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Effect of combined zinc and iron application rates on summer maize yield, photosynthetic capacity and grain quality

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Abstract

Zinc (Zn) and iron (Fe) are important nutrients for both human and plants and their soil deficiency is a worldwide concern. This study investigated the benefits of using different rates of sole Zn and Fe along with their combinations on maize crude protein (CP) concentration, ether extract (EE) and crude fiber(CF). And some agronomic traits including dry biomass, grain yield and photosynthesis were examined as well. The field experiment was carried out at Doukou experimental station in 2016 to investigate the effect of Zn, Fe and Zn-Fe combinations on summer maize agronomic traits and quality. The results showed that the application of the micronutrients did not significantly influence maize dry biomass, total leaf area and chlorophyll content (SPAD). However, the application of Zn-Fe soil spray application had a significant effect on maize grain yield from 3497.01 to 4530.26 kg ha^{-1} and net photosynthetic rate (Pn) from 19.74 to 37.84 CO_2 μm^{-2} g^{-1} being the increases of 30% and 92% respectively. CP content significantly increased from 8.39% to 9.91 % and CF from 10.81% to 15.96%. These results imply that the use of Zn-Fe combination might be an alternative way to improve maize growth and quality in regions with Zn and Fe deficiency.

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Introduction

Micronutrients are essential elements for plants growth that are needed in very little amounts compared to the primary nutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Hänsch, 2009). The micronutrient, zinc (Zn) and iron (Fe), are very important to plant growth and their deficiencies impede plant growth and production(Kanai *et al.*, 2009).

Micronutrients availability depends on many soil factors and crop variety as well (de Valença *et al.*, 2017). In China, 40% of the land is Zn and Fe deficient (B. J. Alloway, 2009b) due to dominance of calcareous and alkaline soils which are characterized by high pH and carbonate content (I. Cakmak *et al.*, 1999; Ma *et al.*, 2014). Soil deficiency in both Zn and Fe is a worldwide problem that is affecting not only crop yield reduction but food quality as well (Kanai *et al.*, 2009; Manzeke *et al.*, 2014).

Zincisan important micronutrient vital to both animals and plants as a structural component of proteins and/or a regulatory co-factor of enzymes taking part in numerous biochemical pathways (García-Bañuelos *et al.*, 2014). For instance, various proteins comprise Znprosthetic groups (zinc finger, zinc twist) and about 300 enzymes necessitateZn as a cofactor (Broadley *et al.*, 2006). Besides, it has been estimated that a number of proteins have the capability ofbinding Zn and more or less of these Zn-binding proteins are transcription factorsrequired for gene regulation (Ismail Cakmak, 2008; Gómez-Galera *et al.*, 2010).

Fe is a necessary trace elementthat take place in several redox states, eagerly accepting and donating electrons, which confer the ability to function as a cofactor for numerous plant proteinsthat contribute in key metabolic pathways (Briat *et al.*, 2007; García-Bañuelos *et al.*, 2014). The role of Fe depend onfrequently on the reversible redox reaction of Fe2+ and Fe3+ ions, its aptitude toform octahedral complexes with several ligands and to diverge its redox potential in reaction to different ligands of the environment. This redox potential(Fe2+/Fe3+) facilitates its use, in the form of heme or Fe sulphur clusters, in anumber of protein complexes, particularly those involved in electron transfer (Hell & Stephan, 2003).

Zinc and iron are extensively used to increase yield and their concentration as well as quality in crops. They can be applied in different way such as foliar spray, soil application (sprayed over soil surface or applied in soil) and seed application method.

Foliar application is considered to be the most efficient method of increasing both grain yield and grain micronutrients contents (George & Schmitt, 2002), it is simple and direct applications to leaves (Hosseini *et al.*, 2007). This technique this has improved millet yield and protein content, lysine acid and soluble sugar content. It has also raised starch content of maize and doubled grain Zn concentration in maize (Leach & Hameleers, 2001; Peck *et al.*, 2008; Zong *et al.*, 2011).

There is another widely used method to apply Zn and Fe fertilizers which is soil application. It is believed to be an efficient way of correcting symptoms of soil Zn or Fe deficiency, significantly improved grain yield, biomass and harvest index in rice as well as enhancing Zn concentration and N in maize grain (Hossain *et al.*, 2008; Orabi *et al.*, 1981; Wang *et al.*, 2012).

Using seed with high Zn and Fe concentration combined with foliar application could be an alternative of the effective way of increasing grain yield and Zn and Fe concentration (Yong Zhang *et al.*, 2010).

Soil Zn application method is considered as not effective as far as increase grain Zn concentration is concerned, especially in severely Zn deficient soils, instead it is recommended to adopt foliar application method in such soils. However, research has proved that among wheat, rice and maize, wheat has been found to be the most promising cereal crop for enhanced Zn and Fe concentration using foliar application while maize seems to be less responsive(Saltzman *et al.*, 2013; Wang *et al.*, 2012). Because of the special growth features (height and girth) of maize, it is hard for farmers to conduct the foliar spray at the late growth stage which is effective stage for Zn and Fe biofortification. Therefore, in case of maize, soil application Zn fertilizer is more practical than foliar application for increasing both yield and grain Zn-Fe concentration(Liu *et al.*, 2017). Our study, therefore aims to investigate the effects of Zn, Fe and their combination, sprayed to the surface of the soil at vegetative stage with different rates, on agronomic traits, yield and quality of summer maize grown in calcareous soil.

Materials and methods

Experimental site and design

The research was conducted at Doukou experimental station (108°52′E, 34°36′N), Northwest A&F University, Shaanxi province, P.R China, 2016.The experimental area is a typical warm temperate semi-humid continental monsoon climate. The annual average precipitation is 595 mm, and the annual average land surface evaporation is 417.6mm. The annual average solar radiation is 247.35 kJ cm^{-2}, the average annual sunshine duration is 2271.6h, the sunshine percentage is 51%; the annual average temperature is 13.2°C. The annual average solar radiation is 247.35 kJ cm-1, the frost-free period is about 220 days, the activity accumulated temperature of 0℃ is 4926.8℃, the activity accumulated temperature of 10℃ is 4337.7℃, the activity accumulated temperature of 20℃ is 2556.4℃ and the average frost-free period is 215 days.

Maize (shandan 609) was planted on 12 June 2016 at 75,000 seeds/ha. Each plot was 7m× 5m with 61.5cm and 21cm row distance and plant distance respectively. An individual plot had eight equal sized rows. The experiment was a factorial design based on completely randomized blocks with three replicates.

The experiment consisted of six treatments and each treatment is assigned two levels: Lone supply of 30 kg $ZnSO_4.7H_2O$ 30 ha^{-1}, 37.5 kg $ZnSO_4.7H_2O$ ha^{-1}, supply of 30 kg ha^{-1} 80% $ZnSO_4.7H_2O$ + 20% $FeSO_4.7H_2O$, 37.5 kg ha^{-1} 80% $ZnSO_4.7H_2O$ + 20% $FeSO_4.7H_2O$, supply of 30 kg ha^{-1} 60% $ZnSO_4.7H_2O$ + 40% $FeSO_4.7H_2O$, 37.5 kg ha^{-1} 60% $ZnSO_4.7H_2O$ + 40% $FeSO_4.7H_2O$, supply of 30 kg ha^{-1} 40% $ZnSO_4.7H_2O$ + 60% $FeSO_4.7H_2O$, 37.5 kg ha^{-1} 40% $ZnSO_4.7H_2O$+ 60% $FeSO_4.7H_2O$, supply of 30 kg ha^{-1} 20% $ZnSO_4.7H_2O$ + 80% $FeSO_4.7H_2O$, 37.5 kg ha^{-1} 20% $ZnSO_4.7H_2O$ + 80% $FeSO_4.7H_2O$, lone supply of 30 kg $FeSO_4.7H_2O$ ha^{-1}, 37.5 kg $FeSO_4.7H_2O$ ha^{-1} and supply of NPK fertilizers with no supplement of Zn and Fe (Control) as presented in Table 1. NPK fertilizers were applied according to the local regulations of fertilizer applications. 0.3% (w/v) $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$ and their combinations were sprayed as aqueous solution to the soil surface at 6 leaves vegetative stage using hand held sprayer between 4-6 pm to avoid evaporation due sunny temperature and when there is less wind to avoid salt damage due to solution high concentration. The control plot was sprayed with the corresponding amount of water. The field was irrigated twice on 18 June and 15 August 2016. The experiment was conducted from June 12 to October 7 in 2016. The maize was harvested at physiological maturity.

Sampling and analysis

Soil samples (0-20) were taken before sowing and analyzed of soil available Zn concentration (DTPA-Zn) and soil available Fe (DTPA-Fe) according to (Lindsay & Norvell, 1978). Soil samples were also analyzed of available nitrogen (N), soil pH, available phosphorus (P) and available potassium (K) (Table 2). Plantsamples were taken at the stage of: vegetative stage, tasseling, silking grain filling and late grain filling. The samples were separated into roots, and shoots (the above ground parts). After flowering the samples were divided into roots, stalks, leaves and ears. At every sampling, 3 random plants were taken from each plot for measurements. All samples were oven dried at 105°Cfor 30 min then heated at 80°Cfor 24-48 hours until constant weight was reached for dry matter determination. At physiological maturity (PM), one eighth of each plot were harvested for maize yield and yield components determination. To avoid the border effect, the harvested area was measured in the middle of the plot.

The leaf area was measured at the same time with sampling by measuring the 3 ear leaves from three plants in each plot. The total leaf area was computed using a potable leaf area analyzer LI-3000C (LI-COR, USA).

Chlorophyll content was estimated using portable Chlorophyll meter (SPAD-502 Minolita, Osaka, Japan) was used as a non-destructive technique to estimate Chlorophyll content in ear leaves by means of absorbance/transmittance measurements. Three random maize plants were selected from every plot for measurement.

Leaf net photosynthetic rate was measured from vegetative stage to the period of late grain filling using a potable gas analysis system LI-6400(LI-COR., USA). Maize leaves were measured at a photosynthetic photon flux density (PPFD) of 1,200µmol m^{-2} s^{-1} and CO_2concentration of 350µmol/mol. Measurements were done from 9:00-11:30 AM and 15:00-17:00 on clear days.

In laboratory, all samples were analyzed for crude protein (CP) was determined by Kjeldahl method (Chemists & Chemists, 1920), the ether extract (EE) was analyzed using mechanical Soxhlet extraction method with petroleum ether (30-60) and crude fiber (CF) was analyzed by filter bag technique (Komarek *et al.*, 1996).

Statistical analysis

All data were analyzed using R 3.3.2 (R Core Team, 2016) and the data were analyzed of variance (ANOVA). Difference among the treatment means were determined for significance using Tukey HSD Test at 5% level.

Results

Biomass accumulation of summer maize

The supply of zinc, iron and their combination as supplement fertilizers had no significance effect on summer maize shoot dry weight at different growing stages.

Table 1. Different fertilizer rates' sources and codes used in the present study.

Fertilizer rate	Source	Code used
30 kg ha^{-1}	$ZnSO_4.7H_2O$	Zn_{30}
37.5 kg ha^{-1}	$ZnSO_4.7H_2O$	$Zn_{37.5}$
30 kg ha^{-1}	80% $ZnSO_4.7H_2O$ + 20% $FeSO_4.7H_2O$	Zn_{24} + Fe_6
	60% $ZnSO_4.7H_2O$ + 40% $FeSO_4.7H_2O$	Zn_{18} + Fe_{12}
	40% $ZnSO_4.7H_2O$ + 60% $FeSO_4.7H_2O$	Zn_{12} + Fe_{18}
	20% $ZnSO_4.7H_2O$ + 80% $FeSO_4.7H_2O$	Zn_6 + Fe_{24}
37.5 kg ha^{-1}	80% $ZnSO_4.7H_2O$ + 20% $FeSO_4.7H_2O$	Zn_{30} + $Fe_{7.5}$
	60% $ZnSO_4.7H_2O$ + 40% $FeSO_4.7H_2O$	$Zn_{22.5}$ + Fe_{15}
	40% $ZnSO_4.7H_2O$ + 60% $FeSO_4.7H_2O$	Zn_{15} + $Fe_{22.5}$
	20% $ZnSO_4.7H_2O$ + 80% $FeSO_4.7H_2O$	$Zn_{7.5}$ + Fe_{30}
30 kg ha^{-1}	$FeSO_4.7H_2O$	Fe_{30}
0 kg ha^{-1}	NPK	Control

The treatments of Zn_{12} + Fe_{18} and Zn_{15} + $Fe_{22.5}$ increased shoot dry matter, not statistically significant, by 10% and 12% from 4607.43 kg ha^{-1} to 4844.19 kg ha^{-1} and 5139.62 kg ha^{-1} respectively at 50 days after sowing (Table 3). At 107 days after sowing (physiological maturity stage), the treatments of Zn_{24} + Fe_6, Zn_{30} + $Fe_{7.5}$, Fe30 and $Fe_{37.5}$ increased dry biomass by13%, 16%, 13% and 14% compared to the control treatment respectively. However $Zn_{7.5}$ + Fe_{30} and Zn_{18} + Fe_{12} accumulated less biomass, not significant, compared to control with 11% and 6% less than the control treatment respectively.

Total leaf area of summer maize

The total leaf area was not statistically different compared to the control but some treatments had higher leaf area measurements than others. Zn_{12} + Fe_{18} had the highest leaf area with 14% at 50 DAS, 12% at 69 DAS and 12% at 89 DAS compared to control treatment (Table 4). All treatment showed almost similar response patterns throughout the growing period. The treatment of $Zn_{37.5}$ had highest total leaf area of 17% recorded at 69 DAS while the treatment of Zn_{30} + $Fe_{7.5}$ exhibited the maximum value At 89 days after sowing with an increase of 12% higher than the control treatment.

Table 2. Selected soil properties from the experimental field sampled before the sowing date (20cm).

pH	Total N (g kg^{-1})	Available K (mg kg^{-1})	Available P (mg kg^{-1})	Extractable Zn (mg kg^{-1})	Extractable Fe (mg kg^{-1})
9.03	150.92	25.21	195.04	0.77	3.91

Chlorophyll content and net photosynthetic rate

The effect of soil spray applied Zn and Fe on chlorophyll content was not statistically different among treatments across the growing stages.

The application Zn_{30} had the higher chlorophyll content (SPAD) with an increase of 7% followed by Zn_{30} + $Fe_{7.5}$ with 6% increase at 50 DAS compared with the control treatment. Zn_{15} + $Fe_{22.5}$ showed the highest increase of 8% higher than the control treatment at 89 days after sowing (Fig. 2).

Fertilization of Zn and Fe on summer maize significantly increased the net photosynthetic rate (P_N) with the highest value of 92% being observed in the treatment of Zn_{12} + Fe_{18} greater than the control at 50 DAS (Fig. 1). The treatments of $Fe_{37.5}$ and Zn_{15} + $Fe_{22.5}$ had also significant different compared to untreated control with an increase of 80% and 68% at 50 DAS. At 69 DAS a statistically significant influence was recorded in the treatment of Zn_{30} with an increment of 22% higher than the control.

Table 3. Shoot dry mass (kg ha^{-1}) of maize at different growth stages throughout the season.

Treatments	50 DAS	69 DAS	107 DAS
Zn_{30}	4994.54 a	10400.85 a	20406.95 a
$Zn_{37.5}$	4831.12 a	9566.94 a	20274.70 a
Zn_{24} + Fe_{6}	4411.15 a	9624.60 a	21250.91 a
Zn_{18} + Fe_{12}	4243.70 a	9211.84 a	21730.47 a
Zn_{12} + Fe_{18}	4844.19 a	9923.97 a	17620.11 a
Zn_{6} + Fe_{24}	4730.29 a	9875.11 a	19649.81 a
Zn_{30} + $Fe_{7.5}$	4815.44 a	9696.18 a	18534.81 a
$Zn_{22.5}$ + Fe_{15}	5061.34 a	10421.80 a	19777.46 a
Zn_{15} + $Fe_{22.5}$	5139.62 a	10007.11 a	19843.33 a
$Zn_{7.5}$ + Fe_{30}	4687.97 a	9220.64 a	16615.82 a
Fe_{30}	4872.18 a	7760.93 a	21186.80 a
$Fe_{37.5}$	4692.41 a	8864.45 a	21287.37 a
Control	4607.43 a	9851.81 a	18726.57 a

DAS: Days after sowing, 50: jointing stage, 69: silking stage, 107: physiological maturity.

Maize grain yield and yield components

The present study showed that the application of Zn, Fe and their combination had a significant effect on the grain yield. The average grain yield of the season was 3252.06 kg ha^{-1}. The treatments of $Zn_{22.5}$ + Fe_{15} and Zn_{30} + $Fe_{7.5}$ had both a significant increase of 19% higher than the untreated control. The highest grain weight was recorded from the treatment of Zn_{15} + $Fe_{22.5}$ which was 30% greater than the control (Table 5). The rest of fertilizer treatments did not exhibit a significant increase grain yield compared to the control treatment. Kernel number per ear was significantly different between the treatments of Zn_{24} + Fe_{6}, $Zn_{22.5}$ + Fe_{15} and aZn7.5+ Fe_{30} and Fe_{30}. While thousand kernel weight was significantly different in the treatment of Zn_{30}, $Zn_{22.5}$ + Fe_{15} and $Zn_{22.5}$ + Fe_{15} with Fe_{30}.

Table 4. Total leaf area of summer maize at different growing stages during the season.

Treatments	50 DAS	69 DAS	89 DAS
Zn_{30}	2097.06 ab	2571.91 a	3042.83 a
$Zn_{37.5}$	2247.30 a	2628.37 a	3194.59 a
Zn_{24} + F_{e6}	1862.19 ab	2329.94 a	3135.91 a
Zn_{18} + Fe_{12}	1967.30 ab	2597.10 a	3046.66 a
Zn_{12} + Fe_{18}	2073.29 ab	2758.70 a	3205.27 a
Zn_{6} + Fe_{24}	2010.90 ab	2549.03 a	3207.26 a
Zn_{30} + $Fe_{7.5}$	2185.84 ab	2718.86 a	3386.36 a
$Zn_{22.5}$ + Fe_{15}	1978.27 ab	2501.93 a	3289.36 a
Zn_{15} + $Fe_{22.5}$	1935.14 ab	2657.41 a	3204.60 a
$Zn_{7.5}$ + Fe_{30}	1649.48 b	2484.11 a	3207.88 a
Fe_{30}	1889.45 ab	2086.60 a	3026.71 a
$Fe_{37.5}$	1693.75 ab	2263.52 a	3050.86 a
Control	1914.32 ab	2437.79 a	3018.70 a

Each value is the mean of three replicates. Different letters in each column indicate significances difference amongst the treatments ($P \leq 0.05$).

Crude protein content, ether extract and crude fiber

The application of Zn, Fe and the combination Zn-Fe as supplement on basal fertilizer had no significant influence on the grain of crude protein content.

The treatments of Zn12 + Fe18 and Zn18 + Fe12 had the higher concentration of crude protein, with 17% and 18% compared to the control treatment respectively (Table 6).

The ether extract in treatment of $Zn_{37.5}$ and Zn_{30} + $Fe_{7.5}$ increased by 9% and 16% higher than the control treatment respectively which is not statistically significant. However, the soil application of Zn and Fe significantly increased the cruder fiber by 47% in the treatment of Zn_{24} + Fe_{6}. The treatment of $Zn_{37.5}$, $Zn_{22.5}$ + Fe_{15} and Fe_{30} have also statistically increased the crude fiber by 29%, 23% and 32% higher the control respectively.

Table 5. Effect of Zn and Fe on maize grain yield (kg ha^{-1}), kernels number per and thousand kernels weight (g).

Treatments	Grain yield	Kernels/ear	Thsd kernels
Zn_{30}	1794.43 b	315.84 ab	332.96 a
$Zn3_{7.5}$	1759.32 b	325.33 ab	314.30 ab
Zn_{24} + Fe_{6}	3549.48 a	441.71 a	320.79 ab
Zn_{18} + Fe_{12}	4155.62 a	337.92 ab	324.86 ab
Zn_{12} + Fe_{18}	3834.32 a	407.68 ab	318.66 ab
Zn_{6} + Fe_{24}	4170.44 a	346.32 ab	315.82 ab
Zn_{30} + $Fe_{7.5}$	4035.08 a	398.93 ab	315.58 ab
$Zn_{22.5}$ + Fe_{15}	4530.26 a	418.13 a	325.97 a
Zn_{15} + $Fe_{22.5}$	4239.15 a	353.07 ab	312.89 ab
$Zn_{7.5}$ + Fe_{30}	1842.75 a	282.19 b	310.31 b
Fe_{30}	3079.38 a	301.76 ab	303.64 b
$Fe_{37.5}$	1867.63 a	259.07 b	321.15 ab
Control	3497.01 a	361.92 ab	318.88 ab

Each value is the mean of three replicates. Different letters in each column indicate significances difference amongst the treatments ($P \leq 0.05$).

Discussion

Biomass and grain yield of summer maize

Zinc, iron and their combination at different rate, supplemented on basal fertilizer NPK, did not significantly increase summer maize biomass in calcareous soil. Our study findings were consistent with that of (Wang *et al.*, 2012) and (Yue-Qiang Zhang *et al.*, 2012) which indicated that the application of zinc did not influence biomass accumulation on maize in soil with 0.63, 0.48 and 0.43 $mgkg^{-1}$ DTPA-Zn.

Table 6. Effect of Zn and Fe on grain crude protein content, ether extract and crude fiber.

Treatments	CP	EE	CF
Zn_{30}	7.77 a	3.39 a	9.61 a
$Zn_{37.5}$	8.99 a	3.10 a	13.93 b
$Zn_{24} + Fe_{6}$	9.54 a	3.01 a	15.96 b
$Zn_{18} + Fe_{12}$	9.91 a	3.29 a	7.93 c
$Zn_{12} + Fe_{18}$	8.18 a	2.10 a	11.97 a
$Zn_{6} + Fe_{24}$	8.58 a	1.32 a	13.34 b
$Zn_{30} + Fe_{7.5}$	9.86 a	2.63 a	8.74 a
$Zn_{22.5} + Fe_{15}$	8.24 a	2.11 a	11.29 a
$Zn_{15} + Fe_{22.5}$	7.82 a	1.87 a	12.24 a
$Zn_{7.5} + Fe_{30}$	9.02 a	2.24 a	10.37 a
Fe_{30}	8.24 a	2.67 a	14.25 b
$Fe_{37.5}$	9.18 a	3.24 a	7.64 a
Control	8.39 a	2.84 a	10.83 a

Each value is the mean of three replicates. Different letters in each column indicate significances difference amongst the treatments ($P \leq 0.05$). C.P: crude protein, E.E: ether extract, CF: crude fiber.

There are several factors that limit the zinc efficiency on maize biomass and yield such as: pH, drought and soil extractable zinc (X Yang *et al.*, 2011a; Xi-wen Yang *et al.*, 2011b).

The critical range of deficiency concentration in soil DTPA extractable Zn being estimated between 0.5 to 1.5 mg kg^{-1}(BJ Alloway, 2009a), our field experiment had the soil DTPA-Zn of 0.77 mg kg^{-1}, it suggests that the field was severely zinc deficient. Soil application of Zn or Fe depends also on the rate applied, in the present study a treatment of Zn12 + Fe18 had raised both biomass and grain yield.

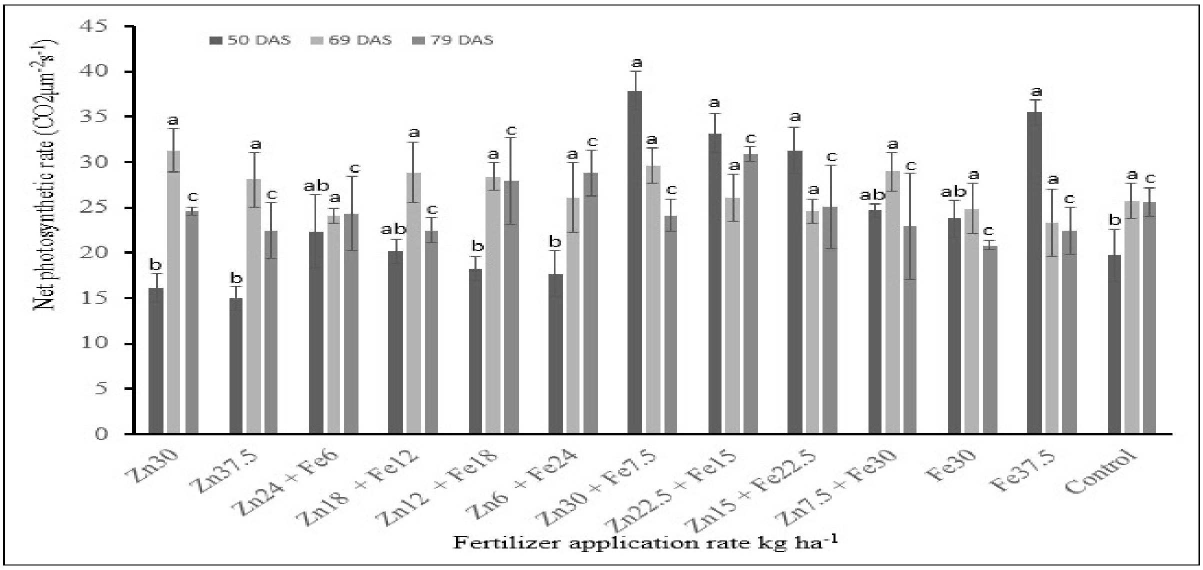

Fig. 1. Effect of Zn and Fe application on net photosynthetic rate (Pn) at different growing stages. Each value is the mean of three replicates (±SE). Different lower case letters indicate significant difference among treatments ($P \leq 0.05$). DAS: Days after sowing, 50: jointing stage, 69: silking stage, 79: grain filling.

The grain yield was statistically increased in our study, which is consistent with the finding of (Manzeke *et al.*, 2014) that showed the significant effect of using zinc as a supplement fertilizer. In our study, the grain yield was increased up to 30% in the field that is severely deficient in soil in DTPA-Zn. It was reported that the optimum soil Zn concentration to achieve high grain yield is 4.7 mg kg^{-1}(Liu *et al.*, 2017) which is quiet higher than that was suggested previously by (Sakal *et al.*, 1981) and (BJ Alloway, 2009a). However, the common thing is, increase both maize grain yield and grain Zn concentration high soil DTPA-Zn is required (Bender *et al.*, 2013).

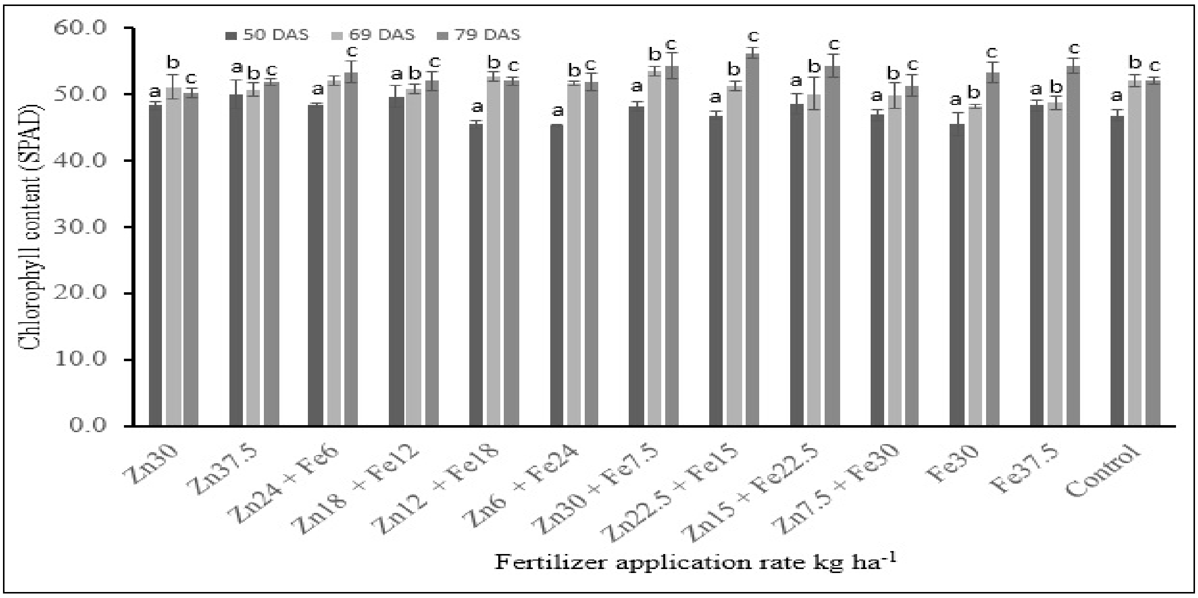

Fig.2. Effect of Zn and Fe application rate on chlorophyll content at different growing stages of summer maize. Each value is the mean three replicates (±SE). Different lower case letters in the same row indicate significant difference among treatments (P≤ 0.05).

DAS: Days after sowing, 50: jointing stage, 69: silking stage, 79: grain filling.

Net photosynthetic rate and chlorophyll content

Iron and zinc play an important role on photosynthesis process of higher plants based on the fact that they are involved in metabolism reactions and catalyzing reaction. Leaves are known to be the central organs of photosynthesis and convert its products to biomass (Horst Marschner, 2011).

In the present study the effect of Zn, Fe and their combination significantly increased net photosynthetic rate (Pn) in the first 50 days after sowing. Later on fertilizers didn't manifest significant difference across the treatments.

This is in consistence with other studies that suggest the decrease of in electron transport which lowers the carbon fixation rate as function of photosynthetic efficiency of photosystem II (Sharma, 2007).

It was suggested also, that after the onset of grain filling, the decrease of Pn was fast.

The decline of net photosynthetic rate is, sometimes, attributed to the decrease of pigments in leaves but it is believed to be other reasons that pigments as plant approaches the maturity period (Li *et al.*, 2012).

Total leaf chlorophyll content is very much influenced by both zinc and iron because these metals are required in biosynthesis of chlorophyll and directly involved in the electron transport reaction (Kobraee *et al.*, 2011).

However in this experiment the application of Zn, Fe and Zn x Fe had no significance influence on SPAD reading throughout the growth stage of maize.

Effect of Zn, Fe and Zn×Fe on Crude protein, ether extract and crude fiber

Provided that zinc plays a crucial role in protein synthesis, enrichment in protein biosynthesis is a result of increasing nitrogen application which may increase the sink strength of Zn (ISMAIL CAKMAK *et al*., 1989; H Marschner, 1995).

A close relationship of protein, Zn and Fe was also found in research conducted in the region of Konya central Anatolia Turkey which is consistent with the results demonstrated by staining Zn, Fe and protein in durum wheat cultivar(Ismail Cakmak *et al*., 2010). Those findings again relates to the biological systems whereby proteins are very depend on zinc ions to maintain their activities which makes zinc useful for lots of proteins by fulfilling both catalytic and structural role (Anzellotti & Farrell, 2008).

Our study did not significantly increase crude protein or ether extract but crude fiber was raised up to 47%. The effect of soil Zn-Fe application on nutrient relate also to DTPA extractable Zn. Enhancing nutrients concentration by soil application was not successful in most cases because of hindered absorption and accumulation due high pH, $CaCO_3$ concentration and insufficient water resources, rather researchers have recommended to use foliar application in such soils (Gonzalez *et al*., 2008; Yue-Qiang Zhang *et al*., 2012).

However, maize seems to be less responsive to the enhancement of grain nutrients through foliar application compared to wheat which is a promising crop in Zn and Fe biofortification(Ismail Cakmak, 2012). Given that maize plants are relatively large and tall, it is difficult for farmers to adopt foliar application at the late growth stages of maize which are effective for grain nutrients enhancement.

Therefore, in case of maize, soil application of Zn and Fe in more doable and effective for enhancing both grain yield and grain nutrient concentration by building up soil available Zn and Fe to requirement of high yield and grain nutrient concentration (Liu *et al*., 2017).

Conclusion

The application of Zn and Fe as soil spray fertilizer was not efficient in increasing maize biomass and chlorophyll content in calcareous soil which is severely deficient of zinc and iron. However, Zinc combined with Iron significantly increased maize grain yield, net photosynthetic rate at jointing stage as well as grain crude fiber content.

It is of great importance to carry out multiple studies on combination of Zn and Fe to ascertain its advantage on achieving high yield and quality of maize through soil application of Zn and Fe given its practicability and effectiveness.

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