sample and the blank (or EU) ÄO.D. mg^{-1} protein.

Lipid Peroxidation (Malondialdehyde Contents; LPO): Lipid peroxidation was determined in terms of malondialdehyde (MDA) to access the membrane damage. It was assayed by the method of (Heath and Packer, 1968). Absorbance was measured by the same spectrophotometer on 532nm and 600nm. The enzyme activity was expressed in nmol MDA 100 mg^{-1} fresh weight.

Photosynthetic efficiency: The pigment contents (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) were estimated by the method of Arnon (1949), and their quantity was expressed in mg g^{-1} fresh weight of tissue. Carotenoid contents were calculated on leaf fresh weight basis according to the 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 weight of 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 GraphPad Prism 7.0. It was followed by the comparison of mean values using Dunnett's multiple comparisons method at pd" 0.05.

Results and Discussion

Soil analysis: The soil used in the study was sandy loam in texture with pH of 7.16; electrical conductivity (EC) of 1.172 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	7.16±0.05	
E C	1.172±0.002 dSm^{-1}	

Growth and biomass: Interpreting the data relating to biomass *i.e.* plant height, leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW), stem dry weight (SDW), root fresh weight (RFW) and root dry weight (RDW) revealed that it showed a significant enhancement on the application of different levels of Fe as shown in fig 1 (A, B, C). It showed an increasing pattern on application of different Fe levels as compared to control. 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 Fe concentration dependent manner. The biomass was sharply increased significantly ($p<0.05$) with increasing Fe concentration in nutrient solution and reached its highest level at T4 (30 mg kg^{-1}Fe soil+pre-flowering foliar spray). The biomass (plant height, LFW, LDW, SFW, SDW, RFW and RDW) was increased by 15.28, 43.45, 55.93, 60.94, 46.94, 229.17 and 9.29% respectively, at T4 Fe levels as compared to control.

Enzyme activity -

Acid Phosphatase: Interpretion of the data relating to acid phosphatase activity revealed that it showed a significant enhancement on the application of different levels of Fe as shown in fig 2. It showed an increasing pattern on application of different Fe

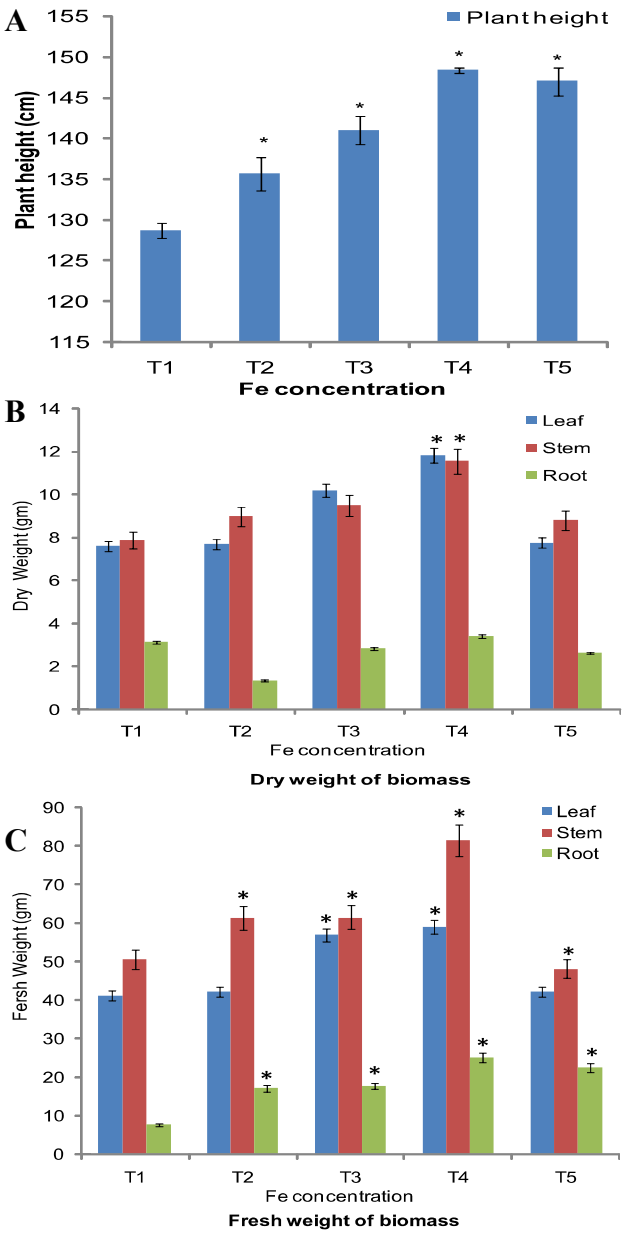

Fig. 1 (A, B, C): Graphical illustration of the effect of Fe on growth and biomass of maize. [T1= control, T2= Fe (15 mg kg^{-1}soil + pre flowering foliar spray), T3= Fe (15 mg kg^{-1}soil+ post flowering foliar spray), T4= Fe (30 mg kg^{-1}soil + pre flowering foliar spray), T5= Fe (30 mg kg^{-1}soil + post flowering foliar spray), plant height (A); fresh weight of biomass (B)= leaf, stem and root; Dry weight of biomass (C)=leaf, stem and root] The values are mean of 3 replicates ± S.E. *Data significant at p<0.05. Multiple comparisons Vs control group (Dunnett's multiple comparisons method). Overall significant level=0.05

levels as compared to control. The acid phosphatase enzyme activity was sharply increased significantly (p<0.05) with increasing Fe concentration in nutrient solution and reached its highest level at T4 (30mg kg^{-1} Fe soil+pre-flowering foliar spray). Greatest activity of acid phosphatase enzyme in plants indicates that highest absorption of iron in leaves. The activity was increased by 127.9, 583.3, 905.4 and 192.6% respectively, with increasing Fe levels as compared to control. Enzyme activity increased by increasing Fe levels in soil and foliar application at particular growth stage i.e. T4 and then was decreased in concentrations.

Catalase (CAT): Catalase enzyme activity showed a significant enhancement on the application of different levels of Fe as shown in fig 3. It showed an increasing pattern on application of different Fe levels as compared to control. The catalase enzyme activity was increased significantly (p<0.05) with increasing Fe concentration in nutrient solution and reached its highest activity at T3 (15 mg kg^{-1}Fe soil+post-flowering foliar spray). Increased activity of catalase enzyme in plants indicates that highest absorption of iron in leaves. The activity was increased by 29.5, 82.9, 61.9% and then decreased by 19.0% respectively, with increasing Fe levels as compared to control. Enzyme activity increased by increasing Fe levels in soil and foliar application at particular growth stage i.e. T3 and then was decreased in concentrations.

Peroxidase (POD): Peroxidase enzyme activity showed a significant enhancement on the application of different levels of Fe as shown in fig 4. It showed an increasing pattern on application of different Fe levels as compared to control. The peroxidase enzyme activity was increased significantly (p<0.05) with increasing Fe concentration in nutrient solution and reached its highest activity at T3 (15 mg kg^{-1}Fe soil+post-flowering foliar spray). Increased activity of peroxidase enzyme in plants indicates that highest absorption of iron in leaves. The activity was increased by 122.2, 131.9, 88.9 and 88.6% respectively, with increasing Fe levels as compared to control. Enzyme activity increased by increasing Fe levels in soil and foliar application at particular growth stage i.e. T3 and then was slightly decreased in concentrations.

Lipid Peroxidation (LPO): It was found to be significantly decreased on the application of different levels of Fe as shown in fig 5. It showed a decreasing pattern on application of different Fe levels as compared to control. The LPO activity was decreased significantly (p<0.05) with increasing Fe concentration in nutrient solution and reached its lowest activity at T3 (15 mg kg^{-1}Fe soil+post-flowering foliar spray). Decreased activity of LPO in plants indicates that highest absorption of iron in leaves. The activity was decreased by 47.9, 78.3, 59.3 and 42.9% respectively, with increasing Fe levels as compared to control. Decreased rate of LPO was recorded as indicated by gradually decreasing MDA contents decreased to 78.3% at T3 level (15 mg kg^{-1} Fe soil+post-flowering foliar spray).

Photosynthetic efficiency: Interpreting the data relating to photosynthetic activity revealed that it showed a significant enhancement on the application of different levels of Fe as shown in fig 6. It showed an increasing pattern on application of different Fe levels as compared to control. The chlorophyll and carotenoid activity were increased significantly (p<0.05) with increasing Fe concentration in nutrient solution and reached its highest activity at T4 (30 mg kg^{-1} Fe soil+pre-flowering foliar spray). The chl a, chl b and total chl activity was increased by 10.8, 18.2, 29.0 and 22.2%; 22.4, 31.4, 62.0 and 41.5% and 39.1, 50.3, 109.6 and 69.3% respectively, with increasing Fe levels as compared to control. The carotenoid activity was increased by 18.5, 28.4, 46.7 and 27.5% respectively, with increasing Fe levels as compared to control.

Overall significant level=0.05. Crop nutrients could be provided through different application methods including soil amendment, seed priming, and foliar application (Khoshgoftarmanesh, 2008). Foliar application of Fe was considered as a short time tool for biofortification with Fe because soil application of most Fe sources are generally ineffective because of the rapid conversion of soluble Fe into plant unavailable solid Fe (III) forms (Fageria, 2009; Aciksoz, 2011) and iron mobility in plant is also low (Grusak and Della Penna, 1999; Zhu *et al.,* 2007; Borg 2009). Foliar applied Fe can be absorbed by the leaf epidermis, remobilized, and transferred into the grain via the phloem (Wei *et al*, 2012). Nutrient deficiency 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 (Yadav *et al.,* 2018).

The element Fe is very abundant in soils. According to Lindsay (1974) most agricultural crops require less than 0.5 ppm iron in the soil in the plough layer whereas the total Fe level is about 2% in the soil. The most luxuriant growth (plant height) and biomass (LFW, LDW, SFW, SDW, RFW and RDW) of plants were observed at T4 (30 mg kg^{-1} Fesoil+pre-flowering foliar spray) with respect to control (fig 1: A-C). As according to Christ (1974) monocots have higher requirements of Fe than dicot plants. Zain *et al*. (2015) and Abbas *et al*. (2009) reported that increase in levels of Fe increased wheat growth and yield. Naz*et al*. (2015) also reported that increase in levels of Fe increased growth, biomass and yield in wheat crop.

Fe as a constituent or a metal cofactor has a specific role in a number of enzymes of metabolic processes. In plant tissue, there are two major groups of iron-containing protein; heme proteins (catalase, peroxidase and acid phosphatase) and Fe-S proteins. The activity of Acid phosphatase was increased significantly with increasing Fe concentration. These results are somewhat similar to Agarwala *et al.* (1965) is recorded increase in acid phosphatase activity in maize and radish by increasing levels of iron.

Catalase (CAT) and peroxidase (POD) enzymes are involved in reducing H_2O_2 to H_2O. In the present study, our results showed that CAT and POD activity increased by increasing Fe levels in soil and foliar application at T3 and then was slightly decreased in concentrations. This increase in CAT and POD activity which was explained by the fact that both the enzymes contain Fe (Fig 3, 4). Fang and Kao in 2000 and Agarwal *et al.* (2006) also reported that there was an increase in POD and CAT activity in leaves of rice and wheat, respectively. Several workers reported that De Santiago *et al.* (2011) and Mohamed *et al.* (2016) are recorded increase in CAT and POD activity by increasing Fe levels.

The activity of Lipid peroxidation (LPO) was reduced significantly at T3, thereafter increased with increasing Fe concentration as compared to control. These results are somewhat similar to Agarwal *et al.* (2006) and Fang *et al.* (2001) who are recorded LPO activity in leaf of wheat and rice, respectively. An decreased in MDA levels was observed in the present study, denotes occurrence of membrane damage resulting from peroxidation of polyunsaturated fatty acid, which causes generation

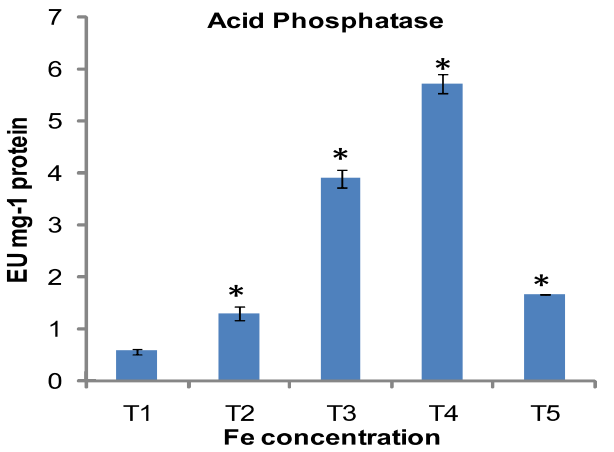

Fig 2: Graphical illustration for the effect of different levels of Fe on enzyme acid phosphatase of maize. [AP=Acid phosphatase (EU mg^{-1} protein)

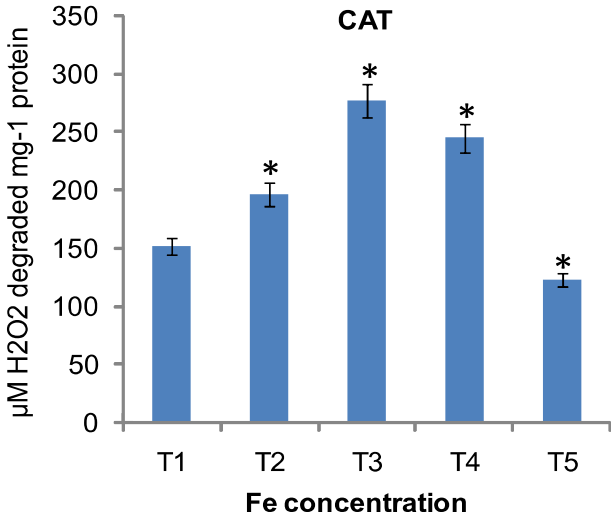

Fig 3: Graphical illustration for the effect of different levels of Fe on enzyme catalase of maize. [Catalase (EU mg^{-1} protein)

In fig. 2 and 3: T1= control, T2= Fe (15 mg kg^{-1}soil+pre-flowering foliar spray), T3= Fe (15 mg kg^{-1} soil+ post-flowering foliar spray), T4= Fe (30 mg kg^{-1}soil+pre-flowering foliar spray), T5= Fe (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 (Dunnett's multiple comparisons method). Overall significant level=0.05.

of ROS and ensuring oxidative stress. This enzyme is involved in providing protection.

Photosynthetic molecule such as Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were increased by increasing Fe concentration as compared to control. Maximum photosynthetic activity was observed in T4 Fe level. The chlorophyll content in maize leaves show great variability at different Fe levels. Similar result was reported by Agarwal *et al.* (2006) and Mohamed *et al.* (2016). In Fe-S proteins, iron is coordinated to the thiol group of cysteine or to inorganic S as clusters, or to both. The most well-known Fe-S protein is ferrodoxin. Other Fe-S proteins have functions in metabolic processes, such as photosynthesis, SO_4 and SO_3 reductions. Heme proteins include the various cytochromes, which are characterized by a heme Fe-

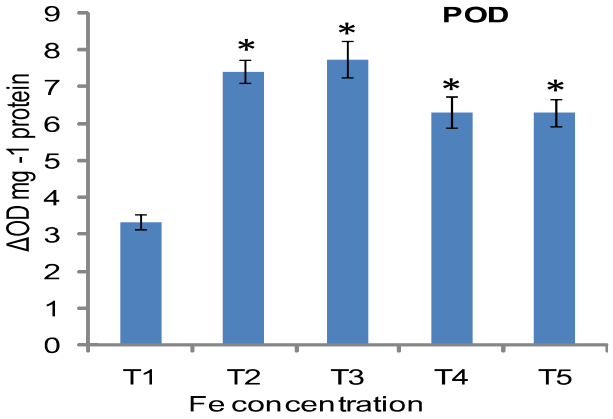

Fig. 4: Graphical illustration for the effect of different levels of Fe on enzyme peroxidase of maize. [Peroxidase (EU mg^{-1} protein)

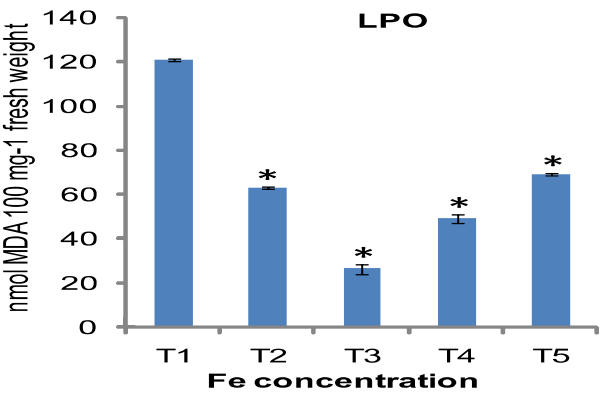

Fig. 5: Graphical illustration for the effect of different levels of Fe on enzyme lipid peroxidation of maize. [Lipid peroxidation (EU nmol MDA 100 mg^{-1} fresh weight)

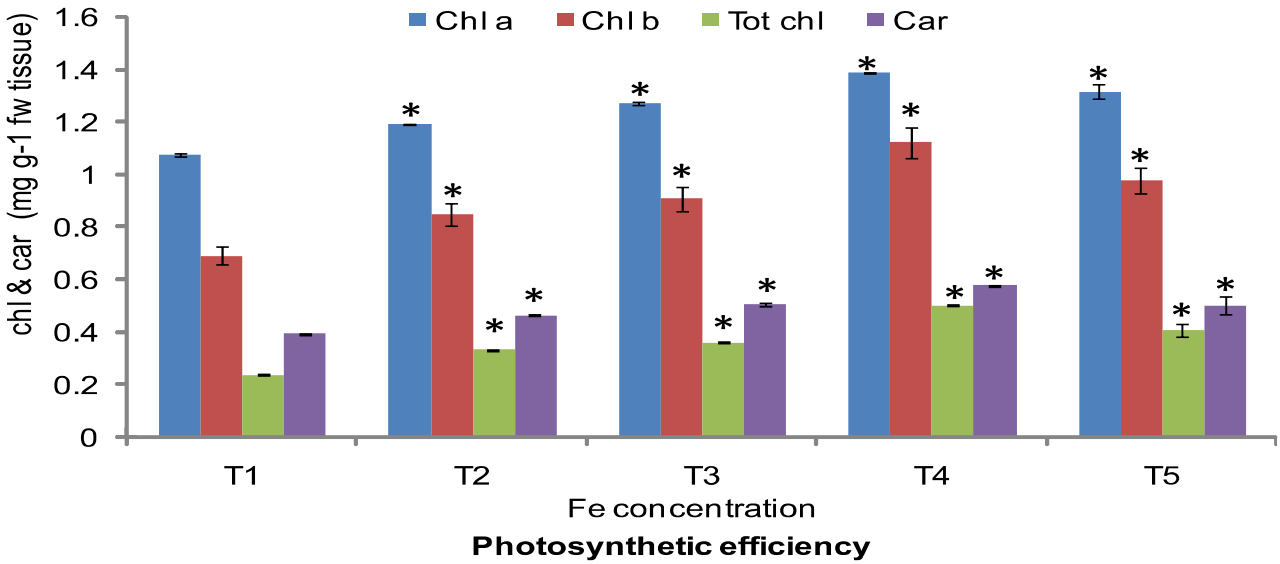

Fig. 6: Graphical illustration for the effect of different levels of Fe on photosynthetic efficiency of maize. [Chl = chlorophyll (mg g^{-1} fresh weight), Chl a= chlorophyll a (mg g^{-1} fresh weight); Chl b= chlorophyll b (mg g^{-1} fresh weight); Tot Chl = total chlorophyll (mg g^{-1} fresh weight), car= carotenoid

In fig. 4,5 and 6: T1= control, T2= Fe (15 mg kg^{-1} soil+pre-flowering foliar spray), T3= Fe (15 mg kg^{-1} soil+ post-flowering foliar spray), T4= Fe (30 mg kg^{-1} soil+pre-flowering foliar spray), T5= Fe (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 (Dunnett's multiple comparisons method). Overall significant level=0.05

porphyrin complex as a prosthetic group. Cytochrome is a constituent of the redox systems in chloroplasts. Fe increases cytochrome b6/f complex, a photosynthetic component of photosystem II (PSII) in thylakoids. It has been studied that excessive dissipation of light might cause photoinhibitory damage (Gogorcena *et al*., 2001; Donnini *et al*., 2003; Siddiqui *et al*., 2017; Katti and Math, 2016; Nath *et al*., 2017).

Iron is an essential metal,Its acquisition; by plant roots from the soil enables its entry into the food chain. Therefore, in this way, essential nutrients reach to the animal and human diets.In the present work a positive relationship was established between iron applications, biomass and the activity of enzymes. The effectiveness of combined application of soil and foliar was observed to be superior over soil application which indicates that foliar iron application might have compensated the iron absorption problem through soil and also its translocation. The timing of foliar application also seemed to have significance in increasing the enzyme activity in the present investigation.An increment has been observed in acid phosphatase, catalase and peroxidase with progressive increment of iron level. The reason for this is that Fe is required as a cofactor in functioning of acid phosphatase, catalase and peroxidase. Due to this reason a drop could be noticed under deficit condition of iron (T1) and improvement with its supply (T2, T3, T4, and T5).

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