The Pharma Innovation Journal 2023; 12(1): 308-315

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(1): 308-315

www.thepharmajournal.com
Received: 09-10-2022
Accepted: 12-11-2022

Srishti Nisha
Department of Soil Science and Agricultural Chemistry, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Uma Shanker Tiwari
Department of Soil Science and Agricultural Chemistry, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Rajendra Krishna Pathak
Department of Soil Science and Agricultural Chemistry, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Corresponding Author:
Srishti Nisha
Department of Soil Science and Agricultural Chemistry, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Biofortification effect of FYM, zinc and iron on yield and quality of wheat (*Triticum aestivum* L.)

Srishti Nisha, Uma Shanker Tiwari and Rajendra Krishna Pathak

DOI: https://doi.org/10.22271/tpi.2023.v12.i1d.17998

Abstract
The present study investigated the biofortification effect of FYM, Zinc and Iron on yield (grain and straw q h^{-1}), of wheat at harvest. Soil application of Zinc and Two foliar applications of Iron (one at heading and the other at milking stage) along with 10 t FYM h-1 gave highest grain yield (53.95 and 56.95 q h^{-1}) and straw yield (71.86 and 75.18 q h^{-1}) for two consecutive years while the highest protein content (12.50 and 12.63%). Yield (grain and straw) significantly increased over control as a result of the combination of the method applied. The protein content increased non significantly over the control. Superior yield of wheat was achieved with the soil application of zinc and two foliar applications of 0.5% Ferrous sulphate along with FYM as compared to sole application of zinc, iron and FYM.

Keywords: Biofortification, malnutrition, quality, zinc, iron, FYM

1. Introduction
Wheat (*Triticum aestivum* L.) is staple and second most important crop after rice in country which contributes nearly one third of total production. The major challenge for the two to three decades of 21st century is food and nutrition security for all specially the families living below poverty line. Wheat contains more nutritive value than other cereals. It has a relatively high content of niacin and thiamine, which is required for human being.The wheat (*Triticum aestivum* L.) is grown all over India. The common bread occupies more than 90per cent of the total wheat area along with 10per cent area under durum cultivation. Considering the importance of wheat in providing people's energy and protein requirements (about 50 percent), it seems that quantitative and qualitative improvement of wheat protein is necessary to reduce the negative effects of malnutrition arising from low protein intake especially among low income and vulnerable segments of society.

1.1 Biofortification
Biofortification is the process of increasing the content and/or bioavailability of essential nutrients in crops during plant growth through genetic and agronomic pathways (Bouis *et al*., 2011). Genetic biofortification involves either genetic engineering or classical breeding (Saltzman *et al*., 2013) [2]. Agronomic biofortification is achieved through micronutrient fertilizer application to the soil and/or foliar application directly to the leaves of the crop. Biofortification is mainly focused on starchy staple crops (rice, wheat, maize, sorghum, millet, sweet potato and legumes), because they dominate diets worldwide – especially among groups vulnerable to micronutrient deficiencies - and provide a feasible means of reaching malnourished populations with limited access to diverse diets, supplements, and commercially fortified foods (Saltzman *et al*., 2013) [2].

1.2 Hidden Hunger
Hidden hunger or micronutrient deficiency retards the growth and development of both crops and humans. Soil micronutrient deficiencies limit crop productivity and nutritional quality of foods, which together affect nutrition and human health (Sanchez and Swaminathan, 2005) [3]. Insufficient micronutrient availability in soils in these regions not only causes low crop productivity, but also poor nutritional quality of the crops and consequently contributes to malnutrition in the human population (Nubé and Voortman, 2011, Hurst *et al*., 2013, Kumssa *et al*., 2015) [4, 5]. Such diets are poor in micronutrients (minerals and vitamins) and consequently micronutrient deficiencies are widespread (FAO, 2015) [7]. The chronic lack of micronutrients can cause severe but often invisible health problems, especially among

women and young children (Black *et al*., 2013) [8]: hence 'hidden hunger'. Worldwide over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies (WHO, 2016, Black, 2003) [10]. The problem is most severe in low- and middle income countries.

1.3 Zinc

Malnutrition arising from micronutrients deficiency such as Zn is considered as one of the present problems of humane societies especially in developing countries like India. Some of the side effects of Zn deficiency in humane societies are stunting in children, high susceptibility to infectious, impaired mental, increased morbidity and mortality (Cunningham-Rundles vd. 2005) [11]. It is estimated that one third of the World's population especially children are suffering from Zn deficiency (WHO 2002) [12] and annually, nearly 450 thousand children below 5 years old die due to the effect of Zn deficiency (Bryce vd. 2005). In Asia, wheat provides about 50 percent of people's daily energy requirements which reaches over 70 percent in rural areas (Cakmak vd. 2004) [14]. Regretfully, this plant naturally has little Zn content and it will be even less if planted in Zn poor soils (Cakmak vd. 2004) [14]. Improvement of Zn content in wheat grain by bifortification is a proper approach for providing Zn requirements of body and reducing the malnutrition (Cakmak vd. 2010; Abdoli vd. 2014; Abdoli vd. 2016; Saha vd. 2017) [15, 17, 18, 19]. Biofortification can be carried out by agronomic and genetic biofortification methods (Sadeghzadeh 2013) [20]. In agronomic method, the amount of plant's Zn requirement is provided Zn application such as soil or foliar application. Providing Zn requirements of plant improves metabolic processes like protein biosynthesis and carbohydrates (Bharti vd. 2013; Nawaz vd. 2015) [21, 28]. For example, Ozturk vd. (2006) [29] and Yang vd. (2011) [30] stated that Zn spraying at grain filling stage had the most effect on increasing Zn content in grain. Furthermore, due to the effect of Zn use in wheat and rice, increase of Zn content in grain, (Mabesa vd. 2013; Imran vd. 2015) [31, 32].

Nowadays, the role of Zn in protein biosynthesis has been proved (Passerini vd. 2007; Shu vd. 2008) [33, 34]. In this regard, increase of free amino acids content in Zn deficiency and decrease of protein biosynthesis content has been reported by Cakmak vd. (1989). Increase of protein due to Zn application has been reported by Bybordi and Malakouti (2007) [35] and Pourgholam vd. (2013) [36]. Zn acts as a cofactor in the structure of RNA polymerase. Furthermore, Zn is from the structural components of ribosome and in its absence, their function and protein biosynthesis is decreased.

Besides the quantitative yield of protein, its quality or its biological value is also important. Some of the factors influencing biological value of wheat protein are amino acids balance and essential amino acids content (Šramková vd. 2009) [37]. Wheat protein has limitation on the amount of Lysine and Threonine amino acids. In order to improve the quality of cereal grain protein, a lot of attempts have been made by reformers to improve the quality of wheat protein and increase Lysine and Threonine amino acids content such as discovering corn and barely with high Lysine and introducing them as a specific genotype. Unfortunately, along with these positive changes, negative characteristics happen like yield reduction and/or sensitivity to pests or disease. Zn foliar application was able to increase essential amino acids content in wheat protein. Moreover, Mishra and Abidi (2010) and Bharti vd. (2013) [21] have reported an increase in Methionine content due to the effect of Zn application in agricultural plants.

1.4 Iron

For all living organism, Iron (Fe) is an essential element as it catalyzes oxidation/reduction reactions. Fe deficiency is one of the most widespread and most severe nutrient deficiencies intimidating human health and affecting approximately two billion people in the world [De Benoist, B., McLean, E., Egli, I. and Cogswell, M., 2008 [22]. Worldwide prevalence of anaemia 1993-2005. ISBN: 978 92 4 159665 7]. Fe deficiency causes various physiological diseases, such as anaemia and neurodegenerative disorders [Sheftela, A.D., Mason, A.B. and Ponka, P.]. A recent report based on the WHO Database described that nearly 1.6 billion people in the world are effected by anemia in which pregnant women and pre-school children are under a greater risk [McLeon E, Cogswell M, Egli I, Wojdyla D, de Benoist B.]. Major health concerns regarding Fe deficiency are impairments of immune system, cognitive function and work capacity as well as maternal mortality and increase in infants [Hunt J R, Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, Lozoff B, Jacobson S W.]. Countries where people have low meat intake and mostly consume staple crops are more likely to be affected by Fe deficiency diseases. Population including young children, pregnant and postpartum are observed to be more severely affected, as high Fe is needed for infant growth and during pregnancy [De Benoist, B., McLean, E., Egli, I. and Cogswell, M.,]. By choosing well balanced diet with bioavailable and sufficient Fe and by giving good attention to the composition of food, human health problem related to Fe can be prevented.

Data relative to Zn biofortification provides conclusive evidence in favor of the soil and foliar applications of Zn fertilizers. These fertilizers play an effective role in improvement of gain concentration of Zn [Peck AW, McDonald GK, Graham RD,Yilmaz A, Ekiz H, Torun B, Gültekin I, Karanlik S, Bagci SA, Cakmak, Zhang Y, Shi R, Md. Rezaul K, Zhang F, Zou C. Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, Arisoy Z, Erdem H, Gokmen O, Ozturk L, Horst W J.]. On the other hand, Fe fertilizers are not exploited to examine their role for improving Fe concentration in cereal gains. All attempts to understand the soil and foliar application of Fe fertilizers are aimed at restoration of Fe levels, improvement of the yield and reversion of Fe deficiency chlorosis. [Tagliavini M, Abadia J, Rombola AD, Abadia A, Tsipouridis C, Marangoni B.] the increase in grain Fe concentration through foliar spray of $FeSO_4$ or Fe chelates has not been recorded to exceed 36% [De Benoist, B., McLean, E., Egli, I. and Cogswell, M.,] whilst the foliar application increases grain Zn concentration to a recorded concentration of 2- or 3-fold depending on the plant availability of Zn in soils [Cakmak I.].

Some independent studies have also showed that plants exhibit a lack of response to Fe fertilization in terms of grain Fe concentration. In more recent studies, it has been exhibited that the N status of plant plays a significant role in enrichment of ceral grains with Fe. This leads to the creation of a positive correlation between grain Fe and N levels [Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T].. Studies employing the greenhouse and field conditions prove that shoot and grain Fe concentrations can be increased through increment in soil N application [Kutman U B, Yildiz B, Ozturk L, Cakmak I.]. Moreover, grain Fe may also be enhanced through foliar sprat of urea [Kutman U B, Yildiz B, Ozturk L, Cakmak I.].

1.5 Organic matter

Organic matter releases organic acids, which that serve as chelators and increase the metal (Fe in this case) availability to plants by solubilizing of nutrients in soil solution (Du Laing *et al*., 2009; McCauley *et al*., 2009) [39, 40]. Poultry manure has high nutrient composition and nitrogen contents, high nitrogen improves Fe concentration in wheat plant by increasing Fe activity and abundance of Fe transporter proteins such as yellow stripe 1 (YS1) in root cell membrane (Bujoczek *et al*., 2000; Curie *et al*., 2009). Biochar (BC) affects soil fertility by changing soil physico-chemical and biological properties leading to nutrient mobilization (Xu *et al*., 2013; Jeffery *et al*., 2015) [41, 42]. In redox mediated reaction, BC affects mineral forms of Fe by acting as an electron shuttle (Kappler *et al*., 2014) [43]. Biochar solubilizes Fe by decreasing soil pH due to its redox catalytic activity (Graber *et al*., 2014) [44]. In addition, soil properties change by BC application may increase nutrient mobilization and uptake in the rhizosphere through increase in the exploratory capacity of the root system and modification of nutrient solubility (Lehmann *et al*., 2011) [45].

2. Materials and Methods

2.1 Site and experimental description

A field experiment on "Biofortification of wheat (*Triticum aestivum* L.) with zinc, iron and organic manure in relation to productivity, quality and soil health in Central U.P. " was conducted at student's Instructional farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during *Rabi* season of the year 2020 and 2021. The soil of the experimental field was sandy loam in texture and alkaline in nature having initial values pH 8.10, E.C 0.36 dsm^{-1}, low in organic carbon 0.427%, available Nitrogen 192.15 kg ha^{-1}, medium in available phosphorus 12.28 kg ha^{-1} and available potassium 162.80 kg ha^{-1} and deficient in available zinc 0.530 ppm, available iron 3.96 ppm.

2.2 Experimental treatment and design

The experiment was laid out in factorial randomized block design and replicated thrice. There are three factors comprising different methods of application of nutrients. For factor -1 two methods of application (No FYM and 10 tonnes of FYM ha^{-1}), for factor 2 four methods of application (No Zn, soil application of 15 kg ha^{-1} Zinc, one foliar application of 0.5% Zinc sulphate at heading stage and two foliar applications of 0.5% Zinc sulphate at heading and milking stage), factor 3 four methods of application ((No Fe, soil application of 10 kg ha^{-1} Iron, one foliar application of 0.5% Ferrous sulphate at heading stage and two foliar applications of 0.5% Ferrous sulphate at heading and milking stage) comprising 32 treatment combinations:. M0 Zn0 Fe0 (Control), M_0 Zn_0 Fe_1 (One foliar application of 0.5% Ferrous Sulphate), M_0 Zn_0 Fe_2 (Two foliar applications of 0.5% Ferrous Sulphate), M_0 Zn_0 Fe_3 (Soil application of 20 kg Fe), M_0 Zn_1 Fe_0 (One foliar application of 0.5% Zn), M_0 Zn_1 Fe_1 (One foliar application of 0.5% Zn + One foliar application of 0.5% Ferrous Sulphate), M_0 Zn_1 Fe_2 (One foliar application of 0.5% Zn + Two foliar applications of 0.5% Ferrous Sulphate), M_0 Zn_1 Fe_3 (One foliar application of 0.5% Zn + Soil application of 20 kg Zn), M_0 Zn_2 Fe_0 (Two foliar applications of 0.5% Zn ha^{-1}), M_0 Zn_2 Fe_1 (Two foliar applications of 0.5% Zn + One foliar application of 0.5% Ferrous Sulphate), M_0 Zn_2 Fe_2 (Two foliar applications of 0.5% Fe + Two foliar applications of 0.5% Ferrous Sulphate), M_0 Zn_2 Fe_3 (Two foliar applications of 0.5% Fe + Soil application of 20 kg Zn), M_0 Zn_3 Fe_0 (Soil application of 15 kg Zn ha^{-1}), M_0 Zn_3 Fe_1 (Soil application of 15 kg Zn + One foliar application of 0.5% Ferrous Sulphate), M_0 Zn_3 Fe_2 (Soil application of 15 kg Zn + Two foliar applications of 0.5% Ferrous Sulphate), M_0 Zn_3 Fe_3 (Soil application of 15 kg Zn + Soil application of 20 kg Zn), M_1 Zn_0 Fe_0 (10 tonnes of FYM), M_1 Zn_0 Fe_1(10 tonnes of FYM + One foliar application of 0.5% Fe ha^{-1}), M_1 Zn_0 Fe_2 (10 tonnes of FYM + Soil application of 20 kg Zn), M_1 Zn_0 Fe_3 (10 tonnes of FYM + Soil application of 20 kg Zn ha^{-1}), M_1 Zn_1 Fe_0 (10 tonnes of FYM + One foliar application of 0.5% Zn), M_1 Zn_1 Fe_1(10 tonnes of FYM + One foliar application of 0.5% Zinc Sulphate + One foliar application of 0.5% Ferrous Sulphate), M_1 Zn_1 Fe_2 (10 tonnes of FYM + One foliar application of 0.5% Ferrous Sulphate + Two foliar applications of 0.5% Ferrous Sulphate), M_1 Zn_1 Fe_3 (10 tonnes of FYM + One foliar application of 0.5% Ferrous Sulphate + Soil application of 20 kg Fe ha^{-1}), M_1 Zn_2 Fe_0 (10 tonnes of FYM + Two foliar applications of 0.5% Zn ha^{-1}), M_1 Zn_2 Fe_1 (10 tonnes of FYM + Two foliar applications of 0.5% Zn + One foliar application of 0.5% Ferrous Sulphate), M_1 Zn_2 Fe_2 (10 tonnes of FYM + Two foliar applications of 0.5% Zinc sulphate + Two foliar applications of 0.5% Ferrous Sulphate), M_1 Zn_2 Fe_3 (10 tonnes of FYM + Two foliar applications of 0.5% Zn + Soil application of 20 kg Fe ha^{-1}), M_1 Zn_3 Fe_0 (10 tonnes of FYM + Soil application of 15 kg Zn ha^{-1}), M_1 Zn_3 Fe_1 (10 tonnes of FYM + Soil application of 15 kg Zn + One foliar application of 0.5% Ferrous Sulphate), M_1 Zn_3 Fe_2 (10 tonnes of FYM + Soil application of 15 kg Zn + Two foliar applications of 0.5% Ferrous Sulphate), M_1 Zn_3 Fe_3 (10 tonnes of FYM + Soil application of 15 kg Zn + Soil application of 20 kg Zn) in factorial RBD with 3 replications on same location

2.3 Crop husbandry

Field preparation was started after harvesting of the previous crop with an object for optimum moisture condition. The experimental field was ploughed once with soil turning plough followed by two cross harrowing. After each operation, planking was done to level the field and to obtain the fine tilth. Finally layout was done and plots were demarcated with small sticks and rope with the help of manual labour in each block. The recommended dose of nutrient i.e. N, P, and K was applied @ 150: 75 : 60 kg h-1 respectively. Half does of Nitrogen and full doses of phosphorus, potash, zinc and Iron were applied as basal dressing {Nitrogen is applied through Urea (contain 46% N), phosphorus is applied through Di-ammonium phosphate (contain 46% P2O5 & 18% N), Potassium is applied through Muriate of Potash (contain 60% K), zinc is applied through zinc sulphate (contain 21% Zn and 14.5% sulphur) and iron is applied through Ferrous sulphate (containing 19% Fe & 10.5% sulphur)}. Then 10 FYM ha^{-1}was added in the field as a source of organic manure on the plots which were supposed to be treated with organic manure. Remaining dose of nitrogen was applied through two split doses of top dressing at stage. Gross plot size of treated plots is 3.0 metre ×4.0

metre in both the years. Wheat variety was sown using HD-2967 on 26th Nov 2020 during 1st year and 1st dec 2021 during 2nd year of the studies. The crop was sown with a seed rate using 100 kg h-1 with the planting distance of 20 cm× 10 cm (Row×Plant), seeds were sown about 5-6 cm depth. To keep the weeds under check, two weeding were done at 25 and 45 days after sowing (DAS) with the help of khurpi (manually). Five irrigations along with three seasonal rains were provided to the crop.

2.4 Yield

With the help of the labours, the crop was harvested at maturity on 13 April 2021 and 18 April 2022 and bundles of each pot were threshold and store separately. Harvested materials of each pot were threshold and stored separately.
The procedure of each pot was collected and the yield of per pot is converted into Q ha^{-1}.

2.5 Quality parameters of wheat

2.5.1 Protein content

In the wheat grain total nitrogen content was estimated by Kjeldahl's method and the value obtained for each sample was multiplied with the factor 6.25 to obtain protein content.
Protein Content (%) =Nitrogen content (%) × 6.25

3. Results and discussion

3.1 Biofortification effect of Zn, Fe and organic manure on grain and straw yield of wheat

Biofortification effect of organic manure, zinc and iron on grain and straw yield of wheat depicted in table 1 revealed that addition of organic manure influenced grain and straw yield significantly over no organic treatment. Highest grain yield 49.11 q ha^{-1} and 51.26 q ha^{-1} and straw yield 66.23 and 68.62 q ha^{-1}was recorded with the application of 10 tonnes of FYM ha^{-1} which was found 8.4 % & 11.9 % higher in grain and 6.8 & 10.2 % higher in straw over no organic treatment during 1st year and 2nd year.

Table 1: Biofortification effect of organic manure, zinc and iron on grain yield (q ha-1) of wheat

		1st year					**2nd year**				
		Zn_0	**Zn_1**	**Zn_2**	**Zn_3**	**Mean**	**Zn_0**	**Zn_1**	**Zn_2**	**Zn_3**	**Mean**
M_0	Fe_0	33.25	35.24	36.57	36.4	35.36	33.27	35.36	36.73	36.4	35.44
	Fe_1	35.57	37.7	39.12	38.94	37.83	35.62	37.89	39.67	39.31	38.14
	Fe_2	37.24	39.47	40.96	40.77	39.61	37.39	40.00	41.87	41.5	40.19
	Fe_3	36.9	39.11	40.59	40.4	39.25	37.06	39.65	41.5	41.13	39.83
	Mean	35.74	37.88	39.31	39.12	38.01	35.83	38.22	39.94	39.58	38.39
M_1	Fe_0	35.24	38.06	40.18	39.83	38.32	35.42	38.96	41.44	41.08	39.22
	Fe_1	38.77	41.87	44.19	43.81	42.16	39.31	43.24	45.99	45.59	43.53
	Fe_2	41.59	44.92	47.41	46.99	45.22	42.5	46.75	49.72	49.3	47.06
	Fe_3	41.24	44.53	47.01	46.6	44.84	42.14	46.35	49.3	48.88	46.66
	Mean	39.21	42.34	44.69	44.3	42.64	39.22	43.53	47.06	46.66	44.12

Mean of the mean Zn	Zn_0	Zn_1	Zn_2	Zn_3		Zn_0	Zn_1	Zn_2	Zn_3
	43.72	46.52	48.16	50.46		44.87	48.02	50.06	51.22

Mean of the mean Fe	Fe_0	Fe_1	Fe_2	Fe_3		Fe_0	Fe_1	Fe_2	Fe_3
	44.74	46.98	48.79	48.34		45.46	48.20	50.49	50.03

	1st year		**2nd year**	
Factors	**SEm ±**	**C.D at 5%**	**SEm±**	**C.D at 5 %**
Organic manure (O.M)	0.239	0.675	0.268	0.759
Zinc	0.338	0.955	0.379	1.073
Iron	0.338	0.955	0.379	1.073
(O.M) X Zinc	0.478	NS	0.537	NS
(O.M) X Iron	0.478	NS	0.537	NS
Zinc X Iron	0.676	NS	0.759	NS
(O.M) X Zinc X Iron	0.956	2.702	1.073	NS

Increase in grain and straw yield with the addition of organic manure may be due to the fact that organic sources of nutrients have the positive effect on yield attributes and cumulative effect of yield attributes mainly responsible for higher productivity with application of organic sources. The increase in grain and straw yield of wheat due to application of FYM might be attributed mainly due to higher content of available nutrients in FYM presence of beneficial microflora such as N-fixer, P-solubilizer, VAM, fungi and higher activity of dehydrogenase enzyme in soil.

3.2 Biofortification effect of Zn on grain and straw yield of wheat:

The different methods of application of zinc also influenced grain and straw yield of wheat significantly during both the years. The maximum yield was recorded with soil application of 15 kg ha^{-1} of Zinc, followed by two foliar applications of 0.5% Ferrous sulphate at heading and milking stage significantly during both the years. Soil application of Zinc ha^{-1} proved its superiority over other methods of application of zinc by producing the highest grain yield 50.46 & 51.22 q ha^{-1} and straw yield 67.80 and 68.29 q ha^{-1} which was found 15.4 & 14.1 % higher in grain and 12.8 % & 11.6 % higher in straw over no zinc application during 1st year and 2nd year respectively.
The favourable influence of applied zinc on yield may be due to its catalytic or stimulatory effect on most of the

physiological and metabolic processes of plants which ultimately led to recognition of higher production of individual plants. The increased availability of zinc and photosynthate might have enhanced yield of wheat.

3.3 Biofortification effect of Fe on grain and straw yield of wheat

Likewise zinc, effect of iron application was also observed on grain and straw yield of wheat during both the years. Grain and straw yield of wheat was influenced significantly with application of distinct methods of application of iron during both the years. It was also observed that the two foliar applications of 0.5% Ferrous Sulphate produced grain yield 48.79 & 50.49 q ha^{-1} and straw yield 66.17 & 67.96 q ha^{-1} which was 9.1 % and 11.1 % higher in grain and 8.6 % & 10.5 % higher in straw over control (Fe_0) during 1st year and 2nd year, respectively.

Enhancement in grain and straw yield of wheat due to the application of iron might be due to increased crop growth and development which dry matter accumulation and yield attributes of plant under better nutritional environment.

Table 2: Biofortification effect of organic manure, zinc and iron on straw yield (q ha^{-1}) of wheat

		1st year					2nd year				
		Zn_0	Zn_1	Zn_2	Zn_3	Mean	Zn_0	Zn_1	Zn_2	Zn_3	Mean
M_0	Fe_0	51.53	54.54	56.53	56.12	54.68	51.57	54.74	56.6	55.94	54.71
	Fe_1	54.77	57.9	60.01	59.6	58.07	54.66	58	60.65	59.83	58.28
	Fe_2	56.6	59.91	62.05	61.6	60.04	56.64	60.52	63.22	62.49	60.71
	Fe_3	56.38	59.68	61.76	61.32	59.78	56.44	60.3	62.95	62.22	60.47
	Mean	54.82	58	60.08	59.66	58.14	54.83	58.39	60.85	60.12	58.54
M_1	Fe_0	54.26	58.53	61.71	61	58.87	54.36	59.72	63.44	62.72	60.06
	Fe_1	59.31	63.89	67.34	66.63	64.29	59.94	65.76	69.85	69.11	66.16
	Fe_2	62.8	67.73	72.07	70.53	68.28	63.96	70.26	74.29	73.75	70.56
	Fe_3	62.6	67.5	71.35	70.27	67.93	63.75	70.03	72.58	73.46	69.95
	Mean	59.74	64.41	67.86	67.1	64.77	60.5	66.44	70.04	69.76	66.68

Mean of the mean Zn	Zn_0	Zn_1	Zn_2	Zn_3		Zn_0	Zn_1	Zn_2	Zn_3
	60.08	63.47	65.20	67.80		61.20	65.04	67.27	68.29

Mean of the mean Fe	Fe_0	Fe_1	Fe_2	Fe_3		Fe_0	Fe_1	Fe_2	Fe_3
	60.94	63.90	66.17	65.64		61.46	65.06	67.96	67.43

	1st year		2nd year	
Factors	SEm±	C.D at 5%	SEm±	C.D at 5 %
Organic manure (O.M)	0.296	0.837	0.279	0.558
Zinc	0.419	1.184	0.395	0.789
Iron	0.419	1.184	0.395	0.789
(O.M) X Zinc	0.592	NS	0.558	1.116
(O.M) X Iron	0.592	1.675	0.558	1.116
Zinc X Iron	0.838	NS	0.789	NS
(O.M) X Zinc X Iron	1.185	NS	1.116	NS

3.4 Combined effect of FYM, zinc and iron on yield of wheat

Maximum yield 53.95 and 56.95 q ha^{-1} and 71.86 and 75.18 q ha^{-1} of grain and straw was obtained with the addition of 10 tonnes of FYM + soil application of 15 kg zinc + two foliar applications of 0.5 % Ferrous Sulphate ha-1 which was found as 25.39 % and 28.71% higher in grain and 28.53 % & 34.39 % straw yield over control during 1st year and 2nd year, respectively.

Impact on grain and straw yield with the combined application of organic manure, zinc and iron might be due to the fact that the soil of the experimental field was deficient in available zinc and iron and its application with FYM improves its availability in soil. Which have enhanced the growth and yield attributes and finally contributed to result in elevated grain and straw yield of wheat.

3.5 Biofortification effect of organic manure on protein content of wheat

Biofortification effect of organic manure, zinc and iron on quality that is protein content in grain of wheat revealed that quality parameter i.e protein content increased significantly in grain with the application of organic manure, zinc and iron during experimentations in both the years. protein content in grain influenced non significantly with the application of organic manure during both the years.

Highest protein content 11.93 and 12.05 per cent was recorded with the application of 10 tonnes of FYM ha^{-1} and minimum 11.67 and 11.84 per cent in control (no organic) during 1st year and 2nd year, respectively.

Addition of 10 tonnes of FYM ha^{-1} showed higher influence in protein content over control during both the years.

3.6 Biofortification effect of Zn, on protein content of wheat

The different methods of application of zinc boost the protein content in grain although the increase in grain protein content with the different methods of application of zinc was found non significant during both the years. Maximum protein content 12.17 and 12.30 per cent was noted with soil application of 15 kg Zinc ha^{-1} and minimum 11.41 and 11.61 per cent at control (no zinc) during 1st year and 2nd year, respectively.

Table 3: Biofortification effect of organic manure, zinc and iron on protein content (%) in grain of wheat

		1st year					2nd year				
		Fe0	**Fe1**	**Fe2**	**Fe3**	**Mean**	**Fe0**	**Fe1**	**Fe2**	**Fe3**	**Mean**
M0	Zn0	11.00	11.25	11.50	11.38	11.28	11.13	12.00	11.63	11.50	11.56
	Zn1	11.38	11.50	11.75	11.63	11.56	11.50	11.63	11.88	11.75	11.69
	Zn2	11.63	11.75	12.00	11.88	11.81	11.75	11.88	12.13	12.00	11.94
	Zn3	11.75	12.00	12.25	12.13	12.03	11.88	12.13	12.38	12.25	12.16
	Mean	11.44	11.63	11.88	11.75	11.67	11.56	11.91	12.00	11.88	11.84
M1	Zn0	11.25	11.50	11.75	11.63	11.53	11.38	11.63	11.88	11.75	11.66
	Zn1	11.63	11.75	12.00	11.88	11.81	11.75	11.88	12.13	12.00	11.94
	Zn2	11.88	12.00	12.25	12.13	12.06	12.00	12.13	12.38	12.25	12.19
	Zn3	12.13	12.25	12.50	12.38	12.31	12.25	12.38	12.63	12.50	12.44
	Mean	11.72	11.88	12.13	12.00	11.93	11.84	12.00	12.25	12.13	12.05

Mean of the mean Zn	Zn_0	Zn_1	Zn_2	Zn_3		Zn_0	Zn_1	Zn_2	Zn_3
	11.41	11.69	11.94	12.17		11.61	11.81	12.06	12.30

Mean of the mean Fe	Fe_0	Fe_1	Fe_2	Fe_3		Fe_0	Fe_1	Fe_2	Fe_3
	11.58	11.75	12.00	11.88		11.70	11.95	12.13	12.00

	1st year		2nd year	
Factors	**SEm±**	**C.D at 5%**	**SEm±**	**C.D at 5 %**
Organic manure (O.M)	0.421	NS	0.387	NS
Zinc	0.595	NS	0.547	NS
Iron	0.595	NS	0.547	NS
(O.M) X Zinc	0.841	NS	0.774	NS
(O.M) X Iron	0.841	NS	0.774	NS
Zinc X Iron	1.190	NS	1.094	NS
(O.M) X Zinc X Iron	1.682	NS	1.548	NS

3.7 Biofortification effect of Fe on protein content of wheat

A critical observation of the data given in table 4.35 and figure 4.35 showed that likewise zinc protein content in wheat was also accelerated non significantly with the application of different methods of iron during both the years. Maximum increase in iron content was recorded with the two foliar application of 0.5 per cent iron at heading and milking stage was observed that is 12.00 and 12.13 per cent and minimum 11.58 and 11.70 per cent at its lowest at Fe_0 during 1st year and 2nd year, respectively.

3.8 Biofortification Effect of interactions on protein content (per cent) in wheat.

Interaction sequel of organic, zinc and iron on protein content in grain furnished in table 4.35 and figure 4.35 revealed similar nonsignificant accelerating effects on protein content to alone application of organic manure, methods of application of zinc and methods of application of iron during both the years. Maximum protein content 12.50 and 12.63 per cent was recorded with the application of 10 tonnes of FYM + soil application of 15 kg Zinc + two foliar applications of 0.5 per cent Fe ha^{-1} at heading and milking stage and minimum 11.00 and 11.13 per cent at control ($M_0Zn_0Fe_0$) during 1st year and 2nd year, respectively. Protein content in wheat grain was recorded higher in all the combinations during 2nd year than the 1st year.

4. Conclusion

Malnutrition among humans is due to micronutrients deficiency due to insufficient zinc and iron dietary intake. The problem is considered prominent especially in vegetarians since they have limited food supplements as compared to non-vegetarian diet. Wheat plays a crucial role in vegetarian diet supply across the world. The result of the present study showed that the different methods of application of zinc and iron with organic manure and 100% RDN produced higher grain and straw yield of wheat in comparison to no organic treatments during both the years. Maximum grain and straw yield of wheat was recorded with the 10 tonnes of FYM + soil application of 15 kg Zinc ha^{-1} + two foliar applications of 0.5% Ferrous Sulphate at heading and milking stage application ha^{-1} along with 100% RDN and minimum at control ($M_0Zn_0Fe_0$) with 100% RDN during both the year. Maximum improvement in quality parameter viz. protein was identified with organic treatment in comparison to no organic treatments. Highest improvement in quality parameters was found with the addition of FYM (10 tonnes of ha^{-1}) + zinc (soil application of 15 kg Zinc ha^{-1}) + iron (two foliar applications of 0.5% Ferrous Sulphate at heading and milking stage). Thus, biofortification of wheat with the use of zinc and iron may help to overcome the micronutrients malnutrition and achieve sustainable yield.

5. References

1. Bouis HE, Welch RM. Biofortification- A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci. 2010;50:20-32.
2. Saltzman Amy, Biro Ekin. Biofortification: Progress toward a more nourishing future. Global Food Security. 2013;2(1):9-17.
3. Sanchez PA, Swaminathan MS. Cutting World Hunger in Half. Science. 2005;307(5708):357-359.
4. Nube M, Voortman RL. Human micronutrient deficiencies: Linkages with micronutrient deficiencies in soils, crops, and animal nutrition. In L. Amoroso, & B. Thompson (Eds.), Combating micronutrient deficiencies: Food-based approaches, 2011.
5. Hurst B, Wallace R, Nixon SB. The Impact of Social Interaction on Student Learning. Reading Horizons: A

Journal of Literacy and Language Arts. 2013;52(4):375-398.
6. Kumssa Diriba B, Edward JM Joy. Valuing increased zinc (Zn) fertiliser-use in Pakistan. Plant Soil. 2017;411:139-150. https://doi.org/10.1007/s11104-016-2961-7
7. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). c2015. BIOFORTIFICATION: An Agricultural Investment for Nutrition. https://glopan.org/sites/default/files/document-files/Biofortification_Policy_Brief_FINAL.pdf
8. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, *et al*., Maternal and child undernutrition and overweight in low-income and middle-income countries (and the Maternal and Child Nutrition Study Group) Lancet. 2013;382(9890):427-451.
9. WHO. Vitamin and Mineral Nutrition Information System, c2016. www.who.int
10. Black R. Micronutrient deficiency - an underlying cause of morbidity and mortality. Bulletin of the World Health Organisation. 2003;81(2):79-79.
11. Cunningham-Rundles C, Ponda PP. Molecular defects in T- and B-cell primary immunodeficiency diseases. Nat Rev Immunol. 2005;5(11):880-92.
12. WHO. The world health report. Reducing risks, promoting healthy life. World Health Organization, 1211 Geneva 27, Switzerland, 2002, p 230. http://www.who.int/whr/2002/en/
13. Bryce J, El Arifeen S, Pariyo G, Lanata CF, Gwatkin D, Habicht J, *et al*. Multi-Country valuation of IMCI Study Group. Reducing child mortality: can public health deliver? The Lancet. 2003;362:159-164.
14. Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, *et al*. Triticumdicoccoides: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Science and Plant Nutrition. 2004;50(7):1047–1054.
15. Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, *et al*. Biofortification and localization of zinc in wheat grain. J Agr Food Chem. 2010;58(16):9092-9102.
16. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil. 2008;302:1-17.
17. Abdoli M, Esfandiari E. Effect of zinc foliar application on the quantitative and qualitative yield and seedlings growth characteristics of bread wheat (cv. Kohdasht). Iranian Journal of Dryland Agriculture. 2014;3(1):77-90.
18. Abdoli M, Esfandiari E, Sadeghzadeh B, Mousavi SB. Zinc application methods affect agronomy traits and grain micronutrients in bread and durum wheat under zinc-deficient calcareous soil. Yuzuncu Yil University Journal of Agricultural Sciences. 2016;26(2):202-214.
19. Saha S, Chakraborty M, Sarkar D, Batabyal K, Mandal B, Murmu S, *et al*. Rescheduling zinc fertilization and cultivar choice improve zinc sequestration and its bioavailability in wheat grains and flour. Field Crops Research. 2017;200:10-17. https://doi.org/10.1016/j.fcr.2016.09.006
20. Sadeghzadeh B. A review of zinc nutrition and plant breeding. Journal of Soil Science and Plant Nutrition. 2013;13(4):905-927.
21. Bharti K, Pandey N, Shankhdhar D, Srivastava PC, Shankhdhar SC. Improving nutritional quality of wheat through soil and foliar zinc application. Plant, Soil and Environment. 2013;59(8):348-352.
22. De Benoist B, McLean E, Egli I, Cogswell M, Peck AW, McDonald GK, *et al*. Zinc nutrition influences the protein composition of flour in bread wheat (*Triticumae stivum* L.). Journal of Cereal Science. 2008;47(2):266-274.
23. Hunt JR. Dietary and physiological factors that affect the absorption and bioavailability of iron. International Journal for Vitamin and Nutrition Research. 2005;75(6)375-84.
24. Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, *et al*. Iron deficiency anemia and cognitive function in infancy. Pediatrics. 2010;126(2):427-434.
25. McLeon E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Public Health Nutrition. 2009;12(4):444–454.
26. Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, *et al*. Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiologia Plantarum. 2007;129(3):635–643.
27. Hunt JR. Dietary and physiological factors that affect the absorption and bioavailability of iron. International journal for vitamin and nutrition research. 2005;75(6):375-384.
28. Nawaz H, Hussain N, Yasmeen A, Arif M, Hussain M, Rehmani MIA, *et al*. Soil applied zinc ensures high production and net returns of divergent wheat cultivars. Journal of Environmental and Agricultural Sciences. 2015;2(1):1-7.
29. Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, *et al*. Concentration and localization of zinc during seed development and germination in wheat. Physiologia Plantarum. 2006;128(1):144-152.
30. Yang XW, Tian XH, Gale WJ, Cao YX, Lu XC, Zhao AQ *et al*. Effect of soil and foliar zinc application on zinc concentration and bioavailability in wheat grain grown potentially zinc deficient soil. Cereal Res. Com Cereal Research Communications. 2011;39:535–543.
31. Mabesa RL, Impa SM, Grewal D, Johnson-Beebout SE *et al*. Contrasting grain-Zn response of biofortification rice (*Oryza sativa* L.) breeding lines to foliar Zn application. Field Crops Research. 2013;149:223-233. https://doi.org/10.1016/j.fcr.2013.05.012
32. Imran M, Kanwal S, Hussain S, Aziz T, Aamer-Maqsood M. Efficacy of zinc application methods for concentration and estimated bioavailability of zinc in grains of rice grown on a calcareous soil. Pakistan Journal of Agricultural Sciences. 2015;52(1):169-175.
33. Passerini A, Andreini C, Menchetti S, Rosato A, Frasconi P. Predicting zinc binding at the proteome level. BMC Bioinformatics. 2007;8(1):1-13.
34. Shu N, Zhou T, Hovmöller S. Prediction of zinc-binding sites in proteins from sequence. BMC Bioinformatics. 2008;24(6):775-782.
35. Bybordi A, Malakouti MJ. Effects of zinc fertilizer on the yield and quality of two winter varieties of canola. Zinc crops. International Congress of Improving Crop Production and Human Health, 24-26 May, Istanbul, Turkey, c2007.
36. Pourgholam M, Nemati N, Oveysi M. Effect of zinc and iron under the influence of drought on prolin, protein and

nitrogen leaf of rapeseed (Brassica napus). Annals of Biological Research. 2013;4(7):200-203.
37. Šramková Z, Gregová E, Šturdík E. Chemical composition and nutritional quality of wheat grain. Acta Chimica Slovaca. 2009;2(1):115-138.
38. Mishra LK, Abidi AB. Phosphorus-zinc interaction: Effects on yield components, biochemical composition and bread making qualities of wheat. World Applied Sciences. 2010;10(5):568-573
39. Du Laing G, Tack FMG. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review Sci. Total Environment. 2009;407(13):3972-3985.
40. McCauley A, Jones C, Jacobsen J. Soil pH and organic matter, Montana State University Extension Service, Bozeman. Nutrient management. 2009;8:1-12.
41. Xu G, Wei LL, Sun JN, Shao HB, Chang SX. What is more important for enhancing nutrient bioavailability with biochar application into a sandy soil: direct or indirect mechanism? Journal of Ecological Engineering. 2013;52:119-124. https://doi.org/10.1016/j.ecoleng.2012.12.091
42. Jeffery S, Bezemer TM, Cornelissen G, Kuyper TW, Lehmann J, Mommer L, *et al*. The way forward in biochar research: targeting trade-offs between the potential wins. Global Change Biology Bioenergy. 2015;7(1):1-13.
43. Kappler A, Wuestner ML, Ruecker A, Harter J, Halama M, Behrens S. Biochar: as an electron shuttle between bacteria and Fe (III) minerals. Environmental Science and Technology Letters. 2014;1(8):339-344.
44. Graber ER, Tsechansky L, Lew B, Cohen E. Reducing capacity of water extracts of biochars and their solubilization of soil Mn and Fe. European Journal of Soil Science. 2014;65(1):162-172.
45. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. Biochar effects on soil biota: a review. Soil Biology and Biochemistry. 2011;43(9):1812-1836.
46. Bhargava VS, Raghupathi HB. Analysis of plant material for macro and micro nutrients. In: Methods of analysis of soil, plant, water and fertilizers: H.L.S. Tandon (Ed). FDCO, New Delhi, c1993, p. 49-82.
47. Bodman GE. Monograph for rapid calculation of soil density, water content and total porosity relationship. Journal American Society of Agronomy. 1942;43(10):883-893.
48. Bouyoucos CJ. Hydrometer method for making particle size analysis of soil. Agronomy Journal. 1962;55:464-465.
49. Chesnin L, Yien CH. Turbidimetric determination of available sulphate. Soil Science Society of America, Proceedings. 1951;15(C):149-151.
50. Jackson ML. Soil Chemical Analysis. Prentice- Hall of India Pvt. Ltd., New Delhi, c1973, p. 1-485.
51. Lindsay WL, Norvell WA. Development of DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal. 1978;42(3):421-428.
52. Magid El AAA, Knany RE, Fotoh El HGA. Effect of foliar application of some micronutrients on wheat yield and quality. Annals of Agricultural Sciences. Cairo. 2000;(1S):301-313.
53. Maralian H. Effect of foliar application of Zn and Fe on wheat yield and quality. African Journal of Biotechnology. 2009;8(24):6795-6798.
54. Narimani H, Rahimi MM, Ahmadikhah A, Vaezi B. Study on the effects of foliar spray of micronutrient on yield and yield components of durum wheat. Archives of Applied Science Research. 2010;2(6):168-176.
55. Olsen SR, Gale CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soil by extraction with sodium bicarbonates, U.S.D.A. Cire, No. 939, Washington, 1954.
56. Ram S, Govindan V. Improving Wheat Nutritional Quality through Biofortification, Wheat Quality For Improving Processing and Human Health. c2020, p. 205-224.
57. Subbiah BV, Asija GL. A rapid procedure for estimation of available nitrogen in soils. Current Science. 1956;25:259-260.
58. Toth SS, Prince AL. Estimation of cation exchange capacity and exchangeable calcium, potassium and sodium contents of soils by flame photometer technique. Soil Science. 1969;67(6):439-448.
59. Walkley A, Black AI. Soil and plant analysis. Hans publications, Mumbai, India, c1934.
60. Yilmaz A, Ekiz H, Torun B, Gültekin I, Karanlik S, Bagci SA, *et al*. Effect of different zinc application methods on grain yield and zinc concentration in wheat grown on zinc-deficient calcareous soils in Central Anatolia. Journal of Plant Nutrition. 1998;20(4-5):461-471.
61. Zeidan MS, Mohamed MF, Hamouda HA. Effect of foliar fertilization of Fe, Mn and Zn on wheat yield and quality in low sandy soils fertility. World Journal of Agricultural Sciences. 2010;6(6):696-699.
62. Zhang Y, Shi R, Md. Rezaul K, Zhang F, Zou C. Iron and zinc concentrations in grain and flour of winte wheat as affected by foliar application. Journal of Agricultural and Food Chemistry. 2010;58(23):12268-12274.