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Effect of zinc and iron application on leaf chlorophyll, carotenoid, grain yield and quality of wheat in calcareous soil of Saurashtra region

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Abstract
Micronutrients like Fe and Zn plays a vital role in growth and development of plant and occupies an important portion by virtue of their essentiality in increasing crop yields. Trend to more intensive crop production with higher yields, heavier usage of fertilizers increases the need for greater consideration and usage of micronutrients. Iron plays an important role in respiration, photosynthesis and the production of healthy green leaves. Keeping in view the important role of iron in crop production, chelated application of Fe and Zn may help to overcome the above problem and helps the increase in crop growth and yield and grain quality, the present study was designed. The results showed that the leaf chlorophyll-a, chlorophyll-b, Total chlorophyll and total carotenoid content in wheat leaves were significantly increase at 45 and 70 DAS in wheat leaves in Fe treated plot. Content also remained higher in Zn treated plot compared to control indicated that Zinc and Iron play important role in improvement of quality, regulation of enzymatic process and the chlorophyll content. Fe and Zn applications, either as soil or foliar application increased the grain yield, protein and gluten content of wheat. This may be the effect of increased in photosynthesis due to higher photo harvesting pigment in leaf as well as increased in nutrient content in plant due to the application of Zn and Fe.

Keywords: carotenoid, chlorophyll, Fe, gluten, micronutrient, protein, *Triticum spp.*, wheat, Zn

Introduction
Wheat (*Triticum spp.*) is the world's leading cereal crop. It is the third most-produced cereal after maize and rice. Trend to more intensive crop production with higher yields, heavier usage of fertilizers increases the need for greater consideration and usage of micronutrients. Micronutrients play a vital role in growth and development of plant and occupy an important portion by virtue of their essentiality in increasing crop yields. In fact, their essential role in plant nutrition and increasing soil productivity makes their importance ever greater. In view of intensive cropping with high yielding varieties and application of high analysis major and secondary nutrient fertilizers, incidence of micronutrient deficiencies have been more pronounced (Dewal and Pareek, 2004) [1]. As farmers strive for top yields and quality, they must give more attention to micronutrient needs like Fe and Zn. It was also documented that soil or foliar application of Fe alone or in combination with other micronutrients like Zn enhanced the plant growth, grain yield and its components and grain quality of wheat compared with non-application. Zinc and Iron play important role in improvement of quality, regulation of enzymatic process and the chlorophyll content. Zinc is needed for the creation of the plant growth hormone auxin and for creation of the green chlorophyll and cytochrome pigments. Wheat is inherently low in Zn deficient soil in concentration of Zn in grain as well as sensitive to Zn deficiency. Iron is mainly involved in the process of plant photosynthesis. Iron plays an important role in respiration, photosynthesis and the production of healthy green leaves. Keeping in view the important role of iron in crop production, chelated application of Fe and Zn may help to overcome the above problem and helps the increase in crop growth and yield and grain quality, the present study was designed.

Materials and Methods
A field experiment was conducted during *rabi* (winter) 2016-17 in an instructional farm, College of Agriculture, JAU, Junagadh. The chemical analysis was carried out at Department of Soil Science and Agril. Chemistry. The details of the methodology followed during the course of study are as below.

Treatments

The experiment comprised of total twelve treatments were laid out in Randomized Block Design with three replications. The detail of treatments is described in following paragraph.

T_1 Control (N-P-K:120-60-60 kg ha^{-1}) RDF
T_2 RDF + Spraying of chelated Zn @ 0.5% at 30 and 60 DAS
T_3 RDF + Spraying of chelated Zn @ 1.0% at 30 and 60 DAS
T_4 RDF + Spraying of chelated Zn @ 1.5% at 30 and 60 DAS
T_5 RDF + Spraying of chelated Fe @ 1.0% at 30 and 60 DAS
T_6 RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS
T_7 RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS
T_8 RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1}
T_9 RDF + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}
T_{10} RDF + Soil application of $FeSO_4$ @ 20 kg ha^{-1}
T_{11} RDF + Soil application of $FeSO_4$ @ 30 kg ha^{-1}
T_{12} RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}

Chemical Parameters

Chlorophyll content in wheat leaves was determined by DMSO method (Hiscox and Israelstam, 1979 [2]; Warwate *et al.*, 2017) [3]. Total carotenoid was measured as per Mahadevan and Shridhar (1986) [4]. Determination of protein and gluten quality-related parameters of wheat was carried out using near-infrared reflectance spectroscopy (Mehmet and Mustafa, 2011) [5]. For interpretation, data was statistically analyzed as per CRD design (Snedecor and Cochran 1956) [6].

Results and Discussion

The results regarding effect of zinc and iron applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of chelated-Zn and Fe on leaf chlorophyll, carotenoid, grain yield, grain protein and gluten content are presented as under.

Chlorophyll Content

The data regarding chlorophyll a and chlorophyll b in leaves as influenced by zinc and iron application at 40 and 70 DAD are presented in Table 1.

Chlorophyll content at 40 DAS

The results for the chlorophyll–a content (mg/g) at 40 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest chlorophyll-a content (3.183 mg/g) was received for the treatment T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}). The lowest chlorophyll A content (1.620 mg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the chlorophyll-a content of wheat.

The chlorophyll-b content (mg/g) at 40 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest chlorophyll-b content (0.958 mg/g) was received for the treatment T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}). The lowest chlorophyll B content (0.356 mg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the chlorophyll B content of wheat.

The results for the total chlorophyll content (mg/g) at 40 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest Total chlorophyll content (4.141 mg/g) was received for the treatment T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}). The lowest total chlorophyll content (1.976 mg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the total chlorophyll content of wheat.

Chlorophyll content at 70 DAS

The results for the chlorophyll-a content (mg/g) at 70 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest chlorophyll-a content (3.603 mg/g) was received for the treatment T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS). It also remained at statistically at par with treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}), T_6 (RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS) and T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}) which contained 3.256, 3.242 and 3.085 mg/g chlorophyll-a, respectively. The lowest chlorophyll A content (2.074 mg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the chlorophyll A content of wheat.

Table 1: Effect of Zn and Fe application on chlorophyll (mg g^{-1}) and carotenoid (µg g^{-1}) content in wheat leaf at 40 DAS and 70 DAS.

Treatment	40 DAS				70 DAS			
	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid
	mg g^{-1} fresh weight			µg g^{-1} fresh weight	mg g^{-1} fresh weight			µg g^{-1} fresh weight
T_1	1.62	0.356	1.976	3.637	2.074	0.419	2.493	4.344
T_2	1.717	0.375	2.092	3.86	2.616	0.548	3.164	4.605
T_3	2.224	0.409	2.633	4.929	2.907	0.641	3.548	5.451
T_4	2.257	0.421	2.678	5.212	2.523	0.541	3.064	5.821
T_5	2.251	0.537	2.788	5.375	2.706	0.632	3.338	6.325
T_6	2.682	0.499	3.181	5.905	3.242	0.545	3.787	6.979
T_7	2.838	0.547	3.385	6.241	3.604	0.774	4.378	7.388
T_8	1.851	0.375	2.226	3.973	2.3	0.498	2.798	4.559
T_9	2.135	0.418	2.553	4.673	2.129	0.384	2.513	5.149
T_{10}	2.505	0.454	2.959	5.601	2.674	0.546	3.22	6.045
T_{11}	3.183	0.958	4.141	7.415	3.085	0.663	3.748	6.761
T_{12}	2.622	0.504	3.126	5.831	3.256	0.67	3.926	6.464
S.Em±	0.106	0.046	0.117	0.193	0.184	0.063	0.252	0.198
C.D. @ 5%	0.312	0.137	0.344	0.57	0.544	0.183	0.744	0.584
C.V.%	7.87	16.46	7.18	6.4	11.65	19.07	13.31	5.89

The chlorophyll-b content (mg/g) at 70 DAS showed significant difference. The highest chlorophyll-b content (0.774 mg/g) was received for the treatment T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS). It also remained at statistically at par with treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}), T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}) T_3 (RDF + Spraying of chelated Zn @ 1.0%) and T_5 (RDF + Spraying of chelated Fe @ 1.0%) which contained 0.670, 0.663, 0.641 and 0.632 mg/g chlorophyll B, respectively. The lowest chlorophyll B content (0.384 mg/g) was received for the treatment T_9 (RDF + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the chlorophyll-b content of wheat.

The total chlorophyll content (mg/g) at 70 DAS also showed significant difference and followed the same trend as per Chlorophyll-a. The highest total chlorophyll content (4.378 mg/g) was received for the treatment T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS). It also remained at statistically at par with treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}), T_6 (RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS) and T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}) which contained 3.926, 3.787 and 3.748 mg/g total chlorophyll, respectively. The lowest total chlorophyll content (2.074 mg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the total chlorophyll content of wheat. Although potassium is not a constituent of chlorophyll, a characteristic symptom of K deficiency is the destruction of chlorophyll. This means that it is suspected that part of the function of K is related to the formation of chlorophyll precursor or to the prevention of the decomposition of chlorophyll (Fagaria, 2002) [7]. In present experiment also, results showed higher K content in such treatment might be the reason for high chlorophyll content.

Carotenoid Content (µg/g)

The data regarding carotenoid in leaves as influenced by zinc and iron application at 40 DAS and 70 DAS are presented in Table 1. The results for the carotenoid content (µg/g) at 40 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest carotenoid content (7.415 µg/g) was received for the treatment T_{11} (RDF + $FeSO_4$ @ 30 kg ha^{-1}). The lowest carotenoid content (6.367 µg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the carotenoid content of wheat.

The carotenoid content (µg/g) at 70 DAS showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest carotenoid content (7.388 µg/g) was received for the treatment T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS). It also remained at statistically at par with treatment T_6 (RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS) which contained 6.979 µg/g carotenoid content. The lowest carotenoid content (4.344 µg/g) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications either as soil or foliar application increased the carotenoid content of wheat. Rehm and Albert (2006) [8] reported that foliar sprays of $FeSO_4$. $7H_2O$ or Fe-chelates were found more effective and efficient than soil application. This result was in agreement with present experiment.

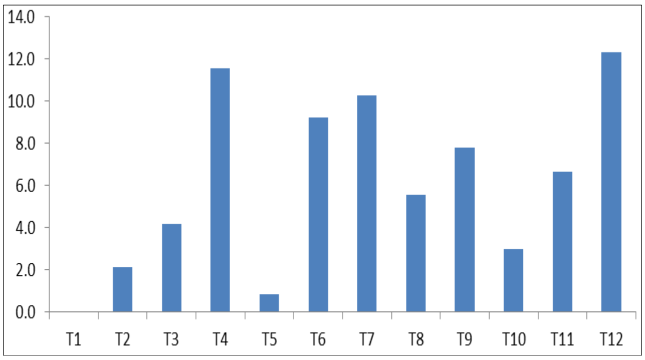
Grain Yield (kg ha^{-1})

The data regarding grain yield per hectare influenced by different levels of zinc and iron applied as a soil as well as foliar application are presented in Table 2. The significantly highest grain yield (4786 kg ha^{-1}) was received for the treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}) which was almost 38.2 per cent higher as compared to control treatment (N-P-K: 120-60-60 kg ha^{-1}) (Fig. 4.3 A). It also remained at statistically at par with treatment T_4 (RDF + Spraying of chelated Zn @ 1.5% at 30 and 60 DAS) yielded 4442 kg ha^{-1} wheat grain which was almost 28 per cent higher as compared to control. The treatment T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS) also recorded 23 per cent higher yield over control treatment i.e. 4264 kg ha^{-1} which remained at par with the treatment T_6, T_9, T_{11} and T_8 produced the wheat grain of 4134, 4060, 3960 and 3852 kg ha^{-1}, respectively. The lowest yield (3462 kg ha^{-1}) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications, either as soil or foliar application increased the grain yield of wheat. This may be the effect of increased in different yield attributes, increase in photosynthetic activity due to higher chlorophyll content as respond to the application of Zn and Fe. According to Ozturk *et al.* (2006) [9], the highest wheat yield is closely related to the cultivar potential to produce fertile tillers, which also influences directly the number of spikes produced per unit area. Zinc application to leaves increased the number of fertile tillers and wheat yield. The above findings were in agreements with the results obtained in present experiment. In general Fe and Zn applications, either as soil or foliar application increased the grain yield of wheat. Habib (2009) [10] also showed the same result for the Zn application and observed the increase in the grain yield of wheat. Kalidasu *et al.* (2008) [11] observed the increase in grain yield due to Fe application in soil. Patel *et al.* (2009) [12] observed the increase in yield by applying $FeSO_4$ as foliar application.

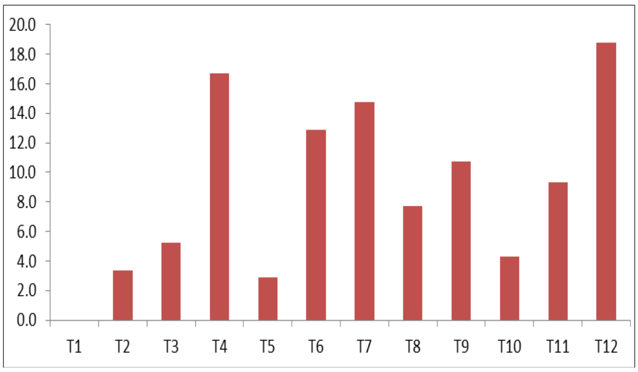
Table 2: Effect of Zn and Fe application on grain yield, protein and gluten content of wheat.

S. No	Treatments	Grain yield (kg ha^{-1})	Protein content (%)	Gluten content (%)
T_1	Control (N-P-K:120-60-60 kg ha^{-1}) RDF	3462	11.70	10.50
T_2	RDF + Spraying of chelated Zn @ 0.5%	3634	11.95	10.85
T_3	RDF + Spraying of chelated Zn @ 1.0%	3768	12.19	11.05
T_4	RDF + Spraying of chelated Zn @ 1.5%	4442	13.05	12.25
T_5	RDF + Spraying of chelated Fe @ 1.0%	3522	11.80	10.80
T_6	RDF + Spraying of chelated Fe @ 1.5%	4134	12.78	11.85
T_7	RDF + Spraying of chelated Fe @ 2.0%	4264	12.90	12.05
T_8	RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1}	3852	12.35	11.31
T_9	RDF + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	4060	12.61	11.63

T_{10}	RDF + Soil application of $FeSO_4$ @ 20 kg ha^{-1}	3736	12.05	10.95
T_{11}	RDF + Soil application of $FeSO_4$ @ 30 kg ha^{-1}	3960	12.48	11.48
T_{12}	RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}	4786	13.14	12.47
	S.Em±	145	0.13	0.22
	C.D. @ 5%	426	0.37	0.65
	C.V.%	6.34	1.75	3.35



[A] Protein



[B] Gluten

Fig 1: Effect of Zn and Fe application on per cent increase over control treatment in [A] Protein and [B] Gluten content of wheat grain

Protein Content (%)

The data regarding protein content in seed analyzed by NIR spectroscopy were influenced by soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe are presented in Table 2, Figure 1A. The highest protein content (13.14 %) was recorded for the treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}) which was 12.3 per cent higher than the control treatment (Fig. 4.4 A). Zeidan *et al.* (2010) [13] indicated that application of Zn and Fe elements was significantly increased the protein content in grain. Mahriya (1997) [14] also found similar result that the protein content in seeds of cowpea was increased significantly with the application of Fe over lower levels of Fe. Treatment T_{12} also remained at statistically at par with treatment T_4 (RDF + Spraying of chelated Zn @ 1.5% at 30 and 60 DAS), T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS) and T_6 (RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS) which contained 13.05, 12.90 and 12.78 per cent protein, respectively. The lowest protein content (11.7 %) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). Marschner (1995) [15] indicated that Zn deficient plant reduces the rate of protein synthesis but increase the accumulation of free amino acid. In general, Fe and Zn applications, either as soil or foliar application increased the protein content of wheat. This may be the effect of increased in nutrient content in plant due to the application of Zn and Fe. Singh (1998) [16] also observed that protein content in seeds of coriander was increased significantly with application of Zn. These results are in agreement with current findings.

Gluten Content (%)

The data regarding gluten content in seed as influenced by zinc and iron application are presented in Table 2, Fig.1 B. The results for the gluten content (%) at harvest showed significant difference for the various treatments applied as soil application as a $ZnSO_4$ and $FeSO_4$ as well as foliar application of EDTA-Zn and EDTA-Fe. The highest gluten content (12.47 %) was received for the treatment T_{12} (RDF + Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + $FeSO_4$ @ 20 kg ha^{-1}) which was 18.8 per cent higher than the control treatment (Fig.1 B). Pallavi and Sudha (2017) [17] observed that the soil and foliar application of zinc increased the protein content and sedimentation value. Micronutrients has enhanced the accumulation of assimilates in the grains (during the grain filling stage) and the resultant seeds had greater protein content which caused the higher development of gluten. Similar results are also observed by Goswami (2007) [18]. Treatment T_{12} also remained at statistically at par with treatment T_4 (RDF + Spraying of chelated Zn @ 1.5% at 30 and 60 DAS), T_7 (RDF + Spraying of chelated Fe @ 2.0% at 30 and 60 DAS) and T_6 (RDF + Spraying of chelated Fe @ 1.5% at 30 and 60 DAS) which contained 12.25, 12.05 and 11.85 per cent protein. The lowest gluten content (10.5 %) was received for the treatment T_1 i.e. (N-P-K: 120-60-60 kg ha^{-1}). In general, Fe and Zn applications, either as soil or foliar application increased the gluten content of wheat. This may be the effect of increased in nutrient content in plant due to the application of Zn and Fe. Keram *et al.* (2012) [19] revealed that total carbohydrate and wet gluten contented on wheat grain increased with Zn application over the control. Stepien and Wojtkowiak (2016) [20] studied the effect of foliar application of Cu, Zn, and Mn on yield and quality indicators of winter wheat grain. Foliar Zn fertilization combined with NPK increased gluten content. These results are in agreement with the present experiment.

Conclusion

Iron and Zinc is needed for the creation of green chlorophyll and cytochrome pigments. In present study, the leaf chlorophyll-A, chlorophyll-B, Total chlorophyll and total carotenoid content in wheat leaves were significantly increase at 45 and 70 DAS in wheat leaves in Fe treated plot. Content also remained higher in Zn treated plot compared to control indicated that Zinc and Iron play important role in improvement of quality, regulation of enzymatic process and the chlorophyll content. Fe and Zn applications, either as soil or foliar application increased the grain yield, protein and gluten content of wheat. This may be the effect of increased in photosynthesis due to higher photo harvesting pigment in leaf as well as increased in nutrient content in plant due to the application of Zn and Fe.

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