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Effect of Combined Zinc and Iron Foliar Fertilizer on Physiological and Agronomic Characteristics of Winter Wheat Nongda399

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Original Research Article

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Abstract: Wheat is one of most important crop in the world contributing up to 28% of the world edible dry matter and up to 60% of the daily calorie intake in several developing country. Nutritional deficiencies are serious problems of public health in developing countries. To increase the productivity and quality of wheat micronutrient plays an important role. This study was conducted to study the effect of combined Zn and Fe fertilizers as foliar fertilizers on wheat agronomic performance and grain yield quantity. A split-plots in a randomized complete block design with 3 replications was used and PASW Statistics 18 software was used for statistical analysis. Results showed that compared to control (0%Zn+0%Fe), net photosynthetic rate was increased with $100\%Zn_{13}$, $100\%Zn_{9.5}$ and $100\%Zn_{5.5}$ at 45.28, 73.07 and 39.54% respectively while $20\%Zn+60\%Fe_{13}$ increased up to 37.75% at the early filling stage and $100\%Zn_{9.5}$ by the increase of 29.99% otherwise Zn an Fe had no effect. Flag leaf area increase was observed with $20\%Zn+80\%Fe_{13}$, $20\%Zn+80\%Fe_{9.5}$, $20\%Zn+80\%Fe_{5.5}$ and $100\%Fe_{9.5}$ at booting stage with 15.84, 15.32, 14.67 and 15.40% increase respectively, at early filling stage with $20\%Zn+60\%Fe_{9.5}$ by 36.25% increase and at late filling stage with $20\%Zn+60\%Fe_{13}$ increased by 25.73%. The stalks dry mass show a more significant effect at booting stage and little effect at early filling stage and no effect at late filling stage. The yield was only affected with $20\%Zn+80\%Fe_{13}$.The inference can be drawn that foliar application of combined Zn and Fe fertilizer can enhance the net photosynthetic rate, SPAD and flag leaf area of Nongda399 winter wheat.
Keywords: Zinc, Iron, Wheat, Agronomic biofortification, Nongda399.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world and is the third major cereal produced in the world, following maize and rice and it is the major staple food crop in many parts of the world in terms of cultivated area and food source, contributing 28% of the world edible dry matter and up to 60% of the daily calorie intake in several developing countries [1, 2]. Nutritional deficiencies are serious problems of public health, which affect more than half of the world's population, particularly in developing countries. Particularly deficiency in Zn and Fe afflict more than two billion individuals, or one in three people, globally [2, 3].

China is one of the primary wheat producing countries, with wheat grown in 30 provinces with a total cultivated area 24.3 million ha [4]. Ma [5] indicated that iron and zinc deficiencies affect over 300 million people in China, most of them being women and children living in rural areas. It is estimated that the loss in economic productivity due to iron deficiency in China is more than 3.6% of the gross national product [6]. Therefore, the yield quantity and quality of the wheat grain has a significant impact on human health and well-being, especially in the developing world.

To increase the productivity of wheat micronutrient plays an important role [7]. Flag leaf and grain yield can be significantly affected by uptake of Zn, Cu, Fe and Mn [8]. Micronutrients helps in different reactions involved in chlorophyll formation, protein synthesis, enzymes activities, photosynthesis and respiration [9, 10]. Ayaz K. [11] reported that the rice yield can be limited by improper use of micronutrient.

Zn and Fe are among important micronutrients to enhance crop performance and improve yield quality of wheat [12]. Chaudry [13] reported that Zn, Fe and B fertilization improved better crop productivity in a study on wheat response to micronutrients in rain-fed areas of Punjab. Moreover reports says that in some circumstances foliar application of fertilizers is the most effective methods to correct nutritional disorder [14]. Many studied about micronutrients foliar application to plants have been carried out but the combination of only Zinc and Iron has not been reported. Considering the above mentioned facts, this research was conducted to study the effect of combined Zn and Fe fertilizers as foliar fertilizers on wheat crop performance and grain yield quantity.

MATERIALS AND METHODS

Field location

The field experiment was conducted at Doukou wheat and maize demonstration research station (108°52′E 34°36′N) of Northwest A&F University, Shaanxi province, P.R China, during 2016-2017 wheat cropping season. The site is semi-humid with an average annual temperature of $13^{O}C$ and the average annual rainfall is 595 mm. The soil used is classified as Earth-Cumuli Orthic anthrosol [15] with Total N of 151.00 mg kg^{-1} , available K of 26.97 mg kg^{-1}, available P of 198.54 mg kg^{-1} , pH of 8.04, extractable Zn of 1.35 mg kg^{-1} and extractable Fe of 7.87 mg kg^{-1}.

Experimental design and treatments

The experimental design consisted of split-plots in a randomized complete block design with 3 replications of 2.3× 5m plot size with 21 cm row spacing of Nongda 399 cultivar sowed using a manual seed drill machine at a seeding rate of 225 kg ha^{-1}. Before sowing all plots received 850 kg ha^{-1} of N-P_2O_5-K_2O 24-15-5.

Combined and non-combined Zn and Fe fertilizers were used as foliar application fertilizers sprayed thrice at different growth stages; booting stage (Fekees 10), anthesis (Fekees 10.51) and early filling stage (Feekes 10.54) using a hand pressure sprayer. Sprays were done evening after sunset under windless conditions. The main plots received different fertilizer ratio treatments of combined or non-combined of Zn used as $ZnSO_4.7H_2O$ and Fe used as $FeSO_4.7H_2O$ as follow: (1) 100%Zn, (2) 80% Zn + 20% Fe, (3) 60%Zn +40%Fe, (4) 40%Zn +60%Fe, (5) 20%Zn + 80% Fe, (6) 100%Fe. Every main plot had sub-plot treatments that received fertilizer quantity of 0.5% (w/v) as follow: 13, 9.5, 5.5 and 0(control) kg ha^{-1}. The control plots (no foliar Zn or Fe application) were treated with a corresponding amount of water

Sample preparation and nutrient analysis

Every plot, at the booting stage, early filling stage and late filling stage, 20 plant vegetative parts were harvested for dry mass and flag leaf area analysis. Spikes were separated from vegetative organs, both oven dried at 105℃ for 30 minutes then heated at 80℃ for 24-48 hours until constant weight was reached. Dry samples of stalks were weighted with an electronic scale and the dry mass recorded. At physiological maturity stage, the wheat plants were harvested in a $2m^2$ area (2×1 m) twice in each plot. After threshing and harvesting cleaned grains were weighed with an electronic scale and converted into grain yield kg ha^{-1}at 13% moisture content. Net photosynthetic rate was measured using a portable open flow gas exchange system LI-6400 (LI-COR. USA) on the flag leaf at early filling stage and late filling stage taken on 6 plants in each plot. The SPAD was measured using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, inc) taken on flag leaf on 6 plants in each plot at booting stage, early filling stage, mid filling stage and late filling stage.

STATISTICAL ANALYSIS

The ANOVA of the effect of Zn and Fe fertilizer ratio and fertilizer quantity and their interactions was performed while the means comparison were done by a two sided Dunnett test that compared all means against the control using PASW Statistics 18 software.

RESULTS AND DISCUSSION

To increase the amount of nutrients in the wheat grain and vegetables, agronomic biofortification has been identified as a good and promising short time solution. Besides increasing the nutrition value of edible part, biofortification also can play a big role in influencing plant growth to improve the crop yield and productivity [16]. It has been proved that adding micronutrient to plant either by soil or foliar fertilizer can improve yield [17]. Micronutrients stimulate growth by helping in chlorophyll formation, protein synthesis, nucleic acid formation, and play also a big role in enzymes activity of photosynthesis and respiration [9] .

Table-1: Analysis of Variance of the effect of Zn and Fe fertilizer ratio and fertilizer quantity and their interactions on SPAD, Net photosynthesis rate and flag leaf area

Source of variation	SPAD				Net photosynthesis rate		Flag leaf area		
	Booting stage	Early Filling Stage	Mid Filling Stage	Late Filling Stage	Early Filling Stage	Late Filling Stage	Booting Stage	Early Filling stage	Mid filling stage
Fertilizer Ratio (R)	ns	ns	ns	**	ns	*	*	***	***
Fertilizer Quantity (Q)	ns	*	*	**	ns	ns	ns	*	*
R×Q	ns	ns	ns	ns	ns	ns	***	***	*

*, **, ***, ns indicate significance at p<0.05, 0.01, 0.001 or not significant effect, respectively

SPAD

SPAD was taken at different growth stages i.e.: booting stage, early filling stage, mid filling stage and late filling stage, Zn and Fe fertilizer quantity had a significant effect at early filling stage and mid filling at $p<0.05$ and at late filling stage at $p<0.01$ while Zn and Fe fertilizer ratio was only significant at late filling stage at $p<0.01$. Their interaction had no statistically significant difference at any of the growth stages (Table 1). Compared against the control (Table 2), for all treatments foliar application of Zn and Fe fertilizer did not affect significantly the SPAD at botting stage, early filling stage, mid-filling stage. A significance difference was observed at the late filling stage with 100%$Zn_{5.5}$, 80%Zn+20%$Fe_{5.5}$, 20%Zn+60%$Fe_{5.5}$ and 100%$Fe_{9.5}$ with an increase of 9.40, 10.06, 9.77 and 9.97%. Other treatments had no significant effect on SPAD at the late filling stage. SPAD was not significant at booting, early filling and mid-filling stage this might be attributed to the fact that application of Fe was done at a late stage to affect chlorophyll. The highest value was observed with 80%Zn+20%$Fe_{9.5}$ of 53.96 at the late filling stage but mostly the Zn and Fe foliar application did not increase SPAD value thus chlorophyll. These findings reflect those of Rosen [22] that mentioned the negative interaction of Zn and Fe in corn, it was found that Zn inhibited chlorophyll production by interfering with Fe metabolism, but not by lowering the Fe content of the leaves. These findings are consisted also with those of Brown [23] and Giordano [24].

Table-2: Means comparison of SPAD against the Control at different growth stages of Winter wheat affected by different treatments of Zn and Fe foliar fertilizers

Foliar applications[a]	Booting stage	Early Filling Stage	Mid Filling Stage	Late Filling Stage
100%Zn_{13}	50.68	54.07	55.54	50.68
100%$Zn_{9.5}$	52.26	55.08	55.17	52.92
100%$Zn_{5.5}$	55.23	54.48	55.28	53.64*
80%Zn+20%Fe_{13}	51.58	57.11	54.58	50.54
80%Zn+20%$Fe_{9.5}$	51.79	55.87	55.33	53.96*
80%Zn+20%$Fe_{5.5}$	53.01	56.77	56.82	51.68
60%Zn+40%Fe_{13}	52.46	55.18	54.48	50.21
60%Zn+40%$Fe_{9.5}$	51.67	55.43	55.86	51.76
60%Zn+40%$Fe_{5.5}$	52.51	55.60	56.07	53.01
40%Zn+60%Fe_{13}	49.82	55.51	55.42	49.68
40%Zn+60%$Fe_{9.5}$	50.70	54.11	54.93	48.00
40%Zn+60%$Fe_{5.5}$	52.50	55.97	57.02	52.88
20%Zn+80%Fe_{13}	51.99	54.06	54.72	51.37
20%Zn+80%$Fe_{9.5}$	52.00	54.72	55.81	52.04
20%Zn+80%$Fe_{5.5}$	52.85	57.42	56.65	53.82*
100%Fe_{13}	51.53	55.86	55.73	50.98
100%$Fe_{9.5}$	52.78	56.27	57.02	53.92*
100%$Fe_{5.5}$	49.12	54.40	56.03	52.93
Control	53.06	57.53	56.01	49.03

[*] Significant at $P<0.05$, [a] : $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ fertilizers were used as source of Zn and Fe micronutrients

The net photosynthetic rate

The net photosynthetic rate was measured at early filling stage and late filling stage. Zn and Fe fertilizer ratio was only significant a late filling stage at $p<0.05$, however Zn and Fe fertilizer quantity and their interaction had no significance difference at any of the two stages (Table 1). A 2 sided comparison all means against the control, shows that the net photosynthetic rate at early filling stage was increased from 12.88 CO2μmol s^{-1} in control (0%Zn+0%Fe) to 18.72-22.30 CO2μmol s^{-1} in 100%Zn_{13} and 100%$Zn_{9.5}$ which translate into 45.28-73.07% increase respectively. 100%$Zn_{5.5}$ increased up to 39.54% while 20%Zn+60%Fe_{13} increased up to 37.75% otherwise Zn and Fe foliar fertilizer had no significant effect to the net photosynthetic rate at early filling stage. At the late filling stage the foliar application of Zn and Fe fertilizer only showed a significant effect at 100%$Zn_{9.5}$ by the increase of 29.99%, otherwise means were not significantly different from the control (Table 3). Net photosynthetic rate showed a significant increase at early filling stage with 100%$ZnSO_4.7H_2O$ levels (100%Zn_{13}, 100%$Zn_{9.5}$, 100%$Zn_{5.5}$). At this stage another significant effect was found with 20%Zn+60%Fe_{13}. At the late filling stage, the effect on net photosynthetic rate was not much noticeable; the effect observed was much controlled by the ratio of Zn and Fe in the sprayed fertilizer as in the early filling stage where the high amount of Zn in Zn and Fe ratio corresponded to the high photosynthetic rate. These results are consistent with those reported by Ohki [18] in a study on Zn nutrition on photosynthesis and carbonic anhydrase activity in cotton where net photosynthesis rate increased with an increase in Zn concentration. Fe also was significant for net photosynthetic rate due to the main reason that in the photosynthetic apparatus, molecules reacted directly to photosystem II hosts two or three iron atoms, 12 atoms in photosystem I, five in the cytochrome complex, and two in the ferredoxin molecule [19]. These distributions illustrate that iron straightly contribute in the photosynthetic activity of plants, thus, their productivity [20]. The deficiency of one of these nutrients can inhibit net photosynthetic rate of the crop [21].

Flag Leaf area

Flag leaf area was much influenced where the Zn and Fe fertilizer showed a significant effect at booting stage at $p<0.05$ and at early filling stage and late filing stage at $p<0.001$. The fertilizer quantity effect was seen at all stages except booting stage while their interaction was significant at booting stage and early filling at $p<0.001$ while at mid filling stage it was significant at $p<0.05$(Table 1). Considering treatment means against the control, at booting stage, 20%Zn+80%Fe_{13} was highly significant at $p<0.001$ with an increase of 15.84%, while 20%Zn+80%$Fe_{9.5}$ and 100%$Fe_{9.5}$ with an increase of 15.32 and 15.40% respectively, significant at $p<0.01$ and 20%Zn+60%$Fe_{5.5}$ with an increase of 14.67% was significant at $p<0.05$. Other treatments had no statistically significant difference with the control. At early filling stage, only 20%Zn+60%$Fe_{9.5}$ had a significant increase of 36.25% while other treatments were not statistically different from the control. Foliar application of Zn and Fe did not influence the flag leaf area at the late filling stage except in 20%Zn+60%Fe_{13} where it was increased by 25.73% (Table 3). Surveyed at different growth stages, 20%Zn+60%$Fe_{9.5}$ and 20%Zn+60%Fe_{13} showed a significant increase of the flag leaf area. As it can be observed, Fe is the most important portion of this treatment but also the small amount of Zn might have played a role in helping Fe reacting positively to the leaf area. Fe is very important for crops and play a great role in enzymes, as well as cytochrome that is involved in electron transport chain, chlorophyll synthesis and keeping the structure of chloroplasts [25]. Similar results was reported by Rawashdeh [26] and Nadim [27] in the studies on effect of some micronutrients and their application methods on growth and yield of wheat and its leaves.

Table-3: Photosynthesis and flag leaf Area means comparison against the Control at different growth stages of Winter wheat affected by different treatments of Zn and Fe fertilizers

Foliar applications [a]	Net Photosynthetic rate (CO2µmol s^{-1})		Flag Leaf area(cm^2 $leaf^{-1}$)		
	Early filling	Late filling	Booting	Early Filling	Late filling
100%Zn_{13}	18.72**	12.11	12.05	13.29	13.66
100%$Zn_{9.5}$	22.30***	17.26*	12.30	12.45	13.67
100%Zn5.5	17.98*	15.60	13.63	14.63	12.66
80%Zn+20%Fe_{13}	12.12	15.04	12.98	12.45	13.58
80%Zn+20%$Fe_{9.5}$	12.13	13.20	12.59	13.50	12.90
80%Zn+20%$Fe_{5.5}$	12.41	16.03	13.13	12.64	12.26
60%Zn+40%Fe_{13}	12.24	15.71	11.58	10.91	13.39
60%Zn+40%$Fe_{9.5}$	13.35	14.66	13.64	11.92	12.89
60%Zn+40%$Fe_{5.5}$	11.77	15.25	13.19	12.39	13.63
40%Zn+60%Fe_{13}	13.90	16.53	11.84	12.66	15.43
40%Zn+60%$Fe_{9.5}$	15.29	16.53	11.43	11.08	11.39
40%Zn+60%$Fe_{5.5}$	15.30	15.27	13.81	12.72	14.65
20%Zn+80%Fe_{13}	17.75*	10.95	15.84***	14.01	16.73**
20%Zn+80%$Fe_{9.5}$	12.86	12.22	15.32**	17.66***	15.07
20%Zn+80%$Fe_{5.5}$	12.64	15.15	14.67*	12.98	14.37
100%Fe_{13}	13.06	11.75	13.82	15.15	13.77
100%$Fe_{9.5}$	14.80	11.89	15.40**	15.01	14.51
100%$Fe_{5.5}$	12.44	15.65	13.53	13.73	14.08
Control	12.88	13.28	11.96	12.96	13.31

*, **, *** indicate significance at p<0.05, 0.01, 0.001 significant effect, respectively, [a]: $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ fertilizers were used as source of Zn and Fe micronutrients

Stalks dry mass

Stalks dry mass was analyzed at booting stage , early filling stage and mid-filling stage, fertilizer ratio of Zn and Fe foliar fertilizer show the influence only at booting stage where fertilizer ratio significantly influenced the dry mass at $p<0.01$ whereas fertilizer quantity effect was pronounced at $p<0.05$, their interaction had no effect at this stage. For other growth stages, fertilizer ratio and quantity and their interaction showed no significant influence on stalks dry mass (Table 4). Averaged across replicates then comparing treatment means, the foliar application of Zn and Fe fertilizer at booting stage affected negatively the stalks dry mass to a significant decrease in the following treatments 100%$Zn_{9.5}$, 80%Zn+20%$Fe_{9.5,}$ 60%Zn+40%$Fe_{13,}$ 60%Zn+40%$Fe_{9.5}$ and 40%Zn+60%$Fe_{5.5}$ of 25.83, 28.87, 29.94, 21.61 and 26.83% respectively. For other treatments the effect had no significant difference from the control. At the early filling stage treatment means did not differ significantly from the control except 40%Zn+60%$Fe_{5.5}$ that significantly decreased the stalks dry mass by 20.59%. At the late filling stage, all treatments did not influence significantly the stalk dry mass. According to the results, as wheat grows the effect of Zn and Fe fertilizers seem to be decreasing (Table 5). Foliar fertilization of Zn and Fe had more effect on dry mass at the booting stage and not in early and late filling stage. This might be explained by the fact that at late stage of vegetative growth the stalks do not increase in mass instead assimilates are remobilized to spikes [28, 29].

Table-4: Analysis of Variance of the effect of Zn and Fe fertilizer ratio and fertilizer quantity and their interactions on Stalks dry mass and grain yield

	Stalks Dry Mass			Gain yield
	Booting stage	Early filling stage	Late filling stage	
Fertilizer Ratio (R)	**	ns	ns	**
Fertilizer Quantity (Q)	*	ns	ns	ns
R×Q	ns	ns	ns	ns

*, **, ns indicate significance at $p<0.05$, 0.01 or not significant effect, respectively

Table-5: Means comparison against the control of stalks dry mass and grain yield of winter wheat affected by foliar application of Zn and Fe Fertilizers

Foliar Applications[a]	Stalks dry mass (g 20plants^{-1})			Grain yield (Kg ha^{-1})
	Booting stage	Early filling stage	Late filling stage	
100%Zn_{13}	24.4967	37.2833	47.9600	4228.047
100%$Zn_{9.5}$	20.73**	34.1767	54.0033	3828.907
100%$Zn_{5.5}$	24.7867	35.5167	50.4567	3711.493
80%Zn+20%Fe_{13}	23.8167	33.6567	51.1133	3395.187
80%Zn+20%$Fe_{9.5}$	19.88***	33.5867	50.2867	2785.113
80%Zn+20%$Fe_{5.5}$	26.0367	42.2033	49.9167	3331.767
60%Zn+40%Fe_{13}	19.58***	33.0800	53.8967	2919.28
60%Zn+40%$Fe_{9.5}$	21.91*	34.1300	51.0033	2904.533
60%Zn+40%$Fe_{5.5}$	24.8233	33.7200	54.9467	3157.42
40%Zn+60%Fe_{13}	26.9033	40.9367	49.9400	4051.32
40%Zn+60%$Fe_{9.5}$	25.6233	33.2267	50.2867	3603.693
40%Zn+60%$Fe_{5.5}$	20.45**	31.30*	50.6533	4161.453
20%Zn+80%Fe_{13}	26.2133	36.4733	56.6233	5424.67*
20%Zn+80%$Fe_{9.5}$	25.8367	33.8300	56.3333	3685.033
20%Zn+80%$Fe_{5.5}$	23.3967	36.6967	57.2267	4251.473
100%Fe_{13}	25.3900	37.0933	47.6533	4935.14
100%$Fe_{9.5}$	26.2000	35.9400	48.9933	4228.187
100%$Fe_{5.5}$	24.5133	34.4533	56.6733	3941.187
Control	27.9500	39.4167	49.2267	3053.89

*, **, *** indicate significance at $p<0.05$, 0.01, 0.001 significant effect, respectively, [a]: $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ fertilizers were used as source of Zn and Fe micronutrients

Grain Yield

While fertilizer ratio affected the yield at $p<0.01$, fertilizer quantity and its interaction with fertilizer ratio has no significant effect on grain yield (Table 4). Foliar application of Zn and Fe fertilizer increased slightly the grain yield, with significant difference from the control only observed in 20%Zn+60%Fe_{13} with an increase of 77.63%. Other treatments did not influence significantly the grain yield (Table 5). In this study, foliar application of combined Zn and Fe in all treatments did not significantly influence the grain yield of Nongda 399 except in 20%Zn+60%Fe_{13} with an increase of 77.63%, same results were reported by Zhang and Gupta [30, 31] but differ from those reported by Maralian, Peck and Yilmaz [32-34]. The soil used in this study was not deficient in Zn and Fe as per critical levels in China (5 mg kg^{-1} for Fe and 0.5mg kg^{-1} for Zn) [30].

CONCLUSION

According to the results obtained from this study, Zn and Fe Fertilizer ratio have an effect on most of studied variables i.e. SPAD, net photosynthetic rate, flag leaf area and grain yield. The effect of fertilizer quantity was much observed in SPAD where it had a significant effect in 3 of 4 growth stages studied and more effective at late filling stage. Among treatments $100\%Zn_{9.5}$ was the best in improving the net photosynthetic rate of wheat even at late filling stage when all photosynthetic ability was decreasing. The flag leaf area was much enhanced by $20\%Zn+60\%Fe_{13}$ and $20\%Zn+60\%Fe_{9.5}$ in all studied growth stages while stalks dry mass at late filling stage was not affected by foliar application of Zn and Fe fertilizer. Grain yield was only enhanced by $20\%Zn+80\%Fe_{13}$. The inference can be drawn that foliar application of combined Zn and Fe fertilizer can enhance the net photosynthetic rate, SPAD and flag leaf area of Nongda 399 winter wheat.

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