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GRAIN ZINC AND IRON ENRICHMENT THROUGH FOLIAR APPLICATION AUGMENTS WHEAT YIELD UNDER VARYING NITROGEN REGIMES

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Abstract

Nutrients supply at optimum level is essential for the improving growth, yield and quality of wheat grain.Therefore thisstudy was carried to find the effect of Zinc (Zn), iron (Fe) and nitrogen (N) fertilization on wheat performance in field studies, during 2014-15 and 2015-16 at Cereal Crop Research Institute (CCRI), PirsabakNoweshera, Pakistan. The treatments were soil applied N (90, 120 and 150) kg ha^{-1}, foliar applied zinc and Fe (i.e. 1, 2, 3 kg ha^{-1}, each) and two controls unsprayed check (i.e. no micro nutrients, no water) and sprayed check (no micro nutrients + water). Use of 3 kg ha^{-1} Zn, 1 kg ha^{-1} iron and 120 kg ha^{-1} Nhad significantly increased the wheat grain yield by 31% over unsprayed check. However, with increasing N to 150 kg ha^{-1} in combination with 3 kg ha^{-1} both Zn and Fe, the increase in grain yield was 23% over unsprayed check. Application of 3 kg ha^{-1} Zn and Fe each along with 120 kg N ha^{-1} had increased the grain zinc content by 17 mg $kg^{-1}$and grain Fe concentration by 32 mg kg^{-1}. The grains Zn and Fe concentration were further increased to 23% and 13.4%, respectively over unsprayed checkwithincreasing N rate from 120 to150 kg ha^{-1}. In conclusion the higher economical wheat grain yield was achieved with 120 kg N ha^{-1} along with 3 kg Zn ha^{-1} and 1 kg Fe ha^{-1}. However, quality wheat was produced by applying Zn and Fe at 3 kg ha^{-1} of each along with 150 kg ha^{-1} of nitrogen. Hence, ensuring higher wheat productivity with quality grains 3 kg Zn ha^{-1} and Fe each along with 120 or 150 kg N ha^{-1} is recommended.

Key words: Iron, Nitrogen**,** Wheat, Yield, Zinc**.**

Introduction

Wheat (*Triticum aestivum*L.) is used as a staple food crop by the people of Pakistan (Hussain *et al*., 2010). It is grown worldwide over 240 million hectares area. In most Asian countries, 60% of the daily intake of calorie comes from wheat, and is 2nd staple food of Asia (Cakmak, 1999). Although wheat is covering large acreage, but obtainable yield is very less than the potential yield (Mann *et al*., 2004). The low yield of wheat crop is attributed to improper fertilization. Proper amount of fertilizer application had important role in increasing crop productivity (Khan *et al*., 2017). The oversupply of nitrogen results in increased vegetative growth thus affecting the quality of the produce negatively (Randhawa & Arora, 2000). Being key macronutrient element, nitrogen is necessary to boost up the wheat crop yield and improve the quality (Grant *et al*., 2016, Akhtar *et al*., 2018a). Its role in rising the nutritive and baking quality of wheat grain along with increasing tillering ability has long been documented thereby, enhancing yield while on the contrary low supply of the element bears negative effects on production and quality both (Khan *et al*., 2014, Muhammad *et al*., 2018). Apart from N, the micronutrients are also needed in small amounts but essential for normal growth plants and regulation of functions. Nitrogen has the ability to augment the immunity and regulating vitamin A functions (Marschner, 1995) and also its application increases crop yield and quality (Mousavi *et al*., 2013). In Pakistan due to soil salinity, less availability of organic matter (Khan *et al*., 2018), moisture stress (Ali *et al*., 2018) calcareous nature of soils etc. the deficiency of micronutrients is widespread (Narimani *et al*., 2010). However, the uptake of any micro and macro nutrient is also depend on the variety of a crop (Khan *et al*., 2019).

Among the micronutrients, high attention has been placed on zinc and iron as its deficiency is found to be widespread in human beings (Bouis & Welch, 2010). The concentration of zinc in wheat grain in the range of 20 to 30 mg kg^{-1} while iron ranged from 30 to 40 mg kg^{-1} (Gawalko *et al*., 2001). Throughout the world people suffering from malnutrition of Zn and Fe are more than 3 billion (Dwivedi*et al*., 2012). In case of developing countries, zinc deficiency ranks 5th amongst the ten main causes of illnesses. It has been demonstrated that the areas or countries with soils deficient in Zn are in direct analogy with Zn deficiency in its inhabitants and Pakistan is among those countries (Hotz and Brown, 2004).The Zn availability to plants is almost 50 % of the cereal-based cropping system worldwide which is less than the required amount (Cakmak, 2002). According to the data of WHO, anemia (iron deficiency) is affecting about a quarter of the world human beings particularly the woman. Due to less concentration of Fe in wheat and being used as a staple food by the developing world, the intake of iron is less (Cakmak, 2008; Gibson *et al*., 2010). It has become a big challenge to improve Zn and Fe quantities in the human diet via applying these to the wheat.

In order to rectify Zn and Fe deficiencies in human diet, a number of techniques like food fortification, diverse food production and consumption are used worldwide. However, an important and viable option is agronomic biofortification. This approach/concept capitalizes on the enrichment of micronutrients through foliar application along with ensuring their bioavailability in grains. Lack of these micronutrients in wheat plants may be due to the reason that these elements are not dissolved in soil water (Ghasemian *et al*., 2010). In such circumstances when micronutrients availability from soils are less and their deficiency cannot be rectified then foliar application is a sound and useful option (Sarkar *et al*., 2007; Cakmak, 2008; babaeian*et al*., 2011). Zinc foliar application is suggested as an important enrichment strategy of Zn in wheat grains and it has been reported that its uptake by plant foliage increases when applied in combination with nitrogen fertilizer like urea (Mortvedt & Gilkes, 1993). Similarly, Abbas *et al*., (2011) also reported that the combine application of NPK with micronutrient remarkably increased the yield and yield related parameters in wheat. In addition, treating seeds with micronutrients potentially provides a simple inexpensive method for improving micronutrient plant nutrition.

Keeping in view the role of nitrogen and foliar use of zinc and iron for yield improvement and bioavailability of these micronutrients in the edible parts, the present study was conducted to come out with the appropriate dose of nutrients for achieving quality of wheat along with high yield.

Materials and Methods

Location, soil and weather condition: Present study on the role of nitrogen, zinc and iron on the wheat productivity and quality was conducted at CCRI, Pirsabak, Nowshera, Khyber Pakhtunkhwa, Pakistan during 2014-15 and 2015-16. The CCRI is situated in the north of Khyber Pakhtunkhwa of Pakistan. It is situated 34° north latitude and 71.96° East longitude, with 288 m altitude from the sea level. Soil analysis of the site showed that it is deficient in organic matter, N and P contents while K contents were adequate (Table 1). The study site has 0.172 and 0.82 mg kg^{-1} Zn and Fe, respectively, which is considered as deficient amount in the soil. The climate of the site is semi-arid with hot dry season during summer and about 70% of the total rainfall occurs during monsoon, with relative humidity of 71% in April and 87% in October. The temperature of the location ranged from the lowest of 3ºC to the maximum of 48ºC (Fig. 1).

Experimental treatments and layout: The experiment consisted with factorial combination of three levels of N (90, 120 and 150) kg ha^{-1} and 3 levels of micronutrients [i.e., 1, 2, 3 kg ha^{-1} of both Zn and Fe along with two controls (i.e., no micronutrients no water spray (unsprayed check) and no micronutrient but only water spray (sprayed check))]. The N was soil applied half at sowing time and the half at Feekes stage 2. However, micronutrients were used as foliar spray. The foliar application of Zn and Fe were made from $ZnSO_4.7H_2O$ and $FeSO_4.10H_2O$ sources, respectively. The micronutrients solutions were diluted up to extent to make the area wet completely. Unsprayed check i.e., (no Zn and Fe application) were sprayed with equivalent quantity of water. The foliar treatments were applied at the booting stage (Feekes 10.0) in the late afternoon. Full dose of the recommended phosphorus was used as single super phosphate at the time of seeding. Wheat variety Pirsabak 2013 was seeded in both years at @ of 120 (kg ha^{-1}) in 5 x 1.8 m plots consisted of six rows per plot.

Crop husbandry: The plowing was done in the experimental field twice with the help of cultivator and was irrigated 2-3 weeks before sowing. At field capacity moisture condition, the seed bed was prepared using cultivator followed by rotavator. Seed was sown using manual hand drill. Total six irrigations were made during the crop growth season i.e. 1st irrigation after three weeks after sowing, 2nd at tillering stage, 3rd at spike initiation, 4th at anthesis, 5th at milking and last at grain filling stage. During the first year, high rainfall occurred in the months of March and April and thus last irrigation was withheld. Both types of narrow leaf (grasses) and broad leaf weeds were found in wheat crop. Weedicide Buctril Super 60EC (Bromoxynil + MCPA:Bayer] @750mL ha^{-1} was applied after 1st irrigation for the control of broad leaf weeds while for the control of grasses, Puma Super 7.5% EW (fenoxaprop-p-ethyl) was used.

Observation and measurements: Productive tillers were counted in central four rows in each treatment and thus productive tillers (spikes) m^{-2} was calculated. From each treatment ten selected spikes randomly were threshed separately, and the grains were counted and averaged. Thousand seeds of seed bunch from entire treatments were counted and were weighed with electronic balance. In each treatment central four rows were harvested, tied into bundles, sun dried and were weighed with the help of spring balance. The wheat harvested plants, threshed, cleaned and the seed weights were recorded using electronic balance.Cost benefit ratioswere recorded on the basis of current market prices of inputs and agronomic practices (Boehlje & Eidman, 1984). This consisted of production cost, gross income, net income andcost benefit ratio of particular treatment.

Table 1. Physico-chemical characteristics of the soil before sowing of wheat crop at Cereal Crop Research Institute, Noweshera.

Symbol	Values
Textural class	Silty clay
pH	7.6
EC (dSm^{-1})	0.26
Organic matter (%)	0.76
Total nitrogen (%)	0.048
Phosphorus (mg kg^{-1})	4.7
Potassium (mg kg^{-1})	100
Zinc (mg kg^{-1})	0.172
Iron (mg kg^{-1})	0.82

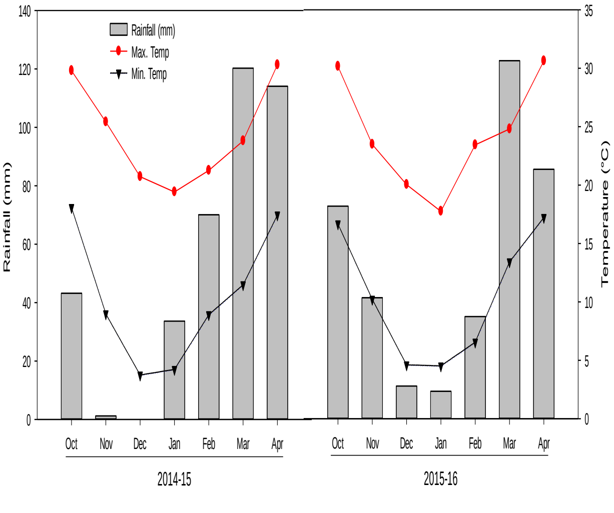

Fig. 1. Average air temperature (ºC) shown with lines and rain fall (mm) with bars at Cereal Crop Research Institute, (CCRI) Pirsabak, Nowshera during the seasons 2014-2015 and 2015-2016.

Zinc and Fe determination: A standard method was used for the determination of Fe and Zn (AOAC, 2000). Briefly, 0.5 g sample was digested in an acid mixture of HNO_3 and $HCLO_4$ (1:1). After digestion the sample was diluted to 50 mL in a volumetric flask with double distilled water. The measurements of Fe and Zn were made with Atomic absorption of Perkins Elmer modal analyst 2000. The atomic absorption was calibrated with standards provided with the instrument by Perkin Elmer (1000 ppm Cu), the hallow cathode lamp were used and reading of the samples were recorded one by one.

Statistical analysis: Randomized complete blocked design with split plot arrangement was used in the experiment. The nitrogen treatments were allotted to main plots where as the micronutrients treatments to sub plots. The N, Zn and Fe were taken fixed factors, year as reparative and also fixed factor, whereas the replications and its interaction with fixed factors as random factor. The treatments mean comparison were made using least significant differences test for significant parameters at 0.05% level of probability.

Result and Discussion

Yield and yield components

Spike m^{-2}: Nitrogen application had significantly increased the spike m^{-2}. Application of 150 kg ha^{-1} had improved the spike by 16% when compared to the spike m^{-2} noted with 90 kg N ha^{-1} (Table 2). Sufficient N availability for crop uptake supports the early vegetative growth that accelerated the crop growth rate and ultimately improved the crop stand, spikes m^{-2} (Rozas *et al*., 1999; Li *et al*., 2001). Nitrogen is the basic structure of chlorophyll (Akhtar *et al*., 2018b), and thus uptake have a major influence on photosynthesis. Zn application @ 3 kg ha^{-1} had more spike m^{-2}, which was statistically non-significant to 2 kg ha^{-1} Zn treated plots. This might be due to its involvement in the tryptophan production which play key role in the production of auxin (Alloway, 2004). More spike m^{-2} was counted in 3 kg ha^{-1} Fe incorporated plots followed by 2 kg ha^{-1} Fe while less spikes were recorded with 1 kg ha^{-1} Fe treated plots. It could be attributed to the iron's dominant role in chloroplast structure and function, and chlorophyll synthesis (Schmidt, 1993); Esfandiari *et al*., (2016); Rawashdeh *et al*., (2014). Narimani *et al*., (2010), argued that spike m^{-2} of wheat significantly increased with the application of Zn. The interaction between Y and MN indicated that increasing Zn and Fe increased the spike m^{-2} in both years, however, the increase was more prominent in 2nd year as compared to 1st year. Residual accumulation of nutrients from the last year might be responsible for such increases in spike m^{-2} which were sharply decreased in unsprayed check and sprayed check in both years. The interaction among Y, N and MN revealed that increase in Zn and Fe increased spikes m^{-2} at all levels of N in both years, however the increase was more prominent with 120 and 150 kg ha^{-1} when compare with 90 kg N ha^{-1}.

Table 2. Influence of nitrogen, zinc and iron on spikes m^{-2}, grains spike^{-1}, thousand grain weight (TGW) and grain yield (kg ha^{-1}) of wheat during winter 2014-15 and 2015-2016.

Year	Spikes m^{-2}	Grains spike^{-1}	TGW (g)	Grain yield (kg ha^{-1})
2015	312 b	52	38 b	4267
2016	333 a	52	42 a	455
LSD (0.05)	**	Ns	**	*
Nitrogen (Kg ha^{1})				
90	287 c	46 b	38 b	4082 c
120	324 b	52 a	41 a	4461 b
150	331 a	53 a	41 a	4879 a
LSD (0.05)	6	4	0.7	229
Zinc (Kg ha^{-1})				
1	316 b	49 b	39 b	4197 c
2	321 a	51 a	39 b	4623 b
3	321 a	51 a	42 a	4832 a
LSD (0.05)	6	1	0.7	130
Iron (Kg ha^{-1})				
1	318	51 a	41 a	4806 a
2	319	50 b	39 c	4359 c
3	321	51 a	40 b	4486 b
LSD (0.05)	Ns	1	0.7	130
Unsprayed check	253 b	48 b	37 b	3974 b
Rest treatments	347 a	56 a	44 a	4992 a
LSD (0.05)	**	**	**	**
Sprayed check	298 b	48 b	36 b	3830 b
Rest treatments	347 a	56 a	44 a	4992 a
LSD (0.05)	**	**	**	**
Interaction				
Zn x Fe	Ns	**(Fig.2a)	**(Fig.2b)	**(Fig.2c)
N x Zn	Ns	**(Fig.3a)	**(Fig.3b)	**(Fig.3c)
N x Fe	Ns	*(Fig.4a)	Ns	*(Fig.4b)
N x Zn x Fe	Ns	Ns	Ns	*(Fig.5a)
Y x N	Ns	Ns	Ns	Ns
Y x MN	**	**	**	**
Y x N x MN	**	Ns	**	*

Means sharing different letter (s) in each category are different statistically at $p \leq 0.05$

Grain spike^{-1}: In wheat crop grains spike^{-1} is also a vital component of yield. N, Zn and Fe had significant effects on grains spike^{-1} of wheat (Table 2). Application of 150 kg ha^{-1} produced more number of (53) grain spike^{-1} followed by 120 kg N ha^{-1} (52), whereas less grains spike^{-1} (46) were with 90 kg N ha^{-1}. Application of nitrogen has strong effects on physiological processes of plants, including plant metabolic activities, photosynthesis affecting the overall plant growth (Ali *et al.*, 2000; Bloom, 2015) and hence the number of grains spike^{-1} (Abedi *et al.*, 2010). Application of either 2 or 3 kg Zn ha^{-1}gave more grains spike^{-1} than 1 kg Zn ha^{-1}. Similarly, Fe applied at 1 or 3 kg ha^{-1} had higher grains of spike than did 2 kg Fe ha^{-1}. Fertilized plots give more grain spike^{-1} over unsprayed checked. The increasing grain spike^{-1} due to micronutrients application might be associated with the enhancement in the rate of photosynthesis and photo-assimilates translocation to the grain as a result of micronutrients-based improvement in enzymatic activity (Marschner, (1995); Lawate *et al*., (2015); Esfandari *et al*., (2016)) and thus might have increased the grains spike^{-1}. Increasing Zn level increased grain spike^{-1} with 1 and 2 kg Fe ha^{-1}, while increasing Zn at 3 kg ha^{-1} reduced the grain spike^{-1} (Fig. 2a). The combined effects of the both micro nutrients might have positively affected the photosynthetic capability, and hence the grains spike^{-1}. Nitrogen levels increasing from 90-120 kg ha^{-1} decreased grain spike^{-1} on all the level of Zn. (Fig. 3a). With the increase in N linearly increased grain spike^{-1} with 2 kg ha^{-}

[1] Fe, but not affected with 1 and 3 kg Fe ha^{-1} (Fig. 4a). The N is a major component of chlorophyll, and thus increasing its photosynthetic capability by micronutrient might have improved the grains spike^{-1}. Low levels of Zn and Fe had higher grains spike^{-1} in following years as compared to first year, the residual effects can better explain such situations.

Table 3. Influence of nitrogen, zinc and iron on harvest index, economic analysis, grain Zn content and grain Fe content of wheat during winter 2014-2015 and 2015-2016.

Year	Harvest index (%)	Economic analysis	Grain Zn content (mg kg^{-1})	Grain Fe content (mg kg^{-1})
2015	44 b	2.6	35	47
2016	46 a	2.8	34	46
LSD (0.05)	*	Ns	Ns	Ns
Nitrogen (kg ha^{-1})				
90	42 b	2.6 c	33 b	45 b
120	44 ab	2.6 b	33 b	47 a
150	46 a	2.9 a	37 a	48 a
LSD (0.05)	3	0.1	1.4	1.3
Zinc (kg ha^{-1})				
1	41 b	2.6 c	34 b	46 c
2	45 a	2.8 b	36 a	47 b
3	46 a	2.9 a	36 a	48 a
LSD (0.05)	2	0.1	1.1	1
Iron (kg ha^{-1})				
1	45 a	2.9 a	36 a	44 c
2	43 b	2.7 b	34 b	48 b
3	44 ab	2.7 b	35 ab	50 a
LSD (0.05)	2	0.1	1.1	1
Unsprayed check	42 b	2.5 b	28 b	41 b
Rest treatments	49 a	3.1 a	38 a	52 a
LSD (0.05)	*	**	**	**
Sprayed check	42 b	2.3 b	30 b	41 b
Rest treatments	49 a	3.1 a	38 a	52 a
LSD (0.05)	*	**	**	**
Interaction				
Zn x Fe	** (Fig.2d)	** (Fig.2e)	**(Fig.2f)	** (Fig.5d)
N x Zn	* (Fig.3d)	Ns	**(Fig.3e)	** (Fig.3f)
N x Fe	** (Fig.4c)	** (Fig. 4d)	**(Fig.4e)	**(Fig.4f)
N x Zn x Fe	Ns	Ns	**(Fig. 5b)	*(Fig.5c)
Y x N	**	**	**	Ns
Y x MN	**	**	**	Ns
Y x N x MN	**	**	**	**

Means sharing different letter (s) in each category are different statistically at $p \leq 0.05$

Thousand grain weight: Nitrogen, zinc and iron had significant effects on thousand grain weight (TGW) of wheat (Table 2). Heavier grains were recorded with higher N doses of 120 or 150 kg ha^{-1} while lighter grains weight was measured with low N dose of 90 kg ha^{-1}.The optimum N-rate probably improved the weight of grains due to greater carbohydrates translocation (Abedi *et al*., 2010; Marino *et al*., 2011). The 3 kg ha^{-1} Zn as foliar application resulted in heavier grains weight, while lighter grains were found in 1 and 2 kg ha^{-1} Zn. Zinc play important role in plant metabolism which might have influenced the functional behavior of enzyme like anhydrase/hydrogenase of C, which consequently stabilized the synthesis the fractions of ribosome and cytochrome (Tisdale *et al*., 1984). Such increases in the ribosomal fractions might have increased the plant dry matter translocation to the grains and hence the grains weight. Heavier grains were measured with 1 kg Fe ha^{-1} and lighter grains were recorded with 2 kg ha^{-1} Fe, concentration. Photosynthetic activities of the plants are directly affected by iron. It is evident from the photosynthetic apparatus; that 2-3 iron atoms are straightly associated to Photosystem-II, 12 atoms in Photosystem-I, 5 in the Cytochrome Complex, and two in the Ferredoxin molecule. Such distribution and role of Fe consequently improved the growth and productivity (Varotto *et al*., 2002; Briat *et al*., 2007) hence might have improved TGW. Our result are in alliance with finding of Zain *et al*., (2015), Jiang *et al*., (2013), Ali *et al*., (2012), Bameri *et al*., (2012), Habib *et al*., (2012), and Zhang *et al*., (2010) who stated that combination of iron and zinc or alone had positive effects on components of grain yield. Increasing zinc doses from 1 to 2 kg ha^{-1} increased TGW both at 1 or 2 kg ha $^{-1}$ Fe, while no changes were noted at 3 kg ha^{-1} Fe (Fig. 2b). Nitrogen levels increasing from 90 to 150 kg ha^{-1} increased TGW at all levels of Zn. However, heavier grains were noted at 150 kg ha^{-1} when combined with 3 kg Zn ha^{-1} (Fig. 3b) in first year linearly at 120 kg ha^{-1} N (Table 2). Similarly increasing Zn and Fe level up to 3 kg ha^{-1} and 1 kg ha^{-1} had increased TGW principally in the following year. Y x N x MN interaction was also found significant and indicated that increasing Zn and Fe level increased TGW in both years with all levels of N, however the increase was more prominent with120 and 150 kg ha^{-1} compare to 90kg N ha^{-1}.

Grain yield: In wheat crop grain yield is directly affected with micronutrients application (Table 3). Applied nitrogen at 150 kg ha^{-1} rate produced higher grain yield (4879 kg ha^{-1}) and minimum grain yield of 4082 kg ha^{-1} was obtained with 90 kg ha^{-1} nitrogen. Nitrogen requirements are in greater amount for optimum photosynthesis of wheat plants, via improved yielding components, leaf area, plant height, biomass, and thus might have increased plant dry mater accumulation and re-mobilization to the reproductive part (Khan *et al*., 2014) and consequently the grain yield. Marino *et al*., (2011) and Abedi *et al*., (2010) reported that use of N improved the spikelet spike^{-1} and grain spike^{-1} that eventually led to high grain yield. Zinc application at 3 kg ha^{-1} resulted in more grain yield of wheat followed by 2 kg ha^{-1}, and lowest grain yield was noted at 1 kg ha^{-1} zinc incorporation plot. Marschner (1995) reported that improvement in yield of grain is due to zinc involvement in enzymes activation during carbohydrate metabolism. Other factors include maintenance of cellular membranes integrity, protein synthesis, flowering and fruiting process, which are greatly affected by Zn (Epstein & Bloom, 2005; El-Bendary *et al*., 2013; Zhao *et al*., 2016), and might have increased the grain yield. Low application of Fe had improved the grain yield, but higher Fe foliar application had negative effects on grain yield. Kerkeb & Connoly (2006) reported that iron is essential component of enzymatic activities that play essential role in the plant various processes. They further reported that iron is enzymes co factor used in plant hormones production and participate in several reactions. Such activities of Fe might have improved the plant performance and hence the grain yield. Same result is also stated by Esfandiari *et al*., (2016); Abdoli *et al*., (2014); Cakmak *et al*., (2010b). Increasing Zn doses from 1 to 2 kg ha^{-1}, grain yield increased at all levels of Fe, however grain yield was not improved beyond 2 kg Zn ha^{-1} (Fig. 2.) Increase in N rate from 90 to 150 kg ha^{-1} improved the grain yield of wheat at all levels of Zn, while increase was more prominent at 2 kg ha^{-1} Zn (Fig. 3). The N, Zn and Fe were also significant (Fig. 2c). Grain yield decreased with increasing Fe levels at increased Zn and N levels. However higher grain yield was at 120 kg ha^{-1} N using highest dose of 3 kg ha^{-1} ZN and lowest rate of 1 kg ha^{-1} Fe. Increasing N levels from 90 to 150 kgha^{-1} grain yield of wheat increased at all levels of Fe, while it was prominent at 1 kg ha^{-1} Fe (Fig. 4c). The interaction Y x MN indicated that increasing Zn and Fe levels increased grain yield across both years with grater changes in first year (Table 2). The interaction Y x N x MN revealed that increasing Zn and Fe level increased grain yield at all levels of N in the 1st year, while in 2nd year increasing Zn 3 kg ha^{-1} along with 1 kg Fe ha^{-1}, improved grain yield at 150 kg N ha^{-1}. In both years, additional increases in Fe concentration beyond 1 kg ha^{-1}, wheat yield decreased (Fig. 5a). In earlier studies Arif *et al*., (2007) observed that the soil application of Zinc treatment produce maximum wheat grain yield as compared to their folier application.

Harvest index: Wheat harvest index (HI) was statistically affected by N, Fe and Zn (Table 3). Greater harvest index of 46% was found in 150 kg ha^{-1} of N incorporated plots being at par with 120 kg ha^{-1} N treated plots (44%). Low HI (42%) was noted in 90 kg ha^{-1} nitrogen. The greater translocation and remobilization of photo assimilate in plots having higher N, might have increased the grain yield component compare with plant biomass. The use of 2 and 3 kg ha^{-1} zinc had greater harvest index than plots having 1 kg Zn ha^{-1}. Comparable results were noted by Rezaeei *et al*., (2014) and Zain *et al*., (2015). The low concentration of Fe solution had resulted in greater harvest index compared to either 2 or 3 kg Fe ha^{-1}. Increasing the Fe concentration had decreased the harvest index, a measure of grain yield proportion out of total plant biomass. Increasing Zn level up to 2 kg ha^{-1} decreased HI across 1 and 2 kg Fe ha^{-1}, however, with further increase in Zn level the harvest index increased at 1 and 2 kg Fe ha^{-1} (Fig. 2d). With increase in N dose up to 120 kg ha^{-1} decreased harvest index across 2 kg ha^{-1} Zn (Fig. 3d).The harvest index only increased with higher concentration of Fe with increasing the N rates from 90 to 150 kg ha^{-1}. Regarding the Zn x Fe x Y interaction, it was noted that increasing both Zn and Fe level increased harvest index apparently in 1st year compared to the 2nd year.

Economic analysis: Efficiency of soil N fertilization with foliar Zn and Fe application on wheat was assessed using economic analysis. Statistical analysis of the data generated for economic analysis indicated that N, Zn and Fe levels had significantly (Table 3) affected the value cost ratio (VCR). The VCR is the net income from a production technology, excluded all the expenditure incurred. It was noted that with increasing N levels, the VCR increased. The using of 150 kg ha^{-1} N gave a VCR value of 2.9, which is significantly higher than the VCR value 2.6 calculated for 90 and also 120 kg N ha^{-1}each. Foliar Zn @ of 3 kg ha^{-1} had higher VCR than 1 and 2 kg ha^{-1}. Fe application at 1 kg ha^{-1} had greater VCR than both 2 and 3 kg ha^{-1}. Generally, the fertilized plots higher VCR than both controls (Table 3). Zinc and iron interaction indicated that increasing Zn level increased VCR at all three levels of Fe (Fig. 2e). The interaction between N x Fe (Fig. 3e) indicated that increase in nitrogen level from 90-150 kg ha^{-1} increased VCR at all levels of Fe, however, more prominent increases was observed with 1 kg Fe ha^{-1}, while at 3 kg Fe ha^{-1} the improvement was prominent beyond 120 kg N ha^{-1} (Fig. 4d). The interaction Y x N indicated that increasing N levels from 90 to 150 kg ha^{-1} increased VCR in both years with prominent increase in first year over 2nd years with increased N application beyond 120 kg ha^{-1} (Table 3). The interaction Y x MN indicated that increasing Zn and Fe levels increased grain yield in both years, however the increase was more prominent in the 1st year as compared to 2nd year.

Grain Zn concentrations: Fertilization is done not only for increasing the crop production, but also its effects can be measured in term of how much nutrients did plants obtain. Since micronutrients were applied as foliar, thus it in expected to be easily obtained by the crops, and cause increases in its foliage concentrations. The data concerning wheat Zn concentration was statistically affected by macro and micro nutrients as shown in the Table 3. More grain Zn content (37 mg kg^{-1}) in wheat was recorded at 150 kg ha^{-1} nitrogen incorporated plot followed by120 kg ha^{-1} (33 mg kg^{-1}) and was statistically same with 90 kg ha^{-1} N treated plots (33 mg kg^{-1}). Kutman *et al*., (2010) also noted increases in wheat grain zinc content with increase in N dose. Increasing N supply to plant increases the growth of plants that ultimately leads to increase strength of the sink for the accumulation of Zn (Ozturk *et al*., 2006). Zn incorporation @ 3 kg ha^{-1} showed more concentration of zinc in grain than 2 kg ha^{-1} zinc treated plots of wheat while lower grain Zn content was recorded in 1 kg ha^{-1} of zinc. Outcome of our research are in alliance with the finding of Zhao *et al*., (2016). More concentration of zinc in grain was found in 1 kg ha^{-1} Fe treated plot of wheat, while lower concentration of zinc in grain was assimilated in 2 kg ha^{-1} Fe treated plot. The reason will be different micronutrients having different ways to accumulate in wheat grain, Zn and Fe pathways for storage in the grain was also strongly influence by nitrogen fertilizer and nitrogen depended (Shi *et al*., 2010). In earlier case of investigations, the Zn concentrations in the wheat grains were improved when the nitrogen incorporation was boosted from 0 to 130 kg ha^{-1} (Hao *et al*., 2007; Habib, 2012; Erenoglu *et al*., 2011; Cakmak *et al*., 2010a; Gomez-Becerra *et al*., 2010; Cakmak, 2008). Zn x Fe interaction was found significant Zinc concentration in wheat grain. (Fig. 2f) Increasing zinc level from 1 to 2 kg ha^{-1} enhanced grain zinc concentration across all levels of Fe. The significant N and Zn interaction is shown in Fig. 3e, it was noted that increasing N levels from 90 to150 kg ha^{-1} increased grain Zn concentration across 2 and 3 kg Zn ha^{-1} while decreased across 1 kg Zn. Similarly, increasing nitrogen rate from 90 to 150 kg ha^{-1} improved grain Zn concentration across all Fe levels. However, zinc concentration was apparently increased at 1 kg ha^{-1} Fe (Fig. 3f). Significant interaction of N, Zn and Fe (Fig. 5b) indicated that increasing Fe level up to 2 kg ha^{-1} decreased grain Zn concentration at all levels of Zn and N.

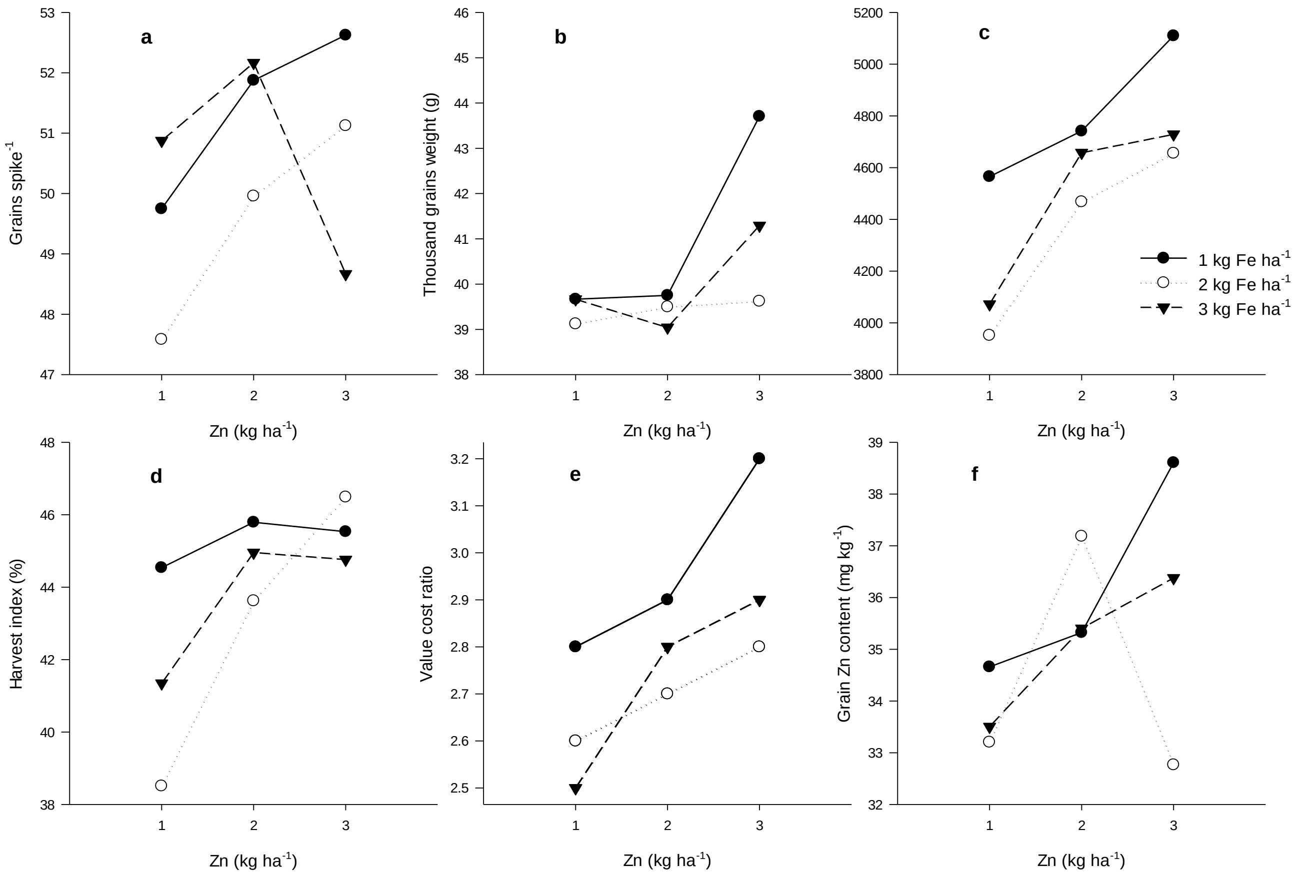

Fig. 2. Interactive response of Zn and Fe during 2014-15 and 2015-16.

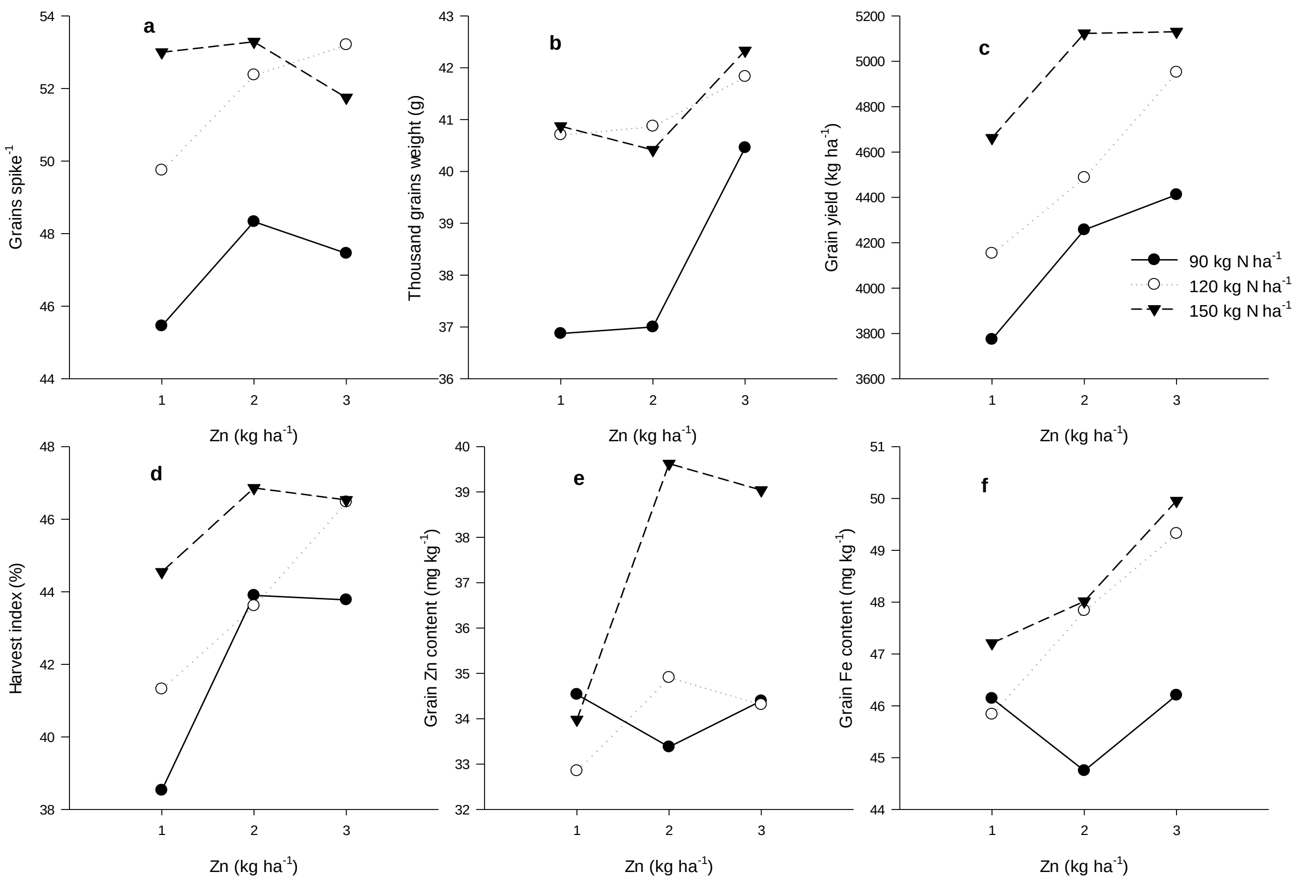

Fig. 3. Interactive response of N x Zn during 2015-16.

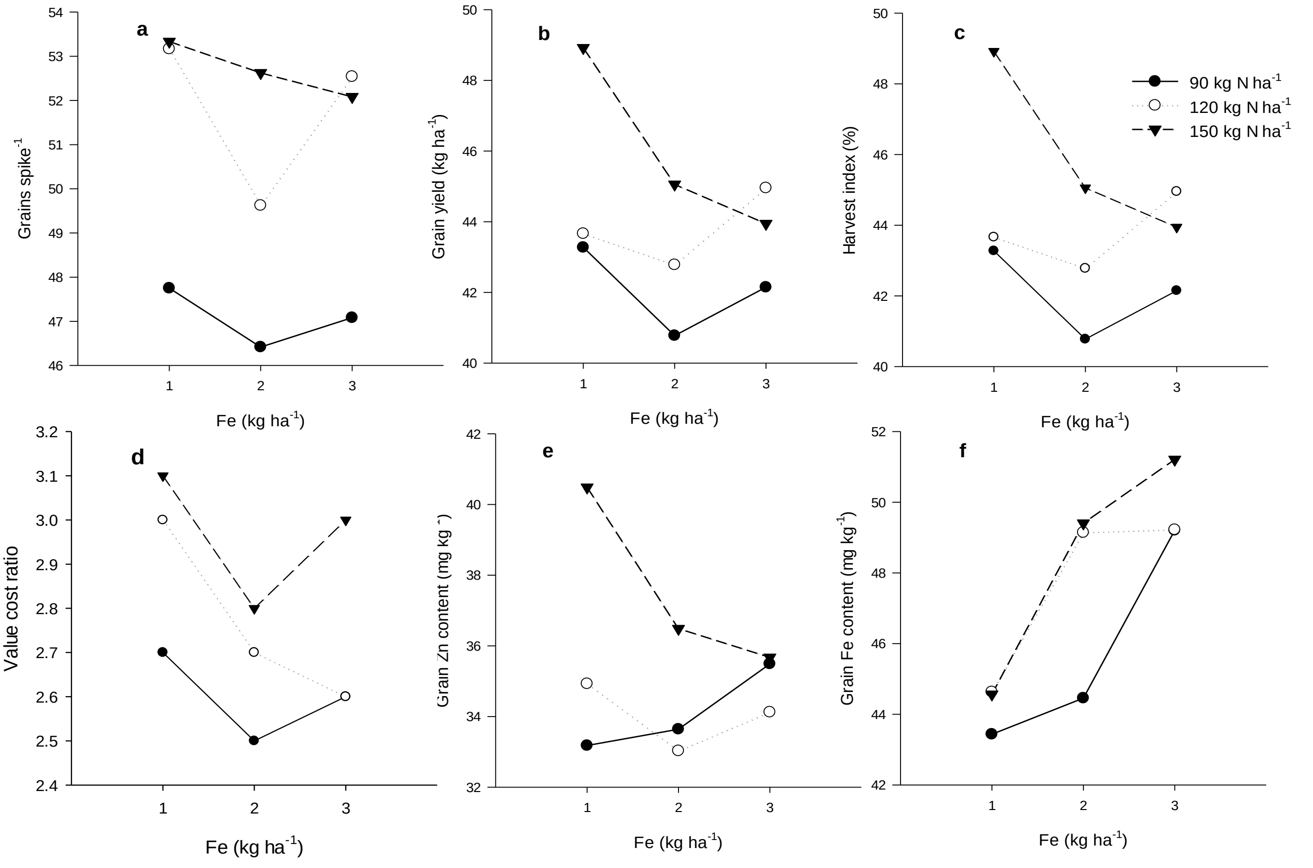

Fig. 4. Interactive response of N x Fe during 2014-15 and 2015-16.

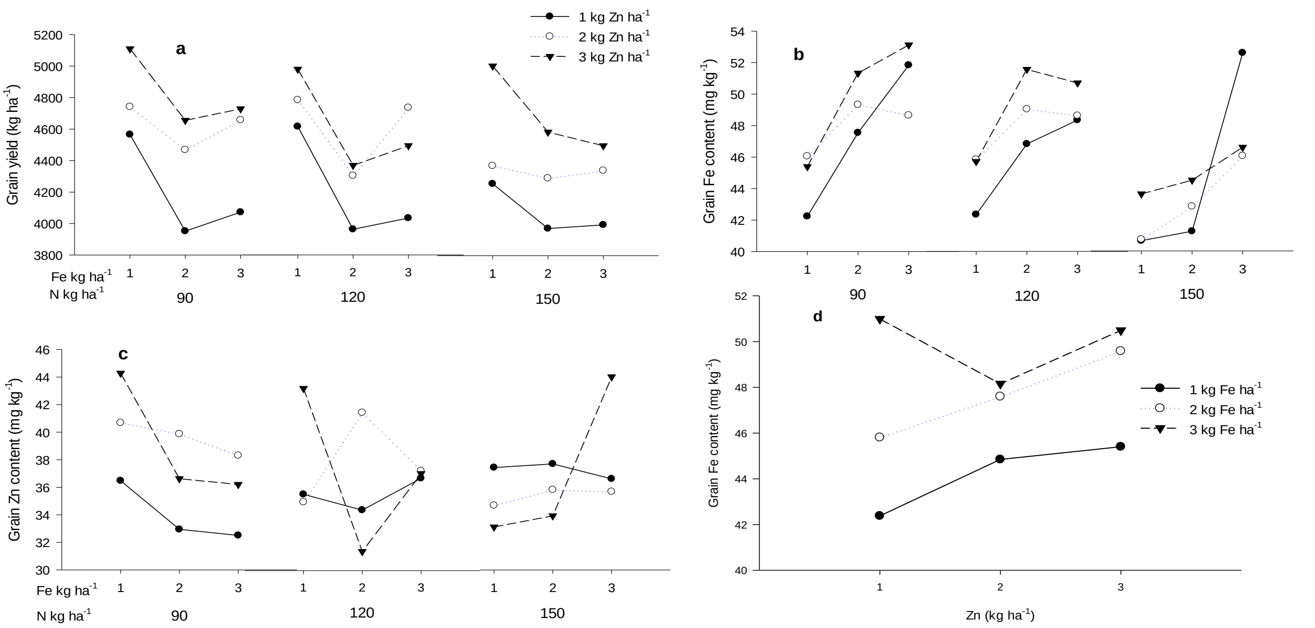

Fig. 5. Three-way interactions among N x Zn and Fe (a, b, c) and two way interaction between Zn x Fe (d) during 2014-15 and 2015-16.

Grain iron concentration: The effect of nitrogen and micronutrients on wheat grain Fe percentage (Table 3), showed that nitrogen, zinc and iron were found to have radically changed wheat grain Fe content. Nitrogen applied @ of 150 kg ha^{-1} resulted in greater Fe concentration (48 mg kg^{-1}) in wheat grain, as compared to low N application i.e., 90 kg ha^{-1} (45 mg kg^{-1}). Nitrogen nutritional status plays a significant role in the allocation of Fe to grain (Kutman *et al*., 2011). Increasing protein concentrations improve the storage capacity of zinc and iron in grain (Kutman *et al*., 2010; Chakmak *et al*., 2010a). More grain Fe content was noted at 3 kg ha^{-1} treated plot of wheat followed by 2 kg ha^{-1} Zn treated plots, while lower grain Fe content was noted at 1 kg ha^{-1} Zn treated plots. Kutman *et al*., (2011) also obtained similar results. Higher grain Fe content was noted at 3 kg

ha^{-1} Fe treated plots followed by 2 kg ha^{-1} Fe treated plots, while lower Fe concentration noted in 1 kg ha^{-1} Fe incorporation. The direct addition of Fe as foliar spray might have enhanced iron contents of plant tissue and subsequently into wheat grains. Another possible explanation might the positive correlations of zinc and iron with protein concentrations in wheat grains (Persson *et al*., 2016; Zhao *et al*., 2009; Gomez-Becerra *et al*., 2010; Aciksoz *et al*., 2011). Increasing the N rate from 90 to 150 kg ha^{-1} improved grain Fe both at 2 and 3 kg ha^{-1} Zn (Fig. 3f). Similarly, the addition of nitrogen rate from 90 to 150 kg ha^{-1}, had increased the grain Fe concentration with increases Fe foliar application from 2 to 3 kg Fe ha^{-1} (Fig. 4f). Increasing Zn from 1 to 3 kg ha^{-1} increased Fe concentration across all levels of Fe, being maximum Fe concentration with 1 kg ha^{-1} Fe (Fig. 5d). This means that addition of Fe > 1 kg Fe ha^{-1} has no positive effects on Fe concentration of grain rather will have negative effects when combined with Zn (Fig. 5d). The direct application of Fe and the positive role of N in plant growth and development can better explain this increased Fe concentration in grains. Interaction of N, Zn and Fe indicated that increasing Fe level increased wheat grain Fe concentration across all levels of zinc and nitrogen (Fig. 5c). However, the increase was more prominent with 1 kg ha^{-1} Zn at 90 and150 kg ha^{-1} nitrogen. According to previous studies iron supplementation increased wheat grain Fe concentrations. Improvement in wheat grain percentage of Fe is elsewhere documented (Kutman *et al*., 2011; Kutman *et al*., 2010). The interaction between Y, N and MN (Table 3) indicated that increasing Zn and Fe levels increased grain iron content at150 N kg ha^{-1} in the 1st year, while in the year second increasing Zn and Fe level increased grain Fe content with supplementation of 120 kg N ha^{-1}.

Conclusions

The application of 120 kg ha^{-1} nitrogen improved wheat yield, yield components and with comparable VCR while quality wheat was produced at 150 kg $ha^{-1}$of N. Foliar application of Zn and Fe enhanced wheat grain uptake of zinc and iron, respectively. Use of nitrogen at the rate of 120 kg ha^{-1} along with 3 kg Zn ha^{-1} and 2 kg Fe ha^{-1} had increased wheat yield while high wheat grain Zn and Fe concentration was achieved with interactive effect of 150 kg ha^{-1} N and 3 kg ha^{-1} each Zn and Fe each. With the increasing nitrogen doses, wheat grain zinc and iron concentration also increased. However, application of 120 kg N ha^{-1} along with 3 kg Zn ha^{-1} and 1 kg Fe ha^{-1} produced greater grain yield and had economically viable VCR and thus is recommended for greater grain yield. Similarly, the nitrogen uses at 150 kg ha^{-1} along with 3 kg Zn ha^{-1} and 1 kg Fe ha^{-1} resulted in better quality grains, thus is recommended to obtain quality grains with optimum Zn and Fe concentration. Though, the values cost ratio was higher for 150 kg N ha^{-1}, however for more profitable levels of N optimization, further studies are needed. It is also recommended that nitrogen assimilation and associated losses would be quantitatively determined for profitable and sustainable production.

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