Biofortification of Maize with Zinc and Iron not only Enhances Crop Growth but also Improves Grain Quality

Zunaira Anwar[1], Zyma Basharat[1], Muhammad Bilal Hafeez [2], Shahbaz Khan[2*], Noreen Zahra[1], Zayrah Rafique[1], Muhammad Maqsood[2]
[1] Department of Botany, University of Agriculture, Faisalabad (38040), Pakistan
[2] Department of Agronomy, University of Agriculture, Faisalabad (38040), Pakistan

*Corresponding author: Shahbaz Khan; shahbaz2255@gmail.com
ORCID 0000-0002-4524-9630, Researcher ID U-2918-2019

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ABSTRACT

Hidden hunger is an emerging challenge for scientists, especially connected to the agriculture sector because over two billion people are facing it globally. This issue is more common in developing countries which have less access to a diverse diet due to their low income. Different potential practices are introduced to minimize the pressure of malnutrition but agronomic biofortification is being considered best practice to improve the contents of micronutrient in grains. A field based study was executed to explore the impact of zinc sulphate ($ZnSO_4$) and iron sulphate ($FeSO_4$) on productivity and grain quality of maize crop. Sole and combined application of $ZnSO_4$ and $FeSO_4$ either via soil or/and plant foliage not only enhanced the yield attributes of maize crop but grain quality was also improved. Soil supplimetation of $ZnSO_4$ (10 kg ha^{-1}) produced maximum plant height and cob weight. Combined treatment of $ZnSO_4$ (10 kg ha^{-1}) and $FeSO_4$ (12 kg ha^{-1}) through soil produced more grains per cob, 1000-grain weight, biological and grain yields. Foliar applied 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ produced highest chlorophyll contents. Foliar treatment of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ improved the concentration of zinc and iron in grains, respectively. Combined treatment of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$ through soil improves the yield attributes while foliar spray of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ enhances quality parameters. Overall, foliar spray approach is more applicable regarding nutrients availability for optimum development and growth of crop and improved grain quality.

Keywords: Chlorophyll; Foliar application; Hidden hunger; Maize yield; Micronutrients.

INTRODUCTION

Micronutrient malnutrition is affecting rural populations residing in developing countries with less access to a diverse diet due to their less purchasing power (Tsakirpaloglou et al., 2019; Kumar et al., 2019). Globally, over two billion people are facing hidden hunger (micronutrients deficiency), and it is an emerging challenge for scientists, especially connected to agriculture sector (WHO, 2016). In human, malnutrition is mainly caused by vitamin A, Zn, Fe, selenium (Se) and iodine (I) deficiency (Haider and Bhutta, 2009; Hess and King, 2009). Particularly Fe and Zn metal deficiencies, are affecting over 50% of world comunity because they depend on cereal crops, chiefly rice, maize and wheat for their regular diet (Ramzan et al., 2020).

Both Zn and Fe are indispensable nutrients for biological systems in plants, humans, and animals (Broadley et al., 2007; Failla, 2003). In human, Fe is needed for haemoglobin formation (oxygen transport), psychomotor development, and resistance to infection (Stoltzfus, 2001). Its clinical deficiency is also associated with pallor (anaemia), dizziness, reduced work capacity and reduced intellectual performance and pregnancy-related issues, i.e., low birth weight and mortality (Lynch, 2003; CDC, 2010). In humans, Zn is needed for activation of over 300 enzymes, maintenance of sensory functions, physical growth and development, immune system, and neurobehavioral development (Gibson, 2012; Levenson and Morris, 2011). Many health impediments, like poor physical growth, damage to DNA, central nervous, gastrointestinal, epidermal, reproductive, skeletal and immune systems may occur due to

Zn deficiency (Hambidge and Walravens, 1982; Prasad, 2006). In Pakistan, more than 40% mothers and one-third children are under Zn malnutrition, with a higher rate in rural communities (MINH, 2009).

Worldwide, maize is growing under wide range of soil and climatic conditions for grain and fodder production (Ranum et al., 2014). Maize ranks third vital cereal crop after wheat and rice that contributes almost 0.5% grand domestic production (GOP, 2019) Maize is also considered as a staple food for greater than 200 million people, and it can be expected that globe population will be eight billion in 2025 (USDA, 2008; Lutz et al., 2001). It is known as a queen of cereals because of high monetary value as it is also treated on commercial scale to make a variety of products for the consumption of human, livestock and poultry industries (Harris et al., 2007). Due to highest yield potential among cereals, it can be paramount crop to overcome global nutrition (Tariq and Iqbal, 2010).

Among different possible agricultural approaches to conquer the malnutrition, the agronomic biofortification is top ranked approach to improve the grain Fe and Zn contents (Borrill et al., 2014; Hassan et al., 2019; Cakmak et al., 2010). It is achieved through the application of micronutrient to crop foliage directly and/or soil (De Valenca et al., 2017; Zahra et al., 2020). It can be implemented easily being more sustainable and economical as compared to other techniques including genetic engineering (Cakmak, 2008). Ngozi (2013) reported that agronomic biofortification of the crops ia an emerging practice to overwhelm malnutrition particularly metal deficiencies in the developing world. Considering the above mentioned rationals, the current study was designed to study the following objective; i) to assess the either sole and/or combined influence of Zn and Fe on quality, growth and yield of maize crop, ii) to compare the efficiency of two application approaches, i.e. foliar and soil application.

MATERIALS AND METHODS

Experimental Particulars: The present field-based trail was conducted to explore the impact of Zn and Fe on grain quality and yield of maize crop. Seeds of maize hybrids (Soni dharti-626) were collected from Sohni Dharti International Seed Company, Sahiwal-Pakistan. Seeds were sown at research area of Agronomy Farm, Universsity of Agriculture, Faislabad-Pakistan during crop growing season of 2018. Site soil was ploughed to a depth of 30 cm and ridges of 30 cm in height and 75 cm of spacing were prepared. Two seeds were planted on the top of all ridges with space of 25 cm between hills. Every experimental unit consist of 4 ridges that were 3 m in length. The experimental soil of area, under study, was sandy loam having following properties; pH 8.1, EC 1.45 dS m^{-1}, organic matter 0.81%, total nitrogen (N) 0.08%, phosphorus contents (P) 8.2 ppm, potassium contents (K) 200 ppm, DTPA-Extractable iron (Fe) 2.54 mg kg^{-1}, zinc (Zn) 0.45 mg kg^{-1}.

Crop Husbandry: After almost 2 weeks of sowing, thinning was done to maintain the plant population and a basal dose of NPK fertilizer (230:145:92) was applied by using urea, DAP and sulphate of potash fertiliser. $ZnSO_4$ and $FeSO_4$ were used as sources of micronutrient. At sowing, one-third of nitrogen and all of the phosphorus, potash, $ZnSO_4$ and $FeSO_4$ doses were applied as a basel dose. The remaining dose of nitrogen was supplimented in equal splits with 1st and 2nd irrigation while foliar application of $ZnSO_4$ and $FeSO_4$ at silking and grain filling stage. Experimental units were weeded twicly in the course of the growing duration.

To study the above mentioned objectives, following treatments were applied;

- Control (foliar spray of water)
- Foliar spray of 0.1% $ZnSO_4$
- Foliar spray of 0.3% $FeSO_4$
- Foliar spray of 0.1% $FeSO_4$ and 0.3% $FeSO_4$
- Soil supplimetation of 10 kg ha^{-1} of $ZnSO_4$
- Soil supplimetation of 12 kg ha^{-1} of $FeSO_4$
- Soil supplimetation of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$
- Foliar spray of 0.1% ZnSO4 & 0.3% $FeSO_4$ and Soil supplimetation of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$

Biochemical Analysis and Quality Attributes: Arnon (1949) method was used for the determination of the chlorophyll pigments (*a* and *b*). Atomic Absorption Spectrophotometer was used to measure the amount of Zn and Fe in maize grains according to method described by AOAC (1990). The total soluble protein was measured according to Bradford method (1976).

Agronomic parameters: Data of yield and its attributes were recorded at crop maturity. Plant population was counted at harvesting (m^{-2}). Ten plants were selected randomly from each experimental unite to recorde the data of plant height. Plant height of individual plant was measured with a meter rod from base to top and then averaged. Number of grain rows per cob, cob weight and its length, and grains per cob were determined manually from the selected cobs. For biological and grain yield, plants harvested from a row of one meter length and converted in to per hectare and weighed with weighing balance. Harvest index (%) was estimated by dividing the grain yield with biological yield.

Statistical analysis:

A randomized complete block design (RCBD) having three replications was adopted. Statistical package (Statistix 8.1) was used to analyse and evaluate the collected data. ANOVA (analysis of variance) technique proposed by Fisher was followed to observe the statistical differences among the variance and means of treatments.

RESULTS

Foliar spray of 0.1% $ZnSO_4$ & 0.3% $FeSO_4$ produced maximum chlorophyll a content in maize (Fig. 1a) that was at par statistically with foliar application of Fe @3% and soil applied Zn @ 10 kg ha^{-1}, whereas minimum in control. Similarly, foliar spray of 0.1% $ZnSO_4$ & 0.3% $FeSO_4$ produced highest chlorophyll b and total chlorophyll contents (Fig. 1b & c). Foliar application of Fe @ 3% produced maximum Fe contents in maize grain while minimum in control (Fig. 2a). Foliar spray of Zn @ 0.1% bring in highest Zn contents in maize grain while lowest in control (Fig. 2b). All the treatments either sole or combined through foliar spray or soil application reduced the protein contents in maize grain (Fig. 2c). Highest protein contents were observed in those grains which received no exogenous treatment.

The number of leaves $plant^{-1}$ were not significantly increased by external treatments of Zn and Fe (Table 1). Soil applied Zn @ 10 kg ha^{-1} produced maximum plant height as compared to other treatments (Table 1). Cob length was not affected by exogenous application of Zn and Fe (Table 1) while cob weight was significantly affected. Soil supplimented Zn @ 10 kg ha^{-1} produced highest cob weight (Table 1) that was statistically at par with soil supplementation of Zn and Fe @ 10 and 12 kg of ZnSO4 and FeSO4 per hectare. Number of grain rows were not affected by treatment application (Table 1). Data regarding number of grains cob^{-1}, 1000-grain weight, biological and grain yield and harvest index are presented in table 2. Application of 10 kg of $ZnSO_4$ and 12 kg of $FeSO_4$ through soil produced maximum grains cob^{-1}, 1000-grain weight, biological as well as grain yield, however, harvest index was statistically non-significant (Table 2).

DISCUSSION

Our outcomes supported the hypothesis that the different amount of zinc and iron treatments either through soil or/and foliar significantly improves the chlorophyll contents, quality of grains, and grain yield in maize crop than control. The improvement in grain yield is associated either the cob weight, grains cob^{-1} and weight of 1000-grains. In this study, maximum grain yield was recorded when combined application of zinc and iron was suplimented (Table 2). The improvement in attributes of yield of maize crop might be due to involvement of zinc and iron in biochemical processes, including photosynthesis. Outcomes of present experimentation are similar to Saleem et al. (2016), who stated the soil supplementation of Zn and Fe (each @ 30 kg ha^{-1}) considerably enhanced yield and quality of grains. Similar outcomes were also described by Kanwal et al. (2010) that more Zn uptake and higher grain yield in corn were noted when Zn soil supplementation was done @ 18 kg Zn ha^{-1}. These findings are also in line with Mugenzi et al. (2018), who stated the collective effect of Zn and Fe enhanced the maize yield, photosynthetic capacity, and grain quality. Eteng et al. (2014) showed a considerable increase in yield of maize with the micronutrients supplementation. Globally, ~50% of the soils under cultivation of cereal have low levels of available Zn for plants (Graham and Welch, 1996).

Combined treatment of Zn and Fe (soil+foliar) increased accumulation Zn and Fe contents (Fig. 2a, b). Maximum Zn content was noted when $ZnSO_4$ applied @ 0.1%, and highest amount of Fe content was recorded when $FeSO_4$ was applied @ 0.3% (Fig. 2a, b). Highest protein contents were analyzed in control while significantly decreased when combined soil Zn and Fe supplementation was done (Fig. 2c). Our results are also in line with the findings of Cakmak et al. (2010), who stated that Zn contents were increased three times in grains by foliar and soil applied Zn, additionally noted that time and method of Zn application is so important to enhance Zn concentration in grains. By application of 0.5% $ZnSO_4$ and 1% $FeSO_4$ zinc and iron contents were also significantly improved (Pahlavan-Rad and Pessarakli, 2009). Saleem *et al.* (2016) noted that Zn and Fe through foliage considerably enhanced the zinc and iron contents in maize grain. These findings are also in line with Zeidan *et al.* (2010), they concluded that zinc and iron concentrations in wheat grains were significantly improved by foliar treatment of zinc and iron. Ozturk *et al.* (2006) also stated that foliar spray improved the zinc content in grain at later growth stages. Sharma and Singh (1990) investigated that zinc absorption through soil to plant was improved by application of ZnSO4 in maize-stover.

Foliar spray of Zn improved the availability of Zn to plants in comparison with soil application (Zhao et al., 2014). The foliar spray of Zn is more productive in enhancing grain Zn concentration of maize crop. Cakmak (2008) reported that the foliar application is better way to improve iron content in grain because clay adsorption and low organic matter reduced the flow of nutrients reported the accumulation of micronutrients in the grain with the addition of their fertilizers. Zuchi et al. (2015) investigated that an insufficient supply of other nutrients might prevent the uptake of iron and its translation to shoots and other parts, that may result in reduction of other nutrients. Among plants, nutrients interaction can be antagonistic, synergistic, Liebig-synergistic and/or zero-interactive. These interactions explained that activities of other nutrients may be affected by the supply of specific nutrient that ultimately adversely affect crop yield and growth (Rietra et al., 2017). Usually, Fe deficiency is seen in calcareous soils having high pH in arid areas (Prasad, 2003). Worldwide, it is estimated that Zn (50%) and Fe (30%) deficiencies are widespread occurring in cultivated soils (Cakmak, 2002). In Pakistan, zinc deficiencies are more extensive, about 70% soils are deficient of zinc (Imtiaz et al., 2010).

Applied micronutrients and their interactions influence physiological and biochemical processes of plants, that substantially affect quality and yield of grains (Wang et al., 2015). Mugenzi et al. (2018) stated that iron and zinc application, either sole or combined had no significant influence on protein content. However, in this study protein content considerably reduced where sole or combined zinc and iron foliar or in soil was applied in comparison with control. Ramzani *et al.* (2017) noted that protein content increased by 64% by Fe application in comparison with control.

Conclusion: Foliar spray of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ improved the contents of zinc and iron in grains, respectively. Combined treatment of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$ through soil improves the yield attributes while foliar application of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ enhances quality parameters. Overall, foliar spray approach is more suitable regarding the availability of nutrients for optimum crop growth and improved grain quality.

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Conflict of Interest

The authors do not declare any conflict of interest regarding current manuscript.

Authors' contribution

Zunaira Anwar, Zyma Basharat, Muhammad Maqsood and MB Hafeez designed and conducted the experiment and collected the data. Shahbaz Khan, Noreen Zahra and Zayrah Rafique analyzed the collected data and prepared first draft. Shahbaz Khan and MB Hafeez finalized the draft. Shahbaz Khan submitted the final draft as a corresponding author.

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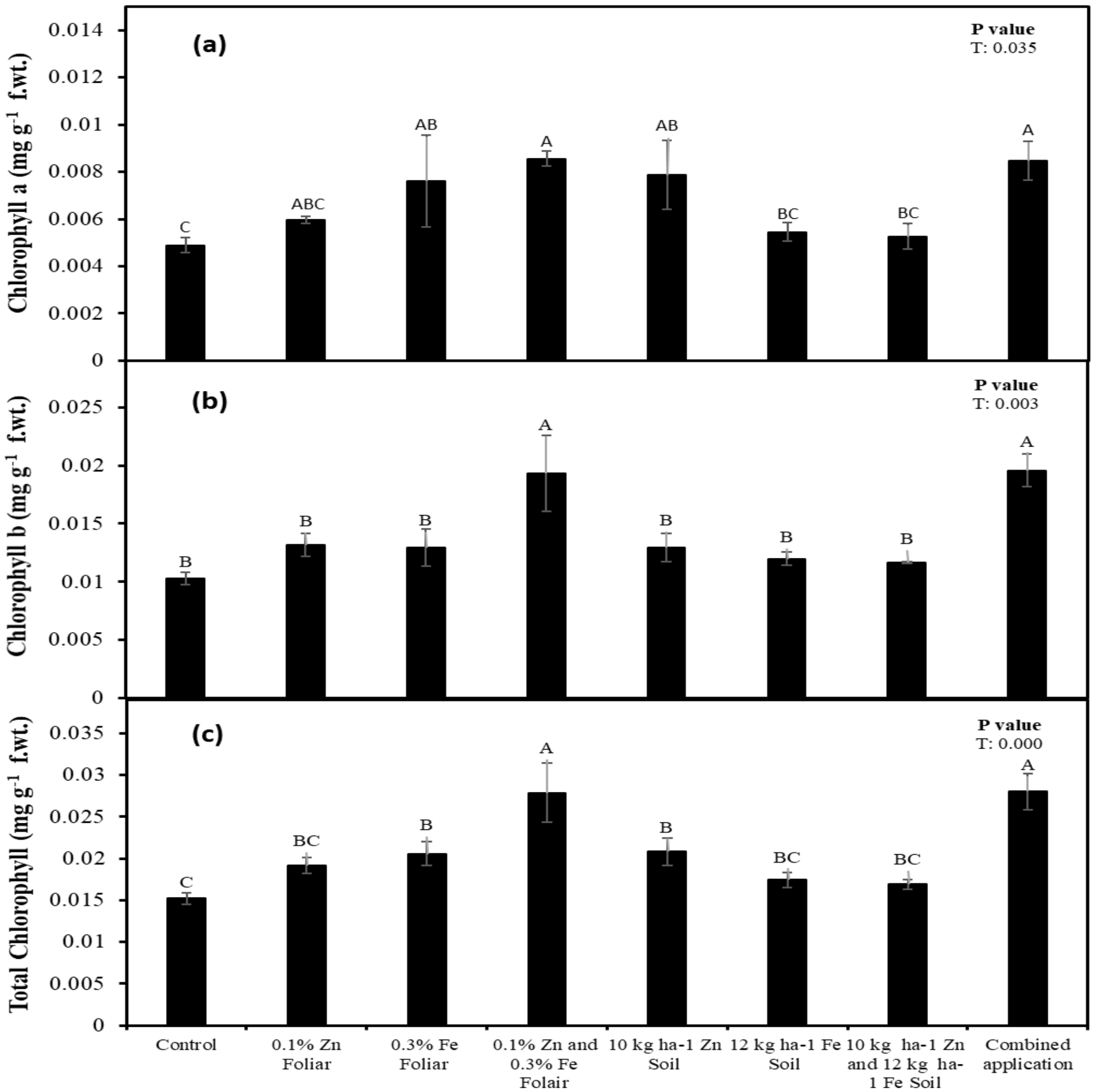

Fig. 1 Influence of foliar and soil supplied $ZnSO_4$ and $FeSO_4$ on chlorophyll *a* (a), *b* (b) and total chlorophyll in maize (c).

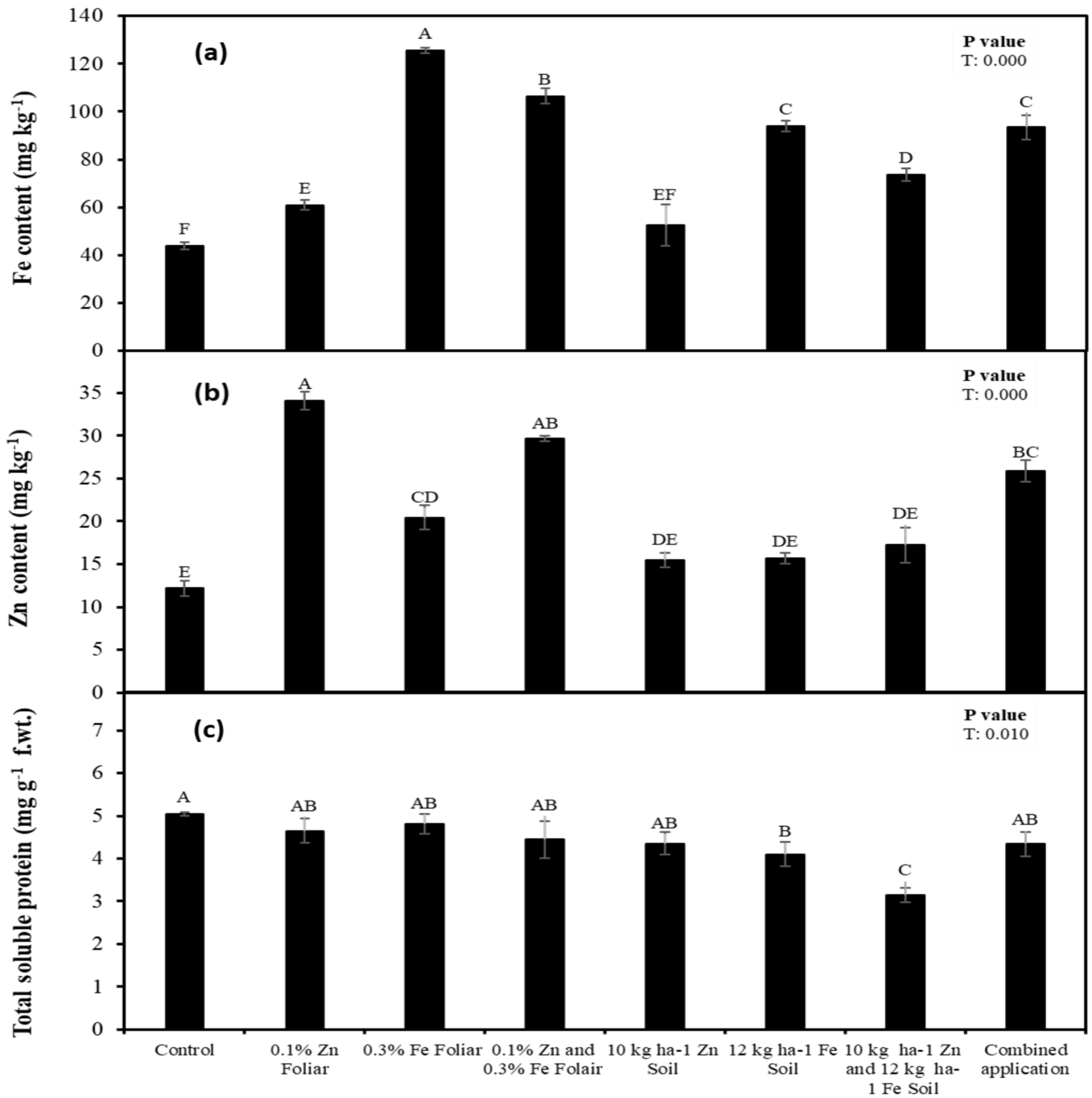

Fig. 2 Influence of foliar and soil supplied $ZnSO_4$ and $FeSO_4$ on Fe contents (a) Zn contents (b) and total soluble proteins in maize grains (c).

Table 1 Influence of foliar and soil supplied $ZnSO_4$ and $FeSO_4$ on number of leaves per plant, plant height, cob length, cob weight and grain rows per cob in maize.

Treatments	Number of leaves per plant	Plant height (cm)	Cob length (cm)	Cob weight (g)	Grain rows per cob
Control (foliar application of H_2O)	14±0.352	181.1±3.78 c	18.15±0.029	230.4±3.600 d	15±0.352
Foliar application of 0.1% $ZnSO_4$	15±0.066	192.4±3.03 ab	18.78±0.450	238.0±4.244 cd	15±0.230
Foliar application of 0.3% $FeSO_4$	15±0.416	190.5±1.99 b	18.24±0.140	241.8±3.120 cd	15±0.417
Foliar application of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$	14±0.133	191.6±2.45 ab	18.36±0.284	242.0±2.017 cd	15±0.230
Soil application of 10 kg ha-1 of $ZnSO_4$	14±0.266	200.0±2.51 a	18.52±0.184	264.3±1.876 a	15±0.666
Soil application of 12 kg ha-1 of $FeSO_4$	14±0.176	197.6±3.16 ab	18.27±0.439	246.3±5.223 bc	16±0.400
Soil application of 10 kg ha-1 of $ZnSO_4$ and 12 kg ha-1 of $FeSO_4$	15±0.266	199.5±2.52 ab	18.54±0.270	260.3±3.186 a	15± 0.581
Foliar application of 0.1% $ZnSO_4$ & 0.3% $FeSO_4$ and Soil application of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$	14±0.176	197.0±4.041 ab	18.40±0.248	253.9±1.931 ab	15±0.266
LSD (P= 0.05)	0.808	9.3123	0.7306	10.411	1.316

Table 2 Influence of foliar and soil supplied $ZnSO_4$ and $FeSO_4$ on grains per cob, 1000-grain weight, biological yield, grain yield and harvest index in maize.

Treatments	Grains per cob	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (kg ha^{-1})	Harvest index (%)
Control (foliar application of H_2O)	511±5.73 d	303.0±4.041 d	22.69±462.9 c	3990±89.5 e	17.62±0.732
Foliar application of 0.1% $ZnSO_4$	528±10.73 cd	330.0±3.000 cd	25.32±201.8 b	4742±244.1 d	18.74±1.107
Foliar application of 0.3% $FeSO_4$	532±11.30 cd	345.0±5.686 bcd	25.00±801.8 bc	4458±38.8 de	17.88±0.728
Foliar application of 0.1% $ZnSO_4$ and 0.3% $FeSO_4$	543±7.62 bc	330.0±4.582 cd	26.39±801.8 b	4903± 338.8 cd	18.61±1.405
Soil application of 10 kg ha^{-1} of $ZnSO_4$	554±8.96 abc	372.0±4.041 abc	31.02±462.9 a	5772±767.5 ab	18.66±0.781
Soil application of 12 kg ha^{-1} of $FeSO_4$	548±9.73 abc	375.0±7.937 abc	31.00±1224.8 a	5378±172.4 bc	17.34±0.207
Soil application of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$	570±6.62 a	405.0±6.429 a	32.41±936.5 a	6156±111.0 a	19.00±0.498
Foliar application of 0.1% $ZnSO_4$ & 0.3% $FeSO_4$ and Soil application of 10 kg ha^{-1} of $ZnSO_4$ and 12 kg ha^{-1} of $FeSO_4$	566±9.25 ab	384.0±6.000 ab	31.48±925.9 a	5473±283.1 bc	17.40±0.923
LSD (P= 0.05)	27.75	16.240	2331.3	578.9	2.608