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Zinc and Iron Enrichment in Wheat Grain Through Soil Amendments

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Abstract. Among staples being consumed globally, wheat (*Triticum aestivum* L.) ranks at top. However, it is low in bioavailable Zn and Fe engendering nutrition problems in the populace consuming it as major calorie intake source. Biofortifcation, a grain enrichment approach, adjudicated as tenable, economical and smartly executed one. A pot experiment was conducted using different levels of Zn and Fe (2.5, 5.0, 7.5 mg/Kg soil) fertilization. Maximum significant grain yield increment over control observed with Zn 7.5 mg/Kg (60.5%) followed by Zn 5.0 mg/Kg (56%). Grain Zn and Fe contents were at maximum (36.20 and 33.75 µg/g over control *i.e*., 21.76 and 17.65 µg/g, respectively) with their highest doses *i.e*. 7.5 mg/Kg soil. Estimated human bioavailability of Zn by trivariate model was enhanced by 67.4 and 46% over control with Zn 7.5 mg/Kg and Zn+Fe 5.0+5.0 mg/Kg, respectively. Estimated Fe bioavailability also increased maximally with Fe 5.0 mg/Kg. The protein content in grain significantly enhanced by the treatments applied in combination of zinc and iron, maximum with Zn+Fe 5.0+2.5 mg/Kg. Conclusively, fertilization approach found feasible in enriching wheat grain with micro minerals Zn and Fe.

Keywords: biofortification, zinc, iron, trivariate model of Zn, wheat (*Triticum aestivum* L.)

Introduction

Malnutrition is posing a major threat to human health and it is worsening day by day in its varying forms (WHO, 2016; FAO, 2014). Over 24,000 people worldwide die daily due to "hidden hunger" and malnutrition (Fiaz *et al*., 2019). Cereals being consumed predominantly in developing world, deficient in Zn and Fe are considered posing major health problems due to Zn, Fe malnutrition (Black *et al*., 2013). People facing higher Zn and Fe deficiencies in rural areas of most of the Asian developing countries rely on cereals diets alone upto 70% of their daily calorie intake (Timmer, 2014; Cakmak, 2008). Zinc is considered as an essential nutrient for accomplishing growth and development in human and plants (Hafeez *et al*., 2013). The plant seed upon germination, grows swiftly into a young seedling requiring enzymes activated by Fe, Zn along with other cofactors (Bastow *et al*., 2018). It is assumed that about one tenth of the total human proteins depend on Zn for its formation (Krezel and Maret, 2016). Zinc is needed for growth and functioning of cell induced immunity, neutrophils and natural killing cells (Prasad, 2008). Zinc ions are vital parts of Zn finger family of transcription factors effecting cell proliferation and differentiation (Guerinot, 2007). Zinc effectuates plants metabolic activities through activation of enzymes (Tisdale *et al*., 1984). It renders vital role in gene expression, stress tolerance (Cakmak *et al*., 2000) and pollen tube formation (Pandey *et al*., 2006). Its insufficiency can lead to impairment of immune system, physical growth retardation and reproductive health in humans (Levenson and Morris, 2011). Zinc insufficiency is a major risk factor in potential mortality each year of 0.80 million people and about 0.45 million kids around the globe (WHO, 2015). The existing Zn status of wheat-cultivated regions range 20-35 mg/Kg, with mean value 28-30 mg/Kg (Cakmak *et al*., 2010; Fardet *et al*., 2008; Graham *et al*., 2007). This reflects a wide gap between existing mean grain Zn concentration and target range for human health, 40-6 mg/Kg (Cakmak *et al*., 2010; Zhao *et al*., 2009; Graham *et al*., 2007).

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Iron being an essential micro-element for humans, regulates various metabolic activities like oxygen transportation, DNA synthesis and electron transport (Abbaspour, 2014). Human body contains about 70% of its iron in form of hemoglobin and myoglobin in RBC and muscle cells respectively (Gupta, 2014). Iron plays pivotal role due to its essentiality in plants as carrying out chlorophyll synthesis, nitrogen fixation and DNA replication (Nouet *et al*., 2001). Iron deficiency is the major factor of anemia, associated with pregnancy malfunctions, lowered immunity and retarded work productivity because of tiredness (WHO, 2017). Iron deficiency induces a number of physiological diseases, as anemia and neurodegenerative disorders (Sheftela *et al*., 2011).

Cereals (wheat, rice, maize) serve as energy intake for 30-35% of the populace in the Great Britain (Bates *et al*., 2014) and close to 60% of daily calorie intake in developing world (Ritchie and Roser, 2018), but much of micronutrients lost during post-harvest processing of whole grains. The cereals contribution towards edible dry matter and daily calorie intake is upto 28 and 60% respectively in developing world (Gross National Income < $12,055) (World Bank, 2019; FAO, 2015). Cereals meet about 60 and 52% of human daily micronutrient requirement for iron and zinc respectively (Boius and Welch, 2010). Wheat (*Triticum aestivum* L.) is dominant cereal cultured globally along with maize and rice. Global wheat production in the year 2018-2019 was 730.50 million ton cultured on 215.24 million hectares of area (USDA, 2019). Wheat is major crop in Pakistan with production of 25.1 million ton on an area of 8.80 million hectares (Pakistan Economic Survey, 2019).

There are many feasible measures that can be adopted to enhance micro-nutrient enrichment of human diet as diet diversification, pharmaceutical supplements, post-harvest fortification and biofortification (Borril *et al*., 2014). Plant breeding (conventional breeding and genetic engineering) and fertilization approach (agronomic biofortification), are two important agricultural tools to enhance the grain density of Zn and Fe. Contrary to breeding approaches, agronomic biofortification is potentially more sustainable, economical and more feasible in execution than other strategies (Cakmak, 2009). Fertilization of crops with microminerals enhances their density in edible parts of plants, has been proven by various studies (White and Broadley, 2011; Cakmak, 2008). Therefore, it seems more practical to focus on cereals that sustain human life in most of the developing countries including Pakistan. Biofortification of wheat grains through fertilization seems to be an feasible and effective approach to reduce Zn and Fe deficiency problems in Pakistan.

Zinc and iron are mainly found in embryo and aleurone layer of wheat grain, while endosperm comprises meager quantities of these micro-minerals. The embryo and aleurone layer are mostly removed during milling process making their bioavailability to humans at minute levels. Adding to the issue, phytate (phytic acid or inositol hexakisphosphate; $C_6H_{18}O_{24}P_6$) abundantly found compound in wheat grain governing phosphorus provision to young seedlings, is also considered as anti-nutrient due to its chelation with Fe, Zn restricting their absorption into intestines. In plants, is dominant storage reservoir of grain P that chelates micronutrients (Ca, Zn, Mg, Fe, Mn, etc.) and hence, lowers their bioavailability (Raboy, 2009; 2001; Sandberg and Andersson, 1988). Phytate forms compounds with proteins under both the acidic and alkaline conditions, effecting proteins structure, thus reducing enzymatic activity, protein solubility and proteolytic digestibility (Yao *et al*., 2011; Kies *et al*., 2006). Phytate: micronutrients molar ratios are taken into consideration in estimating potential bioavailability of the micronutrients. Generally, mineral bioavailability is maximized at lower molar ratio. The Phytate: Fe molar ratio should have been <1 or preferably <0.4 for significant enhancement in Fe absorption (Hurrel and Egli, 2010). The phytate: Zn molar ratios <5, 5-15 and >15 have been designated as causing high, moderate and lower zinc human bioavailability (Gibson, 2006). To gauge the estimated bioavailability of Zn in human nutrition, zinc absorption trivariate model can also be utilized effectively and efficiently (Bouis and Welch, 2010). A trivariate model of Zn absorption as a function of dietary zinc and phytic acid (Miller *et al*., 2007) comprehensively got evaluated in a labeled Zn absorption study on adult women (Rosado *et al*., 2009). Iron bioavailability was estimated using [phytate]:[Fe] ratio. Zinc and Fe bioavailability estimation is pivotal for a viable ferti-fortification project.

The present study executed to assess fertilization approach through soil for enrichment of wheat grain in order to improve its quality for reducing micronutrient malnutrition.

Materials and Methods

The loam soil belonging to Udic Haplustalfs, Haplustalfs, Ustalfs and Alfisols order was collected form farmer field at village Ghayye Wala (32.26497° N, 74.98041° E) Tehsil Wazirabad, District Gujranwala, Pakistan, air dried, ground and added to pots (10 Kg/pot). Zinc and iron were applied at levels 2.5, 5.0 and 7.5 mg/Kg soil alone and in combinations (2.5+2.5, 2.5+5.0, 5.0+2.5 and 5.0+5.0 mg/Kg soil) in the form of sulphate salts. Recommended dose of NPK fertilizers (urea, di-ammonium phosphate, sulphate of potash) were applied @110-84-62 Kg/ha. The experiment was laid out maintaining three replications and sowing six seeds of wheat variety Faisalabad-2008 during November-April, 2017-2018 using Completely Randomized Design at Soil and Water Testing Laboratory, Gujranwala, Pakistan. After germination thinning was carried out maintaining three healthy and vigorous plants. Weeding was done manually. Irrigation was carried out at sowing, tillering, booting, milking and grain formation stages. The agronomic parameters like plant height, number of tillers and spike length data were recorded at maturity. At harvesting grain yield data was recorded as g/pot.

Soil analysis. The soil samples were taken pre-sowing and post-harvest, ground and sieved through 2 mm sieve and analyzed for fertility status. Soil pH was estimated in saturated paste and electrical conductivity EC from 1:1 soil water suspension (Page *et al*., 1989). Soil organic carbon was measured using dichromate reduction method given by (Rayan *et al*., 2001). Available P was determined by Olsen's method (Rowell, 1994) and extractable K by NH_4 acetate extraction method given by Sheldrich and Wang (1993). Zinc and iron concentration in soil was determined using DTPA extraction on an atomic absorption spectrophotometer (Lindsay and Norvell, 1978). The pre-sowing soil analysis of pot experiment evinced soil as loamy, devoid of salinity and sodicity (EC 1.20 ΔS/m) with pH 7.46. The organic matter was low, while it was moderate in phosphorus (11.34 µg/g) and potassium (114 µg/g). The DTPA extractable Zn and Fe in soil was insufficient as 0.87 µg/g and 3.44 µg/g, respectively.

Plant analysis. The crop was harvested in last week of April, 2018 and plant samples were collected. These were air dried and then oven dried at 65 °C to get reduce the moisture. Then finely ground with a Willey micro mill (Staufen, Germany). The dried ground grain samples (0.5 g) were digested in a di-acid (2:1 ratio of nitric acid: perchloric acid) mixture (Rashid, 1986). The Zn, Fe contents in digested material was determined using Atomic Absorption Spectrophotometer. Phytate in extract was estimated by the method illustrated by Haug and Lantzsch (1983) on spectrophotometer. Whole grain sample was dried, sixty mg from each sample was extracted with ten mL of 0.2 N HCl at ambient temperature for two hours and then measured on a spectrophotometer (Zuzi).

Human zinc and iron bioavailability in grain. Zinc and phytate density in food has impact on human Zn bioavailability. Therefore, in wheat grain, [phytate]:[Zn] molar ratio was exploited for estimation of human Zn bioavailability (Brown *et al*., 2002). A [phytate]:[Zn] ratio <20 is generally considered for bettering human nutrition (Weaver and Kannan, 2002). Having knowledge of a quantifiable value of Zn bioavailability, it was tabulated by trivariate model of Zn absorption (Miller *et al*., 2007).

$$TAZ = 0.5 \left[A_{MAX} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_P}\right) - \sqrt{\left(A_{MAX} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_P}\right)\right)^2 - 4 \cdot A_{MAX} + TDZ} \right]$$

where:

A_{MAX} = maximum zinc absorption (0.091); K_R = equilibrium dissociation constant of zinc-receptor binding reaction (0.680); K_P = equilibrium dissociation constant of zinc-phytate binding reaction (0.033) and correlated with human intestinal Zn homeostasis (Hambidge *et al*., 2010); TAZ = Total daily absorbed Zn (mg Zn d^{-1}); TDP = Total daily dietary phytate (mmol phytate d^{-1}); TDZ = Total daily dietary Zn (mmol Zn d^{-1})

The TAZ was determined for 300 g of wheat grain and termed as 'estimated bioavailable Zn to human' basing on assumption as an adult feeding solely on 300 g wheat grain/day.

The bioavailability of Fe from grain is estimated by [phytate]:[Fe] ratio as Fe bioavailability is also affected by phytate in wheat grain. Iron bioavailability may be estimated by tabulating [phytate]:[Fe] and the ratio >1 is considered as indicative of poor Fe bioavailability (Halberg *et al*., 1989).

Total protein. Total protein content in wheat grain was gauged using method illustrated by (Chapman and Parker, 1961). The grains were ground and digestion carried out using digestion mixture (K_2SO_4: $CuSO_4$ in 9:1) and concentrated sulfuric acid as catalysts at digestion assembly. The digested material was distilled with NaOH, capturing released ammonia in 2% boric acid solution. Total nitrogen content in grain were determined by titrating released ammonia against 0.1 N H_2SO_4. Protein content (%) was determined by multiplying total nitrogen with a value of 6.25.

Statistical analysis. The data were analyzed using Analysis of Variance (ANOVA) technique (one way

ANOVA) by SPSS version 16 (SPSS for Windows, Version 16.0. Chicago, SPSS Inc., 2007). Difference among treatment means in pot experiment were compared by Tukey's Honestly Significance Difference Test (Brillinger, 1984) while in field experiment by Least Significance Difference Test at 5% level of probability (Steel *et al*., 1980).

Results and Discussion

Growth and yield attributes. Plant height was significantly increased with four treatments as compared to control (Table 1). Maximum increase of 11.6% was with treatment Zn+Fe@ 5.0+2.5 mg/Kg followed by 10.8% with Fe@ 5.0 mg/Kg. The other treatments enhancing plant height significantly were Zn+Fe@ 2.5+2.5 mg/Kg and Zn@ 2.5 mg/Kg. None of the treatments affected tillers/plant significantly (Table 1). The spike length was affected significantly by most of the treatments as compared to control (Table 2). The maximum enhanced spike length was 13.03 cm followed by 12.63 cm with Fe@ 2.5 mg/Kg and Zn@ 2.5 mg/Kg respectively over control (10.56 cm). Grain yield of wheat (g/pot) was increased significantly over control by three treatments being highest by Zn@ 7.5 mg/Kg (60.5% increase over control) followed by Zn@ 5.0 mg/Kg and Fe@ 2.5 mg/Kg showing 56 and 50.6% increase over control respectively (Table 2). All the other treatments enhanced grain yield but it was statistically non-significant.

The treatments comprising sole doses of Zn and Fe have more pronounced effect on growth and yield attributes than the combined treatments (Table 1). Although, the Zn and Fe salts when applied in combination did affect growth and yield of wheat significantly but it was incomparable with sole treatments of Zn and Fe. It might be due to the reason that zinc application improves yield and yield components through various mechanisms, for example, it improves chlorophyll content and triggers photosynthetic activity and auxin synthesis which lead to better growth and development of the crop, thus efectively amplifying yield and yield components (Rakesh and Jitendra, 2014). These findings are in consistence with the previous researches indicating that soil application of Zn increased grain yield in various cereal crops such as rice, wheat, maize and soya bean (Joy *et al*., 2015). The results are in consistence with Khan *et al*. (2009) stating that soil application substantially improves the translocation of nutrients from soil, which leads to better stand establishment and grain yield. Similar results were observed by Cakmak, 2008 trial, soil application of Zn increase maximum plant height of wheat.

Zinc concentration in wheat grain. Different treatments significantly improved grain Zn concentration (Table 3). It ranged from 21.76-36.20 µg/g in various treatments. Maximum increase was with Zn@ 7.5 mg/Kg (36.20 µg/g) followed by Zn@ 5.0 mg/Kg (32.72 µg/g) over control (21.76 µg/g). The maximum increase was 66.36% with Zn@7.5 mg/Kg over control, while the minimum significant increase in grain Zn content was 34.2% with Zn+Fe@ 2.5+5.0 mg/Kg.

Table 1. Effect of fertilization treatments on growth parameters of wheat

Treatments	Plant height (cm)	Number of tillers/plant
Control = No Zn, Fe	81.23 ±0.78 C*	15 C
Zn@2.5 mg/Kg soil	88.1 ±0.97 B	17 C
Zn@5.0 mg/Kg	86.9 ±0.98 C	17 C
Zn@7.5 mg/Kg	86.53 ±0.67 C	17 C
Fe@2.5 mg/Kg	85.8 ±1.32 C	17 C
Fe@5.0 mg/Kg	90.03 ±1.44 A	16 C
Fe@7.5 mg/Kg	87.56 ±1.50 A	16 C
Zn+Fe@2.5+2.5 mg/Kg	89.36 ±0.71 B	17 C
Zn+Fe@5.0+2.5 mg/Kg	90.66 ±1.14 A	17 C
Zn+Fe@2.5+5.0 mg/Kg	86.36 ±0.66 C	16 C
Zn+Fe@5.0+5.0 mg/Kg	84.30 ±0.47 C	16 C

* = Differing letters in a column designate significant incongruity by HSD at *pd* 0.05 and ± stipulate std. error.

Table 2. Effect of fertilization treatments on yield parameters of wheat

Treatments	Spike length (cm)	Grain yield (g/pot)
Control = No Zn, Fe	10.56±0.22 C*	23.50±0.65 D
Zn@2.5 mg/Kg soil	12.63±0.18 A	32.20±1.75 D
Zn@5.0 mg/Kg	11.86±0.38 C	36.66±1.98 B
Zn@7.5 mg/Kg	12.40±0.11 B	37.73±1.43 A
Fe@2.5 mg/Kg	13.03±0.14 A	35.40±1.76 C
Fe@5.0 mg/Kg	11.73±0.43 C	32.16±1.75 D
Fe@7.5 mg/Kg	12.53±0.17 A	30.60±3.95 D
Zn+Fe@2.5+2.5 mg/Kg	11.80±0.43 C	32.76±3.16 D
Zn+Fe@5.0+2.5 mg/Kg	11.86±0.55 C	33.80±3.17 D
Zn+Fe@2.5+5.0 mg/Kg	12.13±0.24 C	30.36±1.93 D
Zn+Fe@5.0+5.0 mg/Kg	12.40±0.30 B	30.17±1.31 D

* = Differing letters in a column designate significant incongruity by HSD at pd 0.05 and ± stipulate std. error.

Table 3. Effect of fertilization on nutritional elements in wheat grain

Treatments	Zn concentration (µg/g)	Fe concentration (µg/g)
Control = No Zn, Fe	21.76±0.78 D*	17.65±0.44 F
Zn@2.5 mg/Kg soil	25.48±0.75 D	18.22±1.17 F
Zn@5.0 mg/Kg	32.72±1.52 B	17.67±0.71 F
Zn@7.5 mg/Kg	36.20±1.35 A	16.89±0.74 F
Fe@2.5 mg/Kg	20.48±0.89 D	26.12±0.53 D
Fe@5.0 mg/Kg	19.57±0.82 D	30.75±0.67 B
Fe@7.5 mg/Kg	20.25±0.55 D	33.75±0.67 A
Zn+Fe@2.5+2.5 mg/Kg	26.19±0.77 D	25.85±1.46 D
Zn+Fe@5.0+2.5 mg/Kg	31.95±2.08 B	23.23±1.41 E
Zn+Fe@2.5+5.0 mg/Kg	29.26±1.54 C	28.92±1.15 C
Zn+Fe@5.0+5.0 mg/Kg	33.40±0.59 B	29.98±1.49 C

* = Differing letters in a column designate significant incongruity by HSD at pd 0.05 and ± stipulate std. error.

Grain zinc concentration of wheat was at maximum with Zn@7.5 mg/Kg applied through soil. It might be due to the reason that zinc experiences high mobility through phloem in plants coupled with profound N supply (Mao *et al*., 2014). The results are in consistence with a study expanded over ten African countries focusing upshots of Zn enriched fertilizers on different crops, reported that soil applied Zn augmented its content in maize, rice and wheat grains by 23, 7 and 19% respectively (Joy *et al*., 2015). The fertilization strategy to improve Zn density of wheat is an effective and feasible way not mere for grain enrichment in whole grain but also in endosperm, which is pivotal for target populace depending primarily on white flour for calorie intake (Kutman *et al*., 2011; Cakmak *et al*., 2010). The results are in line with those reported by Cakmak in (2010) that soil applied Zn increased its content in wheat grain. The increase in grain content of wheat by fertilization may be attributed to enhanced plant available Zn level in soil and its active transportation through xylem and phloem to the reproductive parts of plant. Coupled soil and foliar application of Zn, Fe is often the most promising approach for enhancing yield and grain content of cereals (Phattarakul *et al*., 2002). It has been evidenced that cereals get improved in terms of growth, yield, and grain Zn concentration through Zn fertilization (Fageria *et al*., 2011; Shehu and Jamala, 2010).

Iron concentration in wheat grain. Iron content in wheat grain increased significantly with all the treatments having Fe alone or in combinations (Table 3). As compared to control, maximum Fe content increase was 33.75 µg/g followed by 30.75 µg/g with Fe@ 7.5 mg/Kg and Fe@ 5.0 mg/Kg over control (17.65 µg/Kg).

The results are in accordance with (De Valenca *et al*., 2017 reporting that combined application of NPK and Fe fertilizers is viable approach in enhancing Fe concentration in cereal grains. The results are also in consistence with Abbas *et al*. (2009) reporting soil application of Fe significantly increased its content in grain as well as wheat yield. The findings are also in line with (Kutman *et al*., 2011; Cakmak *et al*., 2010) describing the coupling of N fertilizer along with Zn, Fe applied through soil or foliar augments both yield and uptake of these microelements. Soil application of ferrous sulphate increased Fe content by 47% in wheat grain when application was coupled with recommended dose of NPK (Aciksoz *et al*., 2011). However, grain Fe density remained lower than Zn in spite of applying at equal rates in both experiments, might be attributed to poor Fe phloem mobility from leaves to grain (Marschner, 2012). It was observed by Singh *et al*. (2018) that soil and foliar application of zinc and iron with recommended N supply enhanced wheat grain Zn contents by 46% and of Fe by 35%.

Phytate, protein, estimated human bioavailability of Zn and Fe in grain. Phytate concentration in wheat grain was not affected significantly by any of the treatments (Fig. 1). It ranged from 7.60-9.40 mg/g under different fertilizer treatments.

Phytate ($C_6H_{18}O_{24}P_6$), an abundantly found compound in wheat grain governing phosphorus provision to young seedling, is also believed to be an anti-nutrient because of its chelation with Fe and Zn. It restricts their absorption into human intestine. The phytate contents in wheat grain remained unaffected by Zn, Fe fertilization. The results are in consistence with (Zhang *et al*., 2012) reporting non-significant effect of soil applied $ZnSO_4 \cdot 7H_2O$ @ 50 Kg/ha on total phosphorus and phytate contents in wheat seeds.

All the Zn containing fertilizer treatments significantly reduced [phytate]:[Zn] molar ratio (Fig. 2). Minimum [phytate]:[Zn] molar ratio of 21 in wheat grain was attained by Zn@ 7.5 mg/Kg.

Trivariate model based estimated Zn bioavailability in wheat grain was significantly affected by different fertilizer treatments (Fig. 3). The range of estimated zinc bioavailability was 138-2.31 mg/300 g. It was enhanced by 67.4 and 46% over control (1.38 mg/300 g) with Zn @ 7.5 mg/Kg and Zn+Fe@ 5.0+5.0 mg/Kg, respectively.

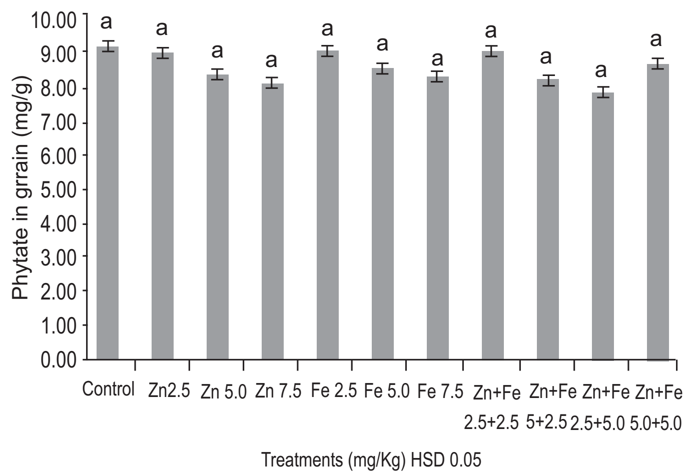

Fig. 1. Effect of fertilization on phytate content in wheat grain.

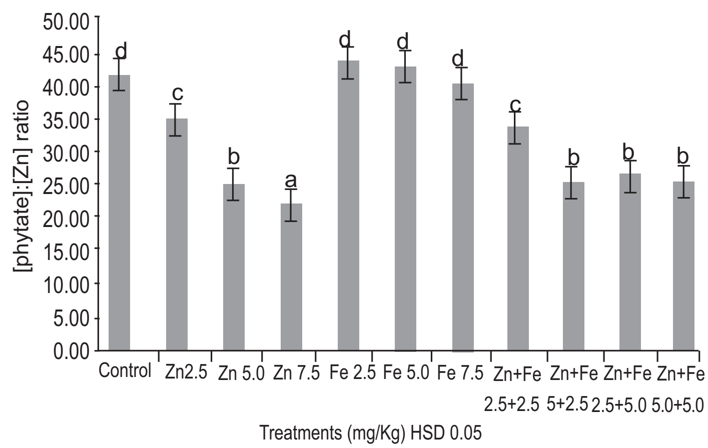

Fig. 2. Effect of fertilization on [Phytate]:[Zn] molar ratio in wheat grain.

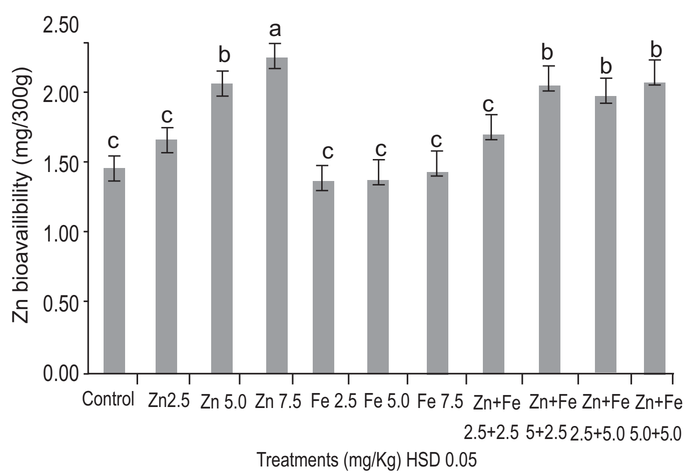

Fig. 3. Effect of fertilization on estimated human Zn absorption in wheat grain.

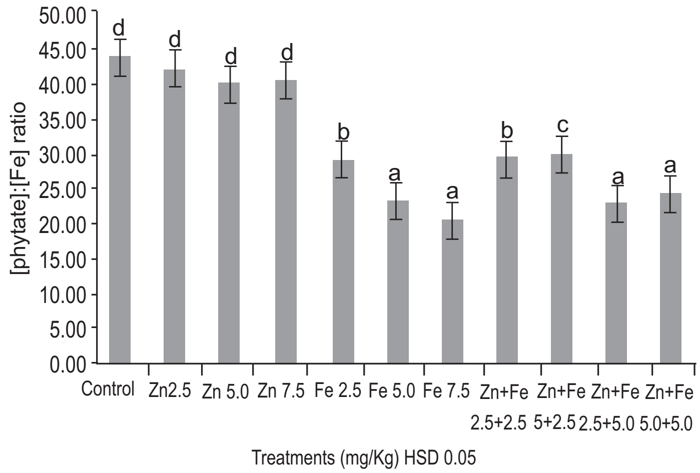

Fig. 4. Effect of fertilization on [Phytate]:[Fe] molar ratio in wheat grain.

Currently phytate. Micronutrients molar ratios are taken into consideration in estimating potential bioavailability of the micronutrients. Generally, mineral bioavailability is maximized at lower molar ratios and vice versa. It was found that the increased rate of Zn fertilization had significant upshot in decreasing the [phytate]:[Zn] molar ratio to bring it at required level that will in turn increase bioavailability of Zn in human. The enhanced Zn grain concentration lowered [phytate]:[Zn], increasing Zn bioavailability. (Hussain *et al*., 2012) reported similar results, giving a range of [phytate]:[Zn] 24-42 in wheat grain by fertilization. Similar result was also reported by (Tavajjoh *et al*., 2011) in durum wheat, finding [phytate]:[Zn] 26.5. The estimated human zinc absorption tabulated using trivariate zinc absorption model was enhanced by zinc fertilizer application alone and in combination with iron ranging from 1.38 (control) to 2.31 mg/300 g. It is ascertained that daily physiological Zn-absorption requirement of an adult human is 3 mg/d (Institute of Medicine, 2001), while this study depicted that consumption of 300 g wheat grain can ensure about 77% of it on daily basis. Thus improved grain Zn concentration through fertilization enhanced Zn bioavailability.

The [phytate]:[Fe] molar ratio significantly influenced by different treatments (Fig. 4). It was reduced from 46 (control) to 24 and 20 with Fe@5.0 mg/Kg and 7.5 mg/Kg, respectively.

Iron bioavailability may be estimated by determining [phytate]:[Fe] molar ratio and the ratio >1 considered lower Fe bioavailability (Halberg *et al*., 1989). Under pot trials, the treatments comprising Fe fertilization alone and in coupled with Zn significantly lowered [phytate]:[Fe] molar ratio thus enhancing Fe bioavailability. The results are in coincidence with (Salunke *et al*., 2014) reporting decreased [phytate]:[Fe] molar ratio by fertilization in bread wheat ranging from 15.5-31.3.

Protein content in grain were significantly improved with different treatments (Fig. 5). Maximum increase was observed with Zn+Fe@ 5.0+2.5 mg/Kg followed by Zn+Fe@ 5.0+5.0 mg/Kg. It was found that treatments in combination affected it more strongly than other ones.

Protein content in grain influenced more profoundly by combined treatments of Zn, Fe. The improved grain quality may be attributed to microelements role in sustained crop physiological growth and enhanced plant enzymes activity. Micronutrients and their interactions influence the physiological activities going on in plants, having significant impact on grain yield and quality (Wang *et al*., 2015). The findings of current study partially reflect, reported by Singh *et al*. (2018) in an experiment carried out in Turkey that treatments in combination of Zn and Fe performed more efficiently than its sole application.

Conclusion

Zinc and iron salts application in aerobic wheat through soil significantly enhanced grain Zn, Fe density, hence, improving nutritional quality of wheat. Fertilization strategy also exerted positive impact on growth and yield attribute, estimated human bioavailability of Zn, Fe and protein content in wheat grain. It may be concluded that biofortification of wheat with Zn and Fe through soil applied fertilizers is helpful in reducing micronutrient malnutrition in human.

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Conflict of Interest. The authors declare no conflict of interest.

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