Research Article

Agronomic Biofortification with Zinc and Iron for the Improvement of Wheat Phenology and Yield

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Abstract | Both zinc (Zn) and iron (Fe) are involved in the biochemical and physiological processes of the wheat plant. Low Zn and Fe contents in soil and/or their low availabilities for plant uptake not only affect the final yield but also decrease their harvestable levels in the wheat grains. The research trials were conducted for two consecutive years (2016-17 and 2017-18) on the foliar supply of Zn and Fe to wheat at different growth stages in the Agronomy Research Farm, the University of Agriculture, Peshawar - Pakistan. Four Zn levels (0, 1, 2, and 3 kg ha^{-1}) and four Fe levels (0, 2, 4, and 6 kg ha^{-1}) at three growth stages (i.e. foliar application of full dosage at the tillering stage, full dosage at the booting stage and their respective half dosages at the tillering stage + half at the booting stage) along with one unsprayed check were used in the experiment. All the treatments' combinations and a control {(4 x 4 x 3) +1} were laid out in a randomized complete block design having three replications. The plot size was 12 m^2 accommodating ten rows. The recommended wheat cultivar 'Pirsabaq 2013' was sown by using 120 kg ha^{-1} seed rate. Nitrogen at the rate of 120 kg ha^{-1} was applied in two splits from urea. Similarly, 90 kg ha^{-1} phosphorus was applied from DAP at the time of sowing. Analysis of variance showed that sole foliar Zn application at 3 kg ha^{-1} significantly improved the flag leaf area (36.9 cm^2) and leaf area $tiller^{-1}$ (97.2 cm^2). While more spike m^{-2} (248), maximum grains $spike^{-1}$ (47.2), spike length (13.1 cm), and thousand grains weight (42.9 g) were observed with foliar application of 2 kg ha^{-1} Zn. Significantly maximum leaf area $tiller^{-1}$ (96.4 m^2), flag leaf area (36.2 cm^2), spike m^{-2} (253), grains $spike^{-1}$ (46.9), spike length (12.9 cm) and thousand grains weight (43 g) were found best with the application of 4 kg ha^{-1} Fe. In case of application stages, more leaf area per tiller (95.9 cm^2) and flag leaf area (36 cm^2) were observed with both the application of Zn and Fe when applied 100 % at tillering stage. Similarly, more $spike^{-2}$ (250.9), maximum grain $spike^{-1}$ (46.3), spike length (12.7 cm), and thousand grains weight (42.6 g) were noted with the split foliar application of both Zn and Fe. It can be concluded from the experiment that application of both foliar Zn and Fe enhanced the phenological development and yield component of wheat crop. Similarly, foliar application of full dosage at tillering stage also hastened the phenological parameters of wheat crop, therefore recommended for the wheat crop cultivation in the locality of Peshawar.

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Introduction

Micronutrients deficiencies are a key challenge for the scientists of agriculture. Deficiencies of micronutrients in human are due to the lack of zinc, iron, iodine and folate (Jawaldeh *et al.*, 2019). Deficiencies of these nutrients, especially of zinc (Zn) and iron (Fe) are strongly suffering more than half people of the world because human being basically depend on the use of cereal crops specially on wheat crop (Kenzhebayeva *et al*., 2019). Micronutrients are as important as macronutrients; however, these are required by crops in very minute quantities. In most cases, both the plants and soils have very small amounts of micronutrients. These micronutrients are important as these play key roles in the plants growth, development and other various physiological processes and functions that occur in their bodies, which improve the crops quality and yield (Tahir *et al*., 2013).

Among these micronutrients, particular attention has been given to the improvement of Zn and Fe concentrations in staple food cereals due to their wide spread deficiency in human beings (Bouis and Welch, 2010). In case of drought stress condition, Zn enhances the content of melatonin and it occurs in different part of plant like root, stem and leave. As a result, the contents of upstream substrate increase, and the gene expression of enzymes involved in melatonin biosynthesis, which in turn enhances the drought tolerance in plants (Sun *et al*., 2020). Zn plays an important role in the activation of enzymes and also used in auxin metabolism (Rehman *et al*., 2018), which are involved in different process including growth and development (Baligar and Fageria, 2005). Zn plays a key role in the formation of protein and amino acids (Cabot *et al*., 2019). It maintains the cellular and physiological function of plants (Hafeez *et al*., 2013). Zn improves grains as it is involved in pollen synthesis (Ziaeyan and Rajeaia, 2009), their fertility and seed set processes (Hafeez *et al*., 2013). It increases the albumin, gliadins, glutenin and globulin content (Liu *et al*., 2015). It also enhances the activity of SOD when plant is under abiotic stress (Noman *et al*., 2018). Zn application increases plant resistance as Zn deficient plants are susceptible to diseases (Helfenstein *et al*., 2015). Zn fertilization significantly enhances yield, yield components and quality related parameters of crop (Chattha *et al*., 2017; Hassan *et al*., 2019). Therefore, Zn supply to crops not only improves the grain protein content but also the overall quality of the crop (Wang *et al*., 2020). As the critical levels of Fe and Zn are 4.8 and 1.1 mg kg^{-1} soil, respectively (Rezaei and Malakoutai, 2001). Unavailability of Zn adversely affects the development of root system, nutrients and water absorption, which results in decreased growth and yield with low grain Zn content (Fageria, 2004). When Zn is severely deficient in plants, it significantly reduces the flowering and fruiting in plants (Epstein and Bloom, 2005).

Similarly, Fe in plants has an essential role in the development and formation of chlorophyll, maintains chloroplast structure and also improves the photosystems (Zayed *et al*., 2011). Fe also helps in the formation and activation of enzymes (Weisany *et al*., 2013). Fe application plays an important role in growth of plant, different cellular functions and in the process of photosynthesis (Kasote *et al*., 2019). Fe supports the process of oxidation. The process of oxidation releases energy from sugars and starches, as a result nitrate convert to ammonium in plants. It is also involved in the metabolism of nucleic acid (Havlin *et al*., 2014). Plant growth positively affects with the application of Fe, if applied at suitable level and time (Hussain *et al*., 2019).

Fe is essential for the completion of crop's life cycle (Cakmak *et al*., 2010b). It exhibits a great reproductive mobility in wheat. Application of Fe significantly improves the content of Fe in wheat (Shahbazi *et al*., 2015). Deficiency symptoms of Fe in plants occur due to low solubility of Fe in soils, as a result its deficiency occurs in human. Deficiency of Fe in calcareous soils reduces growth of plant and yield (Rui *et al*., 2016). Fe deficiency greatly reduces the nutritional quality and yield of crops (Aciksoz *et al*., 2014). The deficiencies of Zn and Fe are the main reasons for low yield in different crops. Their deficiency symptoms generally appear in human beings in a region of Zn and Fe deficient soils, especially in people with more cereal consumptions. Therefore, an adequate supply of Zn and Fe is required for the improvement of wheat quality and yield (Bhatt *et al*., 2020; Jalal *et al*., 2020).

Zn and Fe bioavailability and their content improvement in food crops are the main public health issue and now a day a worldwide challenge. Therefore, this study was aimed to quantify the suitable doses of these micronutrients and their time of applications for the direct foliar supply in the form of spray to plants instead of soil application in order to bypass their uptake from the problem soils. This will not only improve the yield and yield attributes of wheat but also would certainly minimize the Zn and Fe deficiency related health problems for over billion of people while using it as a staple food.

Materials and Methods

The research trials were conducted for two consecutive years (2016-17 and 2017-18) on the foliar supply of Zn and Fe to wheat at different growth stages in the Agronomy Research Farm, the University of Agriculture, Peshawar-Pakistan. Four Zn levels (0, 1, 2, and 3 kg ha^{-1}) and four Fe levels (0, 2, 4, and 6 kg ha^{-1}) at three growth stages (i.e. foliar application of full dosage at the tillering stage, full dosage at the booting stage and their respective half dosages at the tillering stage+half at the booting stage) along with one unsprayed check were used in the experiment. For foliar Zn and Fe application, standard solutions (0.5% and 1%) for their highest levels were prepared in water from $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ sources. The standard solutions of both Zn and Fe were further diluted subsequently as per designed respective levels of factor A and B. All the treatments' combinations and a control {(4 x 4 x 3) +1} were laid out in a randomized complete block design having three replications. The plot size was 12 m^2 accommodating ten rows. The recommended wheat cultivar 'Pirsabaq 2013' was sown by using 120 kg ha^{-1} seed rate. Nitrogen at the rate of 120 kg ha^{-1} was applied in two splits from urea. Similarly, 90 kg ha^{-1} phosphorus from DAP was applied at sowing time. Before experiment, the soil characteristics was evaluated in laboratory and classified the soil as texture silty clay loam having 51.3% silt, 40% clay and 8.7% sand, 14.4% $CaCO_3$, 8.08 pH and having low organic matter of 0.58% g kg^{-1}. Similarly, the mineral contents of soil were i.e. 0.21 ppm Zn, 66.38 ppm Fe, 2.86 ppm nickel, 1.07 ppm lead, .085 ppm chromium and 0.937 ppm was copper. The required range for Zn was from 0.5–0.8 ppm while Zn in the experimental field was 0.2 ppm which was deficient in the experimental field. While Fe in the experimental field was 66 ppm and the required range was from 20–30 ppm, which was sufficient in the experimental site. Summer gap was filled with maize. All other agronomic practices were kept uniform.

Statistical analysis

The experimental data of both the two years were statistically analysed as per the statistical design. Means of the recorded data were compared at $p \leq 0.05$ (LSD test) upon significant F-test (Jan *et al*., 2011).

Results and Discussion

Days to anthesis

Anthesis of wheat as affected by Zn, Fe and their application stages are shown in Table 1. The planned mean comparison between unsprayed check and rest of the treatments revealed significant effect of foliar Zn and Fe application on the days to anthesis, while their application at different stages were found non-significant. The year's effect as a source of variation and all the possible interactions were found non-significant. Delayed anthesis (121 and 119 days) was observed in control plots, while earlier anthesis (118 and 119 days) was noticed in the treated plots during the years 2016-17 and 2017-18, respectively. Regarding the averaged data over years, the anthesis in wheat was delayed by about three days in unsprayed check plots (120 days) over the rest treatments (117 days). Zn as a source of variation affected the days to anthesis significantly during the years 2016-2017 and 2017-18. Earlier anthesis (117 and 115 days) were recorded in those treated plots that received foliar Zn at 3 kg ha^{-1}, similarly delayed (119 and 118 days) anthesis were noted in those plots which were sprayed with water only during the years 2016-17 and 2017-18, respectively. Averaged over the two years, earlier anthesis (116) were observed in plots applied with 3 kg Zn ha^{-1} while delayed anthesis (119) were noted in plots received just water spray. Days to anthesis were decreased with the increasing Zn level because the application of Zn regulates the synthesis of auxin that promotes flowering (Keram *et al*., 2014). Early anthesis in wheat crop might be the role of Zn, which helps in the activation of enzymes that are involved in maintenance of cellular membrane integrity, synthesis of auxin and protein (Keram *et al*., 2014). Hafeez *et al*. (2013) also reported early anthesis with Zn application. Vigorous crop growth rate and their effect on the earliness of anthesis might be attributed to physiological role of Zn in pollen formation and carbohydrate metabolism (Reddy, 2004). Similarly, higher doze of foliar Fe spray also delayed the anthesis across the experimental period. Earlier anthesis (117 and 115) were observed in plots with highest dose of Fe (6 kg ha^{-1}), while in water sprayed treated plots, delayed anthesis (119 and 118) was noticed during the experimental period (2016-17 and 2017-18). Averaged data over years showed that earlier anthesis (116 days) was seen in those plots, which received Fe at 6 kg ha^{-1}, while plots treated with water spray only delayed the anthesis (118 days). Increase in foliar Fe application consequently decreased the number of days to anthesis. Balanced fertilization of Fe and Zn promotes early tillering, early booting and early anthesis (Jalilvand *et al*., 2014). Literature explored that micronutrients supplied as sole or in combination act as a chelating agent and improves the yield growth (Mosanna and

Behrozyar, 2015).

Table 1: *Days to anthesis of wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	120	118	119 a
1	119	117	118 b
2	118	116	117 c
3	117	115	116 d
LSD (0.05)	0.3	0.3	0.2
Fe (kg ha^{-1})			
0 (water spray check)	119	118	118 a
2	118	117	117 b
4	118	116	117 c
6	117	115	116 d
LSD (0.05)	0.3	0.3	0.2
Application timing (AT)			
Full dosage at tillering	118	116	117
Full dosage at booting	118	116	117
Half at tillering + half at booting	118	116	117
LSD (0.05)	NS	NS	NS
Unsprayed check vs rest treatments			
Unsprayed check	121	119	120
Rest treatments	118	116	117
Significance	**	**	**
Year Means	119.9	118.1	**

Leaf area tiller^{-1}

Leaf area per tiller of wheat as affected by Zn, Fe and their application stages is shown in Table 2. Unsprayed check vs rest treatments and year's effects were also found significant, while statistically insignificant effect was found for all the possible interactions. Significantly larger leaf area tiller^{-1} (96.3 cm^2 and 94.6 cm^2) was recorded in treated plots than the control plots (92.5 cm^2 and 88.8 cm^2) during growing seasons of 2016-17 and 2017-18, respectively. Based on two years data, leaf area tiller^{-1} in rest plots were 95.4 cm^2, while 90.7 cm^2 of leaf area tiller^{-1} was measured in non-treated plots. Maximum leaf area tiller^{-1} (97.9 cm^2 and 96.4 cm^2) was recorded in plots with highest foliar applied dosage of Zn (3kg ha^{-1}), while minimum (94.7 cm^2 and 93.4 cm^2) was noted in sprayed check plots during the years 2017 and 2018, respectively. Based on two years data, the higher level of Zn produced more leaf area tiller^{-1} (97.2 cm^2) than water sprayed plots, which produced 94 cm^2 of leaf area tiller^{-1}. Higher level of Zn produced more leaf area tiller^{-1} than the water spray treated plots. Leaf area per tiller increased as foliar Zn supply level increased. This could be the result of its involvement in tryptophan synthesis that had a key role in the cell division and cell elongation. Deficiency of Zn causes crops stunting, which can be improved by application of Zn (Alloway, 2003). Foliar application of Fe had also significant effect on the leaf area tiller^{-1}. Smaller leaf area tiller^{-1} (95.5 cm^2 and 93.8 cm^2) was noted in those plots which received Fe at 6 kg ha^{-1} Fe, while the larger leaf area tiller^{-1} (97.2 cm^2 and 95.6 cm^2) was achieved at the foliar application of 4 kg ha^{-1} in 2017 and 2018, respectively. Average data of both the years showed that lesser leaf area tiller^{-1} (94.6 cm^2) was noted at the higher level of Fe, while 96.4 cm^2 of leaf area tiller^{-1} was recorded at 4 kg ha^{-1} foliar Fe treated plots. Application of Fe increased leaf area tiller^{-1} because Fe improves nitrogen fixation, chlorophyll biosynthesis, chloroplasts, and thus had increased leaf area of the plant (Rawashdeh and Florin, 2014). Similarly, application timing as source of variation also significantly affected the leaf area tiller^{-1}. Smaller leaf area tiller^{-1} (95.9 cm^2 and 94.1 cm^2) was recorded when sole dosage of Fe was applied at booting stage only, while 96.7 cm^2 and 95.1 cm^2 leaf area was recorded when both Zn and Fe were applied as a single dosages at tillering stage only during the years 2017 and 2018, respectively. Average data of both the years showed lower leaf area tiller^{-1} (95 cm^2) with foliar dosages of entire Zn and Fe at booting stage only. Similarly, significantly higher leaf area tiller^{-1} (95.9 cm^2) was noted in plots with sole application of Zn and Fe at the stage of tillering. Smaller leaf area per tiller was recorded at 100% of the foliar Zn and Fe doses application at booting stage, while larger leaf area tiller^{-1} was recorded when both foliar Zn and Fe were applied 100% at tillering stage. Foliar application of these nutrients at tillering stage significantly improves the carotene, chlorophyll and other pigments, hence foliar application 100% at tillering stage significantly improved leaf area tiller^{-1} (Nazran *et al*., 2010).

Table 2: *Leaf area tiller^{-1} (cm^2) of wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	94.7	93.4	94.0 d
1	96.0	94.0	95.0 c
2	96.7	94.4	95.6 b
3	97.9	96.4	97.2 a
LSD (0.05)	0.6	0.7	0.5
Fe (kg ha^{-1})			
0 (water spray check)	95.9	94.1	95.0 c
2	96.8	94.7	95.7 b
4	97.2	95.6	96.4 a
6	95.5	93.8	94.6 d
LSD (0.05)	0.7	0.7	0.5
Application timing (AT)			
Full dosage at tillering	96.7	95.1	95.9 a
Full dosage at booting	95.9	94.1	95.0 c
Half at tillering + half at booting	96.3	94.5	95.4 b
LSD (0.05)	0.6	0.6	0.4
Unsprayed check vs rest treatments			
Unsprayed check	92.5	88.8	90.7 b
Rest treatments	96.3	94.6	95.4 a
Significance	**	**	**
Year Means	94.4	91.7	**

Table 3: *Flag leaf area (cm^2) of wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	34.9	33.8	34.4 d
1	35.5	34.9	35.2 c
2	36.4	35.4	35.9 b
3	37.2	36.6	36.9 a
LSD (0.05)	0.4	0.3	0.3
Fe (kg ha^{-1})			
0 (water spray check)	35.7	35.0	35.4 c
2	36.2	35.3	35.8 b
4	36.6	35.8	36.2 a
6	35.4	34.7	35.1 d
LSD (0.05)	0.3	0.3	0.3
Application timing (AT)			
Full dosage at tillering	36.4	35.5	36.0 a
Full dosage at booting	35.6	34.9	35.2 c
Half at tillering + half at booting	36.0	35.2	35.6 b
LSD (0.05)	0.3	0.3	0.2
Unsprayed check vs rest treatments			
Unsprayed check	34.0	33.7	33.8 b
Rest treatments	36.0	35.2	35.6 a
Significance	**	**	**
Year Means	35.0	34.4	**

Flag leaf area

Analysis of the variance revealed significant differences in flag leaf area (FLA) resulted from foliar application of Zn and Fe at different growth stages (Table 3). Unsprayed check vs rest treatments and the year's effect was found significant on FLA. Interactive effect of all the factors was found non-significant. The lowest FLA (34 cm^2 and 33.7 cm^2) was measured in control plots, while the highest FLA (36.4 cm^2 and 35.2 cm^2) was developed in foliar treated micronutrient plots in the growing seasons of 2016-17 and 2017-18, respectively. Data pooled over years represented the maximum FLA (35.6 cm^2) in foliar Zn and Fe treated plots as compared to unsprayed check plots (33.8 cm^2). Flag leaf area was significantly increased by foliar application of Zn at different stages. Lower FLA (34.9 cm^2 and 33.8 cm^2) was noticed in those plots, which were treated in water check plots, while higher FLA (37.2 cm^2 and 36.6 cm^2) was recorded in 3 kg foliar Zn application ha^{-1} during the years 2017 and 2018, respectively. The two year's average data of FLA revealed the lowest (34.4 cm^2) in water check plots, while the highest FLA in 3 kg ha^{-1} Zn foliar treated plots (36.9 cm^2). Stunted growth, small size of leaves and chlorosis occurred due to Zn deficiency. Zn improved leaf area because Zn helps in the expression of gene maintenance that is required for the tolerance of various types of environmental stresses in plants (Cakmak, 2000). Therefore, foliar Zn might improve the leaf area. Significantly lower FLA was achieved at 6 kg foliar Fe application ha^{-1} (35.4 cm^2 and 34.7 cm^2), while the higher FLA (36.6 and 35.8 cm^2) was recorded at 4 kg Fe treated plots during 2017 and 2018, respectively. Year's average showed that 4 kg ha^{-1} foliar Fe treatment developed higher FLA (36.2 cm^2) as compared to 35.1 cm^2 FLA in the 6 kg ha^{-1} foliar Fe treated plots. Flag leaf area improves with the application of Fe because it is important for chlorophyll synthesis, maintain chloroplast structure, nitrogen fixation and electron transport chain that leads to more crop production and hence might improve leaf

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area (Zayed *et al*., 2011). Similar results were reported by Nadim *et al*. (2012) while studying the micronutrients and their application methods on growth and yield of wheat. Similarly, the studied micronutrients applied at different growth stages had also significant effect on the FLA. Foliar application of Zn and Fe entire dosages at tillering stage developed the higher FLA (36.4 cm^2 and 35.9 cm^2) as compared to their application at the booting stage (35.6 and 34.9 cm^2) during the years 2017 and 2018, respectively. Similarly, data averaged over the years revealed higher FLA at foliar application at the tillering stage (36 cm^2) than the booting stage (35.2 cm^2). Higher flag leaf area was recorded when both Zn and Fe were applied full at tillering stage, while lowest flag leaf area was observed with full dose of foliar Zn and Fe application at booting stage. Our results are in line with Rawashdeh and Florine (2014) who also reported improvement in flag leaf area with foliar Fe application at tillering stage.

Days to physiological maturity

Foliar Zn and Fe application had significantly affected the physiological maturity of wheat (Table 4). The planned mean comparison between unsprayed check and the rest treatments were found significant. Stages of application and all the interactions were non-significant. Delayed physiological maturity (149 days) was observed in unsprayed check plot while early maturity (147 days) was noted in treated plots in both the growing seasons. Pooled data over years indicated 1.08% more-time duration to physiological maturity in unsprayed check as compared to treated plots. Maturity became earlier with the increasing levels of foliar Zn application. Among different levels of Zn, the earliest maturity (146 days) was attained with the application of highest level of foliar Zn as compared with water check (148.5 days). Data averaged over years indicated that water check plots had delayed the maturity by 1.43% in comparison with the highest foliar Zn application. Physiological maturity occurred earlier with the increasing of foliar Zn levels. Among different levels of Zn, application of highest level of foliar Zn promoted physiological maturity, while late maturity was observed in water spray plots. Application of Zn enhances expansion of leaf lamina, early maturity and better ear development of wheat. In wheat crop the early maturity might be the result of Zn because it helps in the growth and functioning of floral tissues in many plant species (Hafeez *et al*., 2013). In case of Fe, early maturity was noted with the increasing level of foliar Fe supply. Early maturity (146 days) was noted in plots treated with 6 kg ha^{-1} foliar Fe treated plots, while delayed maturity (148 days) the water check plots. Fe enhances early maturity because it promotes the activities of enzyme that are involved in photosynthesis, help in the formation of chlorophyll and nitrogen fixation (Masoud *et al*., 2012).

Table 4: *Days to physiological maturity wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	148.5	148.4	148.5 a
1	147.8	147.8	147.8 b
2	147.2	147.0	147.1 c
3	146.2	146.5	146.4 d
LSD (0.05)	0.4	0.3	0.2
Fe (kg ha^{-1})			
0 (water spray check)	148.3	148.7	148.5 a
2	147.5	147.7	147.6 b
4	147.2	147.2	147.2 c
6	146.7	146.1	146.4 d
LSD (0.05)	0.3	0.3	0.2
Application timing (AT)			
Full dosage at tillering	147.4	147.4	147.4
Full dosage at booting	147.4	147.4	147.4
Half at tillering + half at booting	147.5	147.5	147.5
LSD (0.05)	NS	NS	NS
Unsprayed check vs rest treatments			
Unsprayed check	148.3	149.7	149.0 a
Rest treatments	147.4	147.4	147.4 b
Significance	*	**	**
Year Means	147.9	148.6	NS

Spike m^{-2}

Data on spike m^{-2} of wheat as affected by Zn, Fe and their time of application is given in Table 5. Treatments as source of variations showed significant response against the unsprayed check. In comparison to 2018, 7.2 % more spike m^2 were observed in 2017. Averaged data over years (2017-18) indicated more spi ke m^{-2} (238) in the Zn and Fe treat plots than the unsprayed check plots (200), which revealed 18.26 %

more spikes m^{-2} in treated plots over the untreated. Spike m^{-2} (257 and 238) was observed significantly higher at 2 kg ha^{-1} foliar applied Zn over the water sprayed check plots (239 and 222) in 2017 and 2018, respectively. Based on years' average data, significantly higher number of spike m^{-2} (248) followed by 238 spikes m^{-2} and 235 spikes m^{-2} with foliar Zn supplementation at 2 kg ha^{-1}, 3 kg ha^{-1} and 1 kg of Zn ha^{-1}, respectively, while the least spike m^{-2} (231) was found in water sprayed check plots. The data revealed 7.45% increase in spikes m^{-2} due the foliar application of 2 kg Zn ha^{-1} treated experimental units over the water check plots. More spike m^{-2} might be resulted due to its role in the improvement of enzyme functioning (Bameri *et al*., 2012), helps in the production of growth hormones (Kholdebari and Islamzadeh, 2012), spikelet formation and translocation of assimilates to grains (Jan *et al*., 2011). Hassan *et al*., (2019) also proved that number of seedlings and yield contributing parameters improved with the application of Zn. Therefore, more spike m^{-2} was produced with the foliar application of Zn. In case of Fe, maximum spike m^{-2} (261) was recorded at 4 kg ha^{-1} Fe over water sprayed check plot (230) in 2017. Similarly, 246 spike m^{-2} was noticed in foliar applied Fe at 4 kg ha^{-1} as compared to water sprayed check (215 spike m^{-2}) in 2018. Year's average data revealed also less number of spike (223 m^{-2}) in water sprayed check plot, while highest number of spikes m^{-2} (253) were taken in 4 kg Fe ha^{-1} experimental units then 6 kg Fe ha^{-1} (242 spikes m^{-2}) and 2 kg ha^{-1} foliar Fe treatment (235 spikes m^{-2}), respectively. The result elaborated 13.58% increase with the 4 kg foliar Fe application blocks as compared to blank water spray treated plots. Spikes m^{-2} increased with the application of Fe, as Fe plays an important role in the cellular functions and homeostasis of mitochondria that might affected the growth and development (Vigani *et al*., 2013). Timing of foliar micronutrients application had also significant effect on spike m^{-2}. More spike m^{-2} (260 and 242) were recorded with the foliar Zn and Fe supplied half at tillering and half at booting stages, while less number of spike m^{-2} (236 and 222) were noted when nutrients applied at booting stage only in 2017 and 2018, respectively. Application timing pooled data over years reveled the development of less number spike m^{-2} (229) with foliar spray of micronutrients entirely at the booting stage only, while foliar nutrient application, half at tillering and half at booting stage resulted mores more spike m^{-2} (251).

Table 5: *Spike m^{-2} of wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	239	222	231 c
1	241	230	235 bc
2	257	238	248 a
3	246	231	238 b
LSD (0.05)	8.9	8.6	6.1
Fe (kg ha^{-1})			
0 (water spray check)	230	215	223 d
2	243	226	235 c
4	261	246	253 a
6	249	234	242 b
LSD (0.05)	8.6	8.6	6.1
Application timing (AT)			
Full dosage at tillering	242	227	235 b
Full dosage at booting	236	222	229 c
Half at tillering + half at booting	260	242	251 a
LSD (0.05)	7.4	7.4	5.3
Unsprayed check vs rest treatments			
Unsprayed check	208	193	201 b
Rest treatments	246	230	238 a
Significance	**	**	**
Year Means	227.2	211.9	**

Grains spike^{-1}

Data on grains spike^{-1} of wheat as affected by Zn, Fe and their time of application is shown in Table 6. Mean data revealed significant effect of years and treatments against unsprayed check. Significantly higher grains spike^{-1} (47) was recorded in 2017 as compared to less number of grains spike^{-1} (44) in 2018. Both years' average indicated development of more grains spike^{-1} (46) in plots treated with both foliar Zn and Fe than the unsprayed plots (44). This showed an overall increase of 5.03 % grains per spike in treated plots over the unsprayed check plots. During the both growing seasons (2016-17 and 2017-18), foliar application of 2 kg ha^{-1} produced significantly more grains spike^{-1} (49 and 46), while in water sprayed check plots the least grains spike^{-1} (47 and 43) were observed, respectively. Based on average data over years, significantly less grain per spike in wheat (45) was found in water check treated plots, while more number of grains spike^{-1} (47) was noted in foliar Zn treated plots with 2 kg ha^{-1} followed by 3 kg ha^{-1} (46.1) and 1 kg ha^{-1} (45.7), respectively. The data revealed 5.82 % more grains in wheat developed when 2 kg ha^{-1} foliar Zn was applied as compared with water check plots. Increase in grains spike^{-1} due to foliar spray of Zn might be due to the involvement of Zn in pollen tube formation and ultimately more seed set (Ziaeyan and Rajaeia, 2009). Similarly, Zn has a key role in increasing the activities of enzyme (Bameri *et al*., 2012), helps in growth hormones production (Kholdebari and Islamzadeh, 2012) formation of spikelet and assimilates translocation to seed (Jan *et al*., 2011). Sultana *et al*., (2016) studied that application of Zn enhances yield of wheat crop. In case of Fe, significantly higher number of grains spike^{-1} (48 and 46) was recorded in plots treated with 4 kg ha^{-1} foliar Fe than the water sprayed check plots (47 and 45) during 2017 and 2018, respectively. Pooled data over years revealed minimum grains spike^{-1} (45) in the water check plots, while significantly maximum grains spike^{-1} (47) was found in foliar Fe treated plots with 4 kg ha^{-1} followed by 6 kg ha^{-1} (46), respectively. Over control, 4 kg ha^{-1} foliar Fe produced 4.22 % more grains spike^{-1}. Foliar application of Fe significantly increases the number of grain of wheat plant (Hussain *et al*., 2012a). Grains spike^{-1} increased because foliar application of Fe might improve the photosynthetic process, biochemical and physiological activities (Habib, 2009; Zaidan *et al*., 2010). Timing of application had also a significant effect on the grains spike^{-1}. Highest level of wheat grains in spike (48 and 45) was found when foliar Zn and Fe were applied half at tillering and half at booting stages, while significantly less grains spike^{-1} (47 and 44) were observed with complete foliar supplement at booting stage only during 2017 and 2018, respectively. Both years' average data showed less grains spike^{-1} (45) with sole foliar supply at booting stage, while significantly more grains spike^{-1} (46) were noticed at split foliar application of nutrients i.e. half at the stage of tillering and half at the stage of booting. Split application of both Zn and Fe improved the grains spike^{-1} by 1.53% over the sole foliar application at booting stage only. Foliar spray of both Zn and Fe half at each stage (i.e. at tillering and booting) improved grains spike^{-1} due to the pollen synthesis, spikelets formation and their fertility (Hafeez *et al*., 2013). Armin *et al*. (2014) also studied that early application of nutrients increases the grains spike^{-1}. Grains spike^{-1} had showed variation as due to the interactive effect of Zn and AT. Higher number of grains spike^{-1} was noticed when foliar Zn was supplied at vegetative stages i.e. tillering, booting or combined i.e. half at tillering + half at booting stages. Nadergoli *et al*. (2011) showed similar investigation of increased grains number due to vital role of Zn in the stamens formation. Interaction of Zn and Fe was also found significant for grains per spike of wheat. Figure 2 elaborated an increase in the grains spike^{-1} with increase in Zn and Fe application up to 2 kg ha^{-1} and 4 kg ha^{-1}, respectively, while subsequent increase in the dosages of both nutrients showed a significant decrease in grains spike^{-1}.

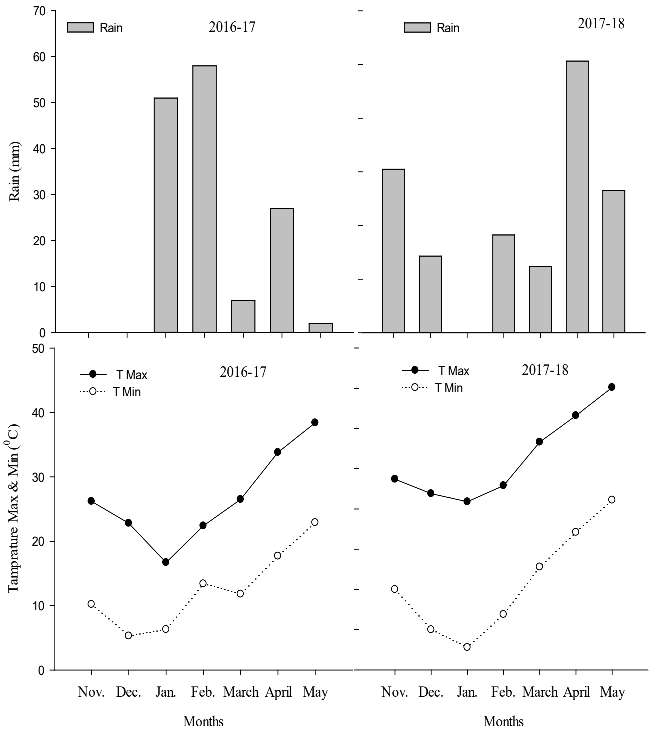

Figure 1: *Mean monthly rainfall (mm), minimum and maximum temperatures (°C) at the experimental site for crop growing seasons (2016-17 and 2017-18).*

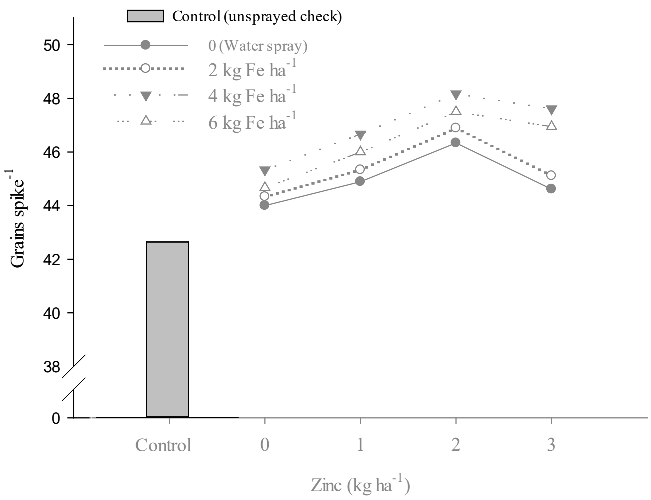

Figure 2: *Interactive effect of foliar zinc (Zn) and iron (Fe) application on the grains spike^{-1} of wheat.*

Table 6: *Grains spike^{-1} of wheat as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	46.6	42.6	44.6 d
1	46.7	44.7	45.7 c
2	48.8	45.7	47.2 a
3	47.6	44.5	46.1 b
LSD (0.05)	0.3	0.3	0.2
Fe (kg ha^{-1})			
0 (water spray check)	46.5	43.4	45.0 d
2	47.0	43.9	45.4 c
4	48.5	45.4	46.9 a
6	47.8	44.8	46.3 b
LSD (0.05)	0.3	0.3	0.2
Application timing (AT)			
Full dosage at tillering	47.4	44.3	45.8 b
Full dosage at booting	47.1	44.1	45.6 c
Half at tillering + half at booting	47.9	44.8	46.3 a
LSD (0.05)	0.3	0.3	0.2
Unsprayed check vs rest treatments			
Unsprayed check	44.7	42.7	44 b
Rest treatments	47.4	44.4	46 a
Significance	**	**	**
Year Means	46.0	43.5	**
Interaction Significance			
Zn x Fe **			

Table 7: *Spike length (cm) of wheat crop as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	12.1	11.8	11.9 d
1	12.6	12.2	12.4 c
2	13.4	12.8	13.1 a
3	13.0	12.6	12.8 b
LSD (0.05)	0.1	0.1	0.1
Fe (kg ha^{-1})			
0 (water spray check)	12.4	12.1	12.3 d
2	12.6	12.2	12.4 c
4	13.1	12.6	12.9 a
6	12.9	12.5	12.7 b
LSD (0.05)	0.1	0.1	0.1
Application timing (AT)			
Full dosage at tillering	12.8	12.4	12.6 b
Full dosage at booting	12.6	12.2	12.4 c
Half at tillering + half at booting	12.9	12.5	12.7 a
LSD (0.05)	0.1	0.1	0.1
Unsprayed check vs rest treatments			
Unsprayed check	11.7	11.6	11.6 b
Rest treatments	12.8	12.3	12.6 a
Significance	**	**	**
Year Means	12.2	12.0	**

Spike length

Data on spike length of wheat as affected by Zn, Fe and their timing of application is given in Table 7. Mean data showed significant effect of years and treatments against the unsprayed check. Significantly lengthy spikes (12.2 cm) were recorded in 2017 than in 2018 (12 cm). In 2017, spike length was 1.66 % more than recorded in 2018. Both the growing seasons' pooled data revealed longer spikes (12.6 cm) with foliar nutrients application than the control (11.6 cm), which represented an overall increase of 8.62 % in treated plots over unsprayed check plot. Longer spikes (13.4 cm and 12.8 cm) were observed in 2 kg ha^{-1} foliar Zn treated plots as compared to water sprayed check plots (12.1 cm and 11.8 cm) in 2016-17 and 2017-18, respectively. Years' based averaged data also revealed significantly smaller spikes (11.9 cm) in water spray treated plots, while lengthy spikes (13.1 cm) were noted in 2 kg Zn ha^{-1} treated plots followed by 3 kg ha^{-1} Zn (12.8 cm) and 1 kg ha^{-1} Zn plots (12.4 cm), respectively. Spike length was observed 10.08 % more in 2 kg ha^{-1} Zn treated plots over control. Spike length of wheat increased with the application of Zn because it might improve the catalytic activity in anther of wheat that increased spike length (Shaheen *et al*., 2007; Abbas *et al*., 2009). Similarly, in case of Fe, 4 kg ha^{-1} foliar application produced longer spikes (13.1 cm and 12.6 cm) as compared to control (12.4 cm and 12.1 cm) in 2017 and 2018, respectively. Data averaged over years, shorter spikes (12.3 cm) were found water sprayed plots, while longer spikes (12.9 cm) were noted in 4 kg Fe treated plots next by 6 kg ha^{-1} (12.7 cm) and 2 kg ha^{-1} (12.4 cm), respectively. The result revealed comparatively 4.84 % lengthy spikes at 4 kg ha^{-1} foliar Fe treatment over control. Maximum spike length might be that Fe enhances the activities of enzyme and photosynthesis. Therefore, foliar application of Fe improved the spike length. Timing of foliar micronutrients application had also significant effect on the spike length. In 2017 and 2018, split dosages of both the micronutrients developed significantly lengthy spikes (12.9 cm and 12.5 cm) as compared to their sole application at booting stage (12.6 cm and 12.2 cm), respectively. Pooled data over years also revealed similar results. Longer spikes were recorded with the application of Zn and Fe when applied 50% at tillering and 50% at booting stages, while smaller spikes were noted in plots with nutrients sprayed at booting. Hemantaranjan and Garg (1988) reported the best spike length with foliar spray at tillering stage.

Table 8: *Thousand grains weight (g) of wheat as affected by foliar application of zinc (Zn), iron (Fe) and their time of application (AT) during 2016-17 and 2017-18.*

Treatment	Year (Y)		Mean
	2016-17	2017-18	
Zn (kg ha^{-1})			
0 (water spray check)	42.6	40.5	41.6 d
1	43.3	41.3	42.3 c
2	43.9	41.9	42.9 a
3	43.3	41.7	42.5 b
LSD (0.05)	0.3	0.3	0.2
Fe (kg ha^{-1})			
0 (water spray check)	42.6	40.8	41.7 d
2	43.0	41.1	42.1 c
4	44.2	41.7	43.0 a
6	43.4	41.8	42.6 b
LSD (0.05)	0.3	0.3	0.2
Application timing (AT)			
Full dosage at tillering	43.3	41.4	42.3 b
Full dosage at booting	43.0	41.1	42.0 c
Half at tillering + half at booting	43.6	41.6	42.6 a
LSD (0.05)	0.3	0.3	0.2
Unsprayed check vs rest treatments			
Unsprayed check	42.0	40.0	41.0 b
Rest treatments	43.3	41.4	42.3 a
Significance	**	**	**
Year Means	42.6	40.7	**
Interaction	Significance		
Zn x Fe	**		

1000 grain weight

Data of 1000 grain weight of wheat as influenced by Zn, Fe and its application timing are reported Table 8. Zn, Fe and their timing of application significantly affected thousand grains' weight. The planned mean comparison of unsprayed check vs rest treatments as well as year's effect was also found significant. Heavier grains (42.6 g) were noted in 2017, while found lighter (40.7 g) in 2018. The unsprayed check plot vs rest comparison revealed significantly heavier grains (43.3 g and 41.4 g) in the treated plots than in the unsprayed plots (42 and 40 g) during 2017 and 2018, respectively. Pooled data over years indicated significantly lower thousand grains' weight (41 g) in unsprayed check plot than the rest plots (42.3 g). Maximum thousand grains' weight was noted in foliar Zn fertilized plots treated at the rate of 2 kg ha^{-1} (43.9 g and 41.9 g) during 2017 and 2018, respectively, while the both years average showed 42.9 g. Similarly, the minimum thousand grains' weight during both years (42.6 g and 40.5 g) was recorded in water spray check plots. The increases in thousand grains weight due to foliar Zn might be due to the involvement of the Zn in activation of enzymes (Aown *et al*., 2012) and nitrogen metabolism. Harris *et al*. (2007) and Karim *et al*. (2012) also observed increase in thousand grains weight with each incremental level of Zn. Niyigaba *et al*. (2019) also find out that both Zn and Fe fertilization significantly improve weight of thousand grain, spike length and grain yield wheat crop. In case of Fe, more thousand grains' weight was noted in both years (44.2 g and 41.7 g) with the foliar supplementation of Fe at 4 kg ha^{-1} over the water check plots (42.6 g and 40.8 g), respectively. Similarly, pooled data over years also revealed best performance of the same Fe dosage (43 g) as compared with the minimum (41.7g) in the water check plots. Thousand grains weight increased with the application of Fe because Fe played a key role in the photosynthetic process of plants. Fe atoms are directly involved in the photosystem I and II (Briat *et al*., 2007) and hence might have increased the mean weight of the grains. In case of application timing, more thousand grains' weight was recorded with split dosages of foliar micronutrients at tillering and booting stages during both years (43.6 g and 41.6 g), respectively, while averaged over years revealed 42.6 g. The minimum was noted for the sole foliar application at booting stage only during both years (43 g and 41.1 g), respectively. Foliar fertilization of Zn at tillering and in splits i.e. half at tillering and half at booting stages accounted for maximum thousand grains weight. Foliar feeding of crop at different timings had a strong relation with growth parameters. Adequate and instant supply of nutrients to crop at their early stages promoted crop features and responded better. Our results are in conformity with Armin *et al*.

(2014). They reported that thousand grains weight increases with foliar application and their later time of application. As regards interaction, only Zn and Fe interaction had significant effect on the thousand grains' weight. Foliar Zn supplementation up to 2 kg ha^{-1} increased the thousand grains' weight, while further supply of Zn decreased the thousand grains' weight. Foliar Fe had a synergistic response in combination with foliar Zn. However, the response pattern remained the same with the increasing levels of both micronutrients (Figure 3).

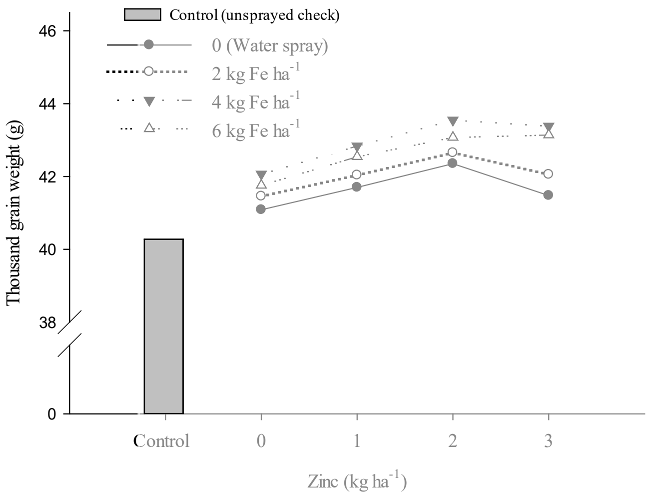

Figure 3: *Interactive effect of foliar zinc (Zn) and iron (Fe) application on the thousand grains weight of wheat.*

Conclusions and Recommendations

Foliar application of 3 kg Zn ha^{-1} significantly enhanced leaf area tiller^{-1} and flag leaf area, while foliar Zn at 2 kg ha^{-1} significantly improved spike m^{-2}, maximum grains spike^{-1}, length of spike and weight of 1000 grain. In case of, foliar Fe at 4 kg Fe ha^{-1} significantly increased the leaf area tiller^{-1}, flag leaf area, spike^{-2}, grains spike^{-1}, spike length and thousand grains weight. Similarly, foliar application of both micronutrients in split foliar application i.e. half at the stage of tillering and half at the stage of booting enhanced significantly the spike^{-2}, maximum grain spike^{-1}, spike length and thousand grains' weight. The study also concluded that phenological parameters of wheat crop increasing with the rates of both Zn and Fe foliar application, therefore recommended for the wheat crop cultivation in the Peshawar locality of Khyber Pakhtunkhwa province, Pakistan.

Novelty Statement

Novelty of this study is to find out the impact of Zn, Fe and their application stages and which improved wheat phenology and yield.

Author's Contribution

Sajid Ali: PhD scholar, who did research, data collection, analysis and wrote draft of the manuscript.
Shahen Shah: Major supervisor, provided guidelines and supervised the whole study.
Muhammad Arif: Co-supervisor, helped in technical guidelines.

Conflict of interest
The authors have declared no conflict of interest.

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