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Effects of Different N Rates and Concentrations of Fe and Zn on Grain Quality Applied at the Booting Stage of Wheat (*Triticum aestivum* L.) in the South-Eastern Zone Tigray, Ethiopia

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Authors' contributions

This work was carried out in collaboration among all authors. Author YG contributed to the collection, data management, analysis, and preparation of the manuscript. Authors BA and AA contributed to the review, editing, and supervision of the research. All authors read and approved the final manuscript.

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ABSTRACT

The application of nitrogen, Fe, and Zn fertilizers corresponding to reproductive development leads to a higher level of protein synthesis and an improvement in grain quality. Therefore, the study was carried out to evaluate the effect of the rate of late nitrogen fertilizer and the application of Fe and Zn in the foliar medium on the quality of bread wheat. The experiment was carried out in split plot

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design, with three replications in two locations in the Southeastern Tigray zone, Ethiopia. Where the main plots were assigned with three rates of late application nitrogen fertilizer treatments: N1= control, N2= 25kg of urea/ha and N3= 50kg of urea/ha at the booting stage and the subplots were assigned with four levels of foliar application of Fe and Zn at the start stage: MI1= Control, MI2 = Fe at 12kg/ha, MI3= Zn at 10kg/ha, and MI4= Fe at 12kg/ha + Zn at 10kg/ha. Data such as grain protein content, grain iron, and grain zinc were collected. Analysis of variance showed that all parameters were significantly affected ($P<0.05$) by the interaction effect of late application N and foliar application Fe and Zn. The combination of N3 with MI4 offered the highest grain protein content (11.82%) and grain iron (31.12 mg/kg); whereas highest grain zinc (24.58 mg/kg) of N3 with MI3. Therefore, it can be concluded that applying 50 kg/ha of urea from the late application of nitrogen fertilizer and foliar application of Fe and Zn in the booting stage in combination considerably improved the grain protein content and grain concentration of Fe, while applying 50 kg/ha of urea from the late application of nitrogen fertilizer and the foliar application of Zn in the booting stage improve the grain concentration of Zn bread wheat.

Keywords: Bread wheat; foliar application; iron; late application; zinc.

1. INTRODUCTION

"Bread wheat (*Triticum aestivum* L.) is the second most important crop after maize in the world and is a staple food for approximately a third of the world's population. China, India, and Russia are the world's three largest individual wheat producers. Ethiopia is the second largest wheat in Africa after Egypt" [1]. "Bread wheat ranked third after tef (*Eragrostis tef*) and maize (*Zea mays*) and also second in total production after maize. The national wheat area coverage was estimated to be 1.86 million ha with a productivity of 3.11 tons per hectare" [2]. In the Tigray region, bread wheat is one of the main crops, grown under a wide range of environmental conditions. It ranks third of area coverage, behind sorghum (*Sorghum bicolor*) and tef (*Eragrostis tef)*. The average area coverage and productivity were estimated at 0.12 million ha and 2.1 t ha^{-1}, respectively [3].

Despite the long history of wheat cultivation and its importance to Ethiopian agriculture, its average yield is still very low, not exceeding 3.11 t ha^{-1} [2] which is below the world's average of 3.4 t ha^{-1} [4,5]. In the Tigray region also the average wheat yield is very low, not exceeding 2.1 t ha^{-1} in which below the national average yield [3]. Many constraints contributed to the reduction in bread wheat yield and quality in the Tigray region. This low productivity and quality of bread wheat in the Tigray region could be attributed to the lack of well-adapted improved varieties, poor soil fertility, pests and diseases, unsuitable soil pH, and drought stresses [4].

"Low soil fertility, especially nitrogen deficiency, is one of the main constraints that limit wheat production in Ethiopian highlands" [5]. "The key role that nitrogen nutrient has played in increasing crop yields and improving the protein quality of grain and straw in bread wheat is widely recognized. Grain protein content (GPC) is important for human health as it is necessary to build and repair tissues. It is also used to make enzymes, hormones, and building blocks of bones, muscles, skin, and blood. The low protein content of grain can be attributed to nitrogen stress" [6]. "The grain protein content can increase whenever nitrogen is available to the plant during grain filling, but it is not likely that enough nitrogen is applied to maximize yield" [7,8]. "Applying nitrogen fertilizer to correspond to reproductive development leads to higher protein synthesis and storage in grains, but delaying fertilization too long may restrict the amount of N that can be converted into quality protein" [9]. Nitrogen fertilizer applied between booting and anthesis could effectively improve grain protein content, although it generally does not increase grain yield. Taking into account different application timings, late N fertilization (application of additional N in the heading or anthesis) resulted in a higher concentration of flour protein [6, 7, and 8]. Ottman [9] and Jones and Olson-Rutz [10] indicated that this late-season soil for nitrogen fertilizer application should be in the 30 to 50 kg urea ha^{-1} range.

Micronutrients such as Fe and Zn play an important role in improving grain quality. They are needed in trace amounts, but their adequate supply improves nutrient availability and positively affects cell physiology, which is reflected in yield [11]. "Micronutrients such as Fe and Zn play an essential role in the plant life cycle and are very essential for the normal growth of plants" [12, 13]. "Iron is play responsibility in many plant functions. These functions include chlorophyll development, photosynthesis, energy transfer, ingredient of sure enzymes and proteins, and involved in nitrogen fixation. It plays an essential role in nucleic acid metabolism" [14, 15, 16, 17]. "As an electron carrier, it is -involved in oxidation-reduction reactions" [18]. "It is required in protein synthesis and is a constituent of hemoprotein. It is also a component of many enzymes and involved in respiratory enzyme systems as a part of cytochrome and hemoglobin" [14]. "The catalytic function of iron depends on its electronic structure, which can undergo reversible changes through several oxidation states differing by one electron. Chlorophyll synthesis, thylakoid synthesis, and photosynthesis are dependent on the integrity of many iron-containing proteins, including heme and iron sulfur proteins" [19]. "The activity of ribulose-1, 5 -biphosphate carboxylase/ oxidase (rubisco) is also reduced under iron deficiency" [20]. "Its deficiency symptoms are first appear on the younger tissues or leaves. The deficiency is exhibited as a chlorosis developing intervenally in the new leaves. Most crop plants are more susceptible to Fe deficiency in the early stage of growth. Plants become stunted in the early seedling stages. Its deficiency delayed flowering and maturity dates in crop plants. For human, Fe is an essential micronutrient critical for cognitive development. Children and pregnant women are more affected by iron deficiency. Low hemoglobin concentration (anemia) affects 43% of children 5 years of age and 38% of pregnant women worldwide. Anemia during pregnancy increases the risk of maternal and perinatal mortality and low birth weight. Maternal and neonatal deaths are the main cause of mortality, together causing 2.5 to 3.4 million deaths worldwide" [21]. Zinc is another essential micronutrient that has many biochemical functions in the plant From these main functions it is necessary for producing chlorophyll and forming carbohydrate and aids plant growth substances and enzyme systems. Zinc along and with Cu, has been shown to be a constituent of the enzyme superoxide dismutase. It also closely involved in the N-metabolism and required for the synthesis of tryptophane. As tryptophane is also a precursor of indoleacetic acid, the formation of this growth substance is also indirectly influenced by zinc. Additionally, improves root development in crop plants. A key role of zinc in gene expression and regulation has been reported [22]. "Plants suffering from Zn deficiency often show chlorosis in the intervenial areas of the leaf. These areas are pale green, yellow, or even white. Zinc-deficient wheat has been reported to develop small anthers and abnormal pollen grains" [23, 13]. "Under severe zinc deficiency, internodal length is reduced, plants are stunted, terminal growth is retarded, and new leaves develop slowly" [24]. "Its deficiency in protein levels are markedly reduced, and amino acids and amides accumulate in plants" [13]. For human, Zn is found in cells throughout the body and is necessary for the proper functioning of the defensive (immune) system, and it plays an important role in cell division, cell growth, wound healing, and carbohydrates breakdown. It is also needed during pregnancy, childhood, and childhood for proper growth and development. Deficiency of Zn leads to frequent infections, hypogonadism in men, hair loss, poor appetite, problems with taste and smell sense, skin sores, slow growth, difficulty seeing in the dark, and delayed wound healing [25].

Due to continuous cultivation and minimal use of fertilizer input, a serious depletion of micronutrients occurs in soils. These problems started from low Fe and Zn concentration food intake due to low bio-fortification of cereal crops, including bread wheat. Different methods are used for micronutrient application, such as seed preparation, soil application, and foliar application. Foliar fertilization (or foliar feeding) is a moderately new and contentious technique for feeding plants by applying liquid fertilizer directly to their leaves [26]. From micronutrients. Recent research reports indicated a significant increase in wheat production through foliar application of small amounts of micronutrients in combination with each other. Especially the foliar spraying of Fe and Zn either separately or in combination with other micronutrients significantly improved growth and yield, yield components and grain quality of wheat [27, 28, 29 and 30]. This research work aims to elucidate the synergetic effect of different rates of late application of nitrogen fertilizer and the foliar application of Fe and Zn on grain protein content, grain Fe, and grain Zn of bread wheat.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The field experiment was carried out during the main cropping season (that is, summer) in 2019 at two different experimental sites: *Mikial abyi in Degua Temben,*district and *K/weyane* in *Enderta* district in farmers field in southeast Tigray, northern Ethiopia.

Mikial abyi is located in the *Degua Temben* district at a latitude of 13º37 ′ N and a longitude of 39º08′E, with an elevation of 2,604 masl. *Kedamay weyane (May Tsedo),* which was the second site, is located in the *Enderta* district at alatitude of 13º25'N and longitude of 39º34'E with an elevation of 2348 masl.

The location of the experimental sites is indicated in Fig. 1. According to MoA and ATA [31], these two study areas are deficient in nutrients for nitrogen, phosphorus, sulfur, iron, and zinc.

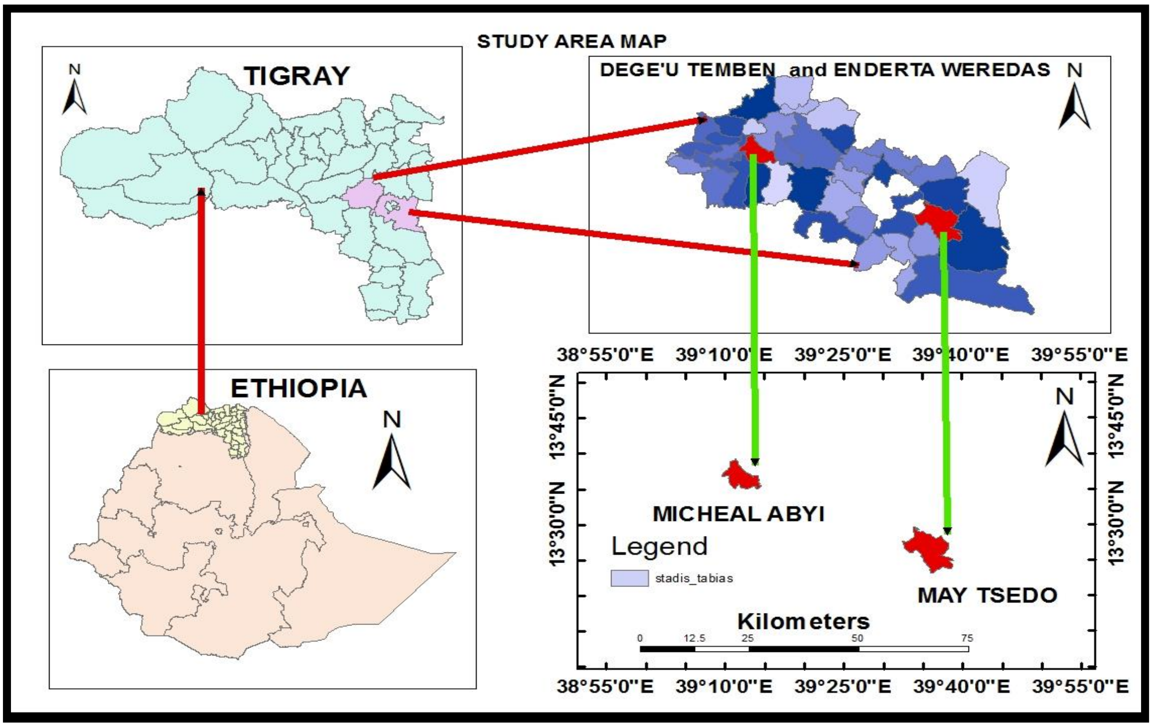

Fig. 1. Location of experimental sites

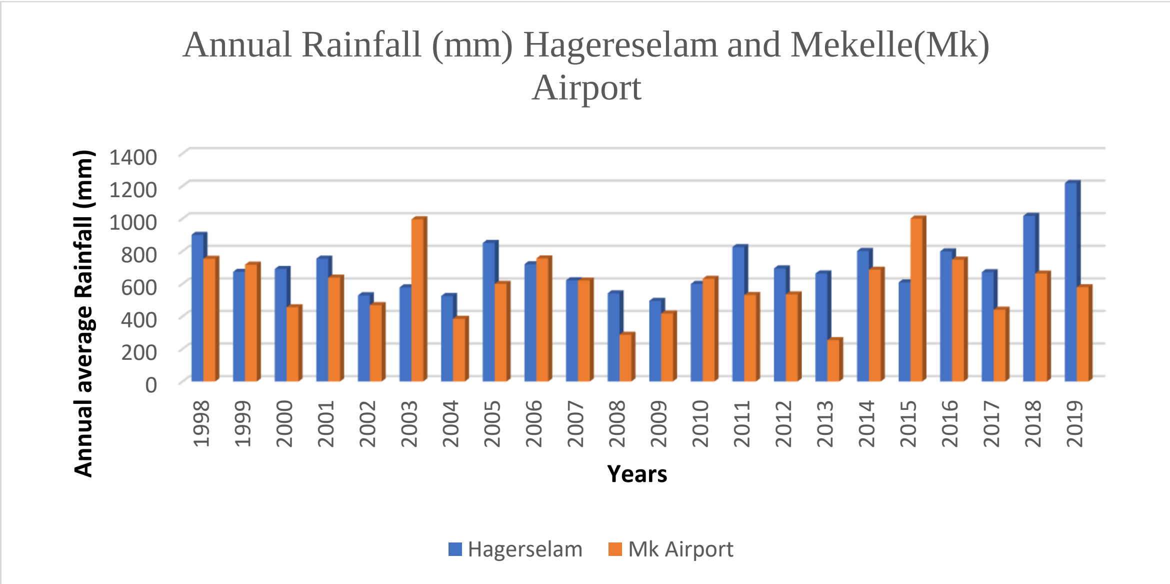

Fig. 2. Annual average rainfall (mm) recorded at Hagereselam and Mekelle airport station from 1998 to 2019

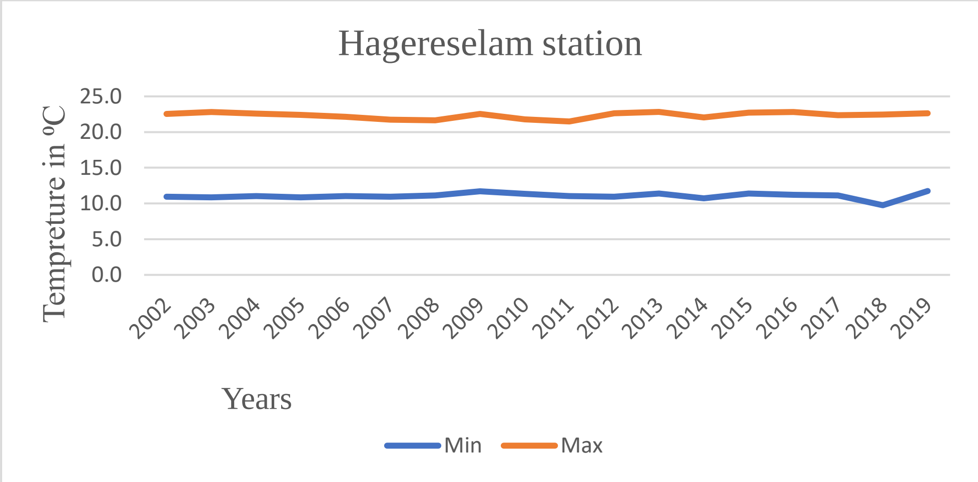

Fig. 3. Minimum and maximum air temperatures (°C) of Hagereselam station from 2002 to 2019

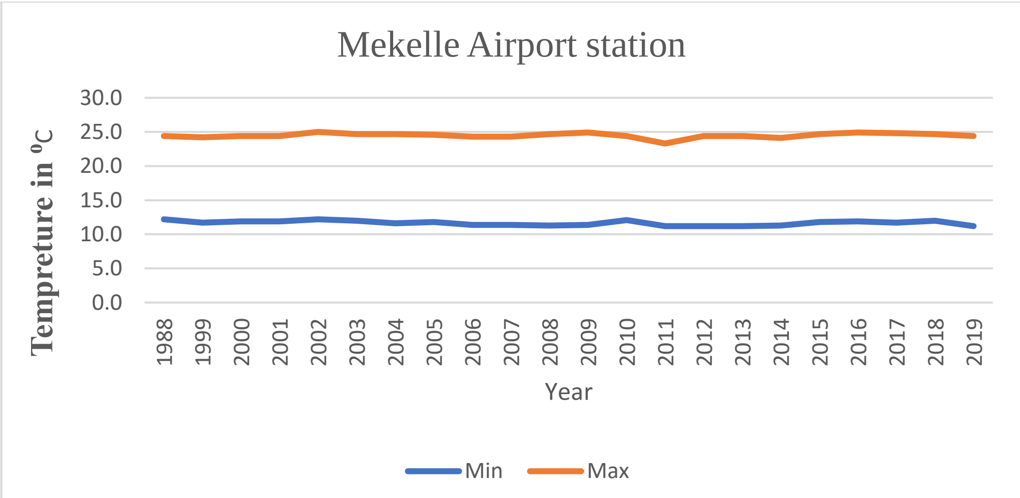

Fig. 4. Minimum and maximum air temperatures (°C) of Mekell airport station from 1988 to 2019

Study areas are characterized by erratic rainfall and follow monomodal. The rainy season is June to September and subsistence agricultural production is almost entirely dependent on this rainy season. The average annual rainfall for the previous 22 years of *Degua Temben (Mikial abyi)* was 717 mm (*Hagereselam* station, 2020), and for *Enderta (K/weyane*) was 598 mm (*Mekelle* airport station, 2020) (Fig. 2).

The mean minimum and maximum air temperature of the last 18 years for *Degua Temben (Mikial abyi)* was 11.1 ° C and 22.3°C, respectively (*Hagereselam* station, 2020) (Fig. 3); and the mean minimum and maximum air temperature of the last 22 years for *Enderta (K/weyane*) was 11.7 ° C and 24.5°C, respectively (*Mekelle* airport station, 2020) (Fig. 4).

2.2 Experimental Design and Treatments

This experimental field was laid out in a split-plot design. Three different rates of nitrogen fertilizer as main plots;

- ✓ N1 = control,
- ✓ N2 = 25 kg of urea / ha (11.5 kg of N / ha) and
- ✓ N3 = 50 kg of urea / ha (23 kg N/ha) applied at the booting stage were assigned.

Four levels of foliar applications of Fe and/or Zn as sub- plot;

- ✓ MI1 = control (water sprayed),
- ✓ MI2= Fe at 12 kg $FeSO_4.7H_2O$ /ha (2.28 kg Fe/ha),

- ✓ MI3 = Zn at 10 kg $ZnSO_4.7H_2O$ /ha (2.3 kg Zn/ha), and
- ✓ MI4 = Fe at 12 kg $FeSO_4.7H_2O$ /ha (2.28 kg Fe/ha) + Zn at 10 kg $ZnSO_4.7H_2O$/ha (2.3kg Zn/ha) at the booting stage.

The booting stage starts from the developing head within the sheath of the flag leaf becoming visibly enlarged and ends when the first awns emerge from the flag leaf sheath and the head. The sources of Fe and Zn were $FeSO_4.7H_2O$ (19% Fe) and $ZnSO_4.7H_2O$ (23% Zn), respectively. The source of nitrogen fertilizer was urea (46% N). Each treatment was replicated three times. The plot size was 2m x 2.5m ($5m^2$) with spacing between blocks, plots, and rows of 1m, 0.5 m, and 0.2 m, respectively. The net plot size was 1.6m x 2.5m ($4m^2$).

2.3 Soil Sample and Analysis

Soil samples were randomly collected from a depth of 0-30 cm in a zigzag pattern, and a composite sample of approximately 1 kg of soil was taken before sowing from the entire experimental field of 15 spots and after harvesting soil samples were taken from each sup plot. Soil samples were air dried and passed through a mesh sieve of 2mm and 0.5mm for physicochemical analysis. The collected soil samples were analyzed for soil texture, soil pH, cation exchange capacity (CEC), available phosphorus, total N, Fe and Zn of the soil were analyzed. All samples were analyzed at the Mekell soil research center and the Mekelle University School of Geology Department. The soil texture was determined using the hydrometer method and the soil pH was determined in a 1: 2.5 soil water suspension using a glass electrode pH meter [32]. Soil cation exchange capacity and total nitrogen were determined using a Kjeldahl procedure. Organic carbon in the soil (%) was determined using the method of Walkely and Black [33]. The available phosphorus was determined using Olsen's methods [34]. The Fe and Zn content was determined using the Atomic Absorption Spectrometer (AAS) method [35].

2.4 Land Preparation and Planting

The experimental plots were well prepared and ploughed three times to ensure uniform and timely seed germination and avoid early weed infestation. The planting time depends on the onset and distribution of the rainfall and varies from location to location. Therefore, seeds were planted on 11 July 2019 at the *Mikial Abyi* site and on 15 July 2019 at the *K/weyane* site. A seed rate of 125 kg ha-1 at a spacing of 20 cm between rows using seeds of the improved variety kakaba (pica flor). The field was uniformly fertilized with 100 kg/ha of NPS fertilizer (19% N + 38%P_2O_5 + 7% S) (50 grams / plot) at sowing and 100 kg of urea / ha (46% N) in split application (half in planting and the rest half in tilling). After emergence, all aspects of agronomic management, such as weeding, were carried out. The hand weeding was performed three times to avoid competition for soil moisture, nutrients, and light.

2.5 Grain Quality Analysis

Grain protein content*:* The nitrogen content of the bread wheat grain harvested from each plot was determined using the micro Kjeldahl method, as stated in the European Society for Agronomy (ESA) method ES 669-2001. The process involved 3 major steps: First, the sample was digested in boiling concentrated H_2SO_4 at 380 °C for 3 hours, with the addition of a catalyst, until complete dissolution and oxidation. The nitrogen was transformed into ammonium sulfate. Then, distillation was done by adding an excess of NaOH solution. The percentages of grain protein were calculated as the percentage of grain nitrogen multiplied by 5.70, that is, grain protein (% GP) = grain nitrogen (% GN) *5.70 [36].

Concentration of Fe and Zn grains: The Fe and Zn content of the wheat grain in each plot was determined by the Atomic Absorption Spectrometer (AAS) method. The grain samples (5 g) were digested a mixture of chloridric acid (HCL) and perchloridric acid ($HclO_4$) [35]. The digested samples were analyzed for iron and zinc using a flame atomic absorption spectrometer (SpectrAA50B-Varian Ltd) at wavelengths of 248.3 and 213.9 nm for iron and zinc, respectively.

2.6 Harvesting and Threshing

The timely harvest of wheat bread is important to prevent cracking and reduce grain and seed quality. The experimental fields were harvested on November 26, 2019 for the Mikial Abyi site and December 02, 2019, for the *K/weyane* site, when the crop was physiologically mature and the grain moisture was approximately 14-15%.

2.7 Statistical Data Analysis

Analysis of variance (ANOVA) for yield and yield components and quality parameters was performed using Gene-Stat version 18 (used for ANOVA analysis) [37]. The least significant difference (LSD) test with a probability of 5% was used for mean separation when the analysis of variance indicated the presence of significant treatment differences.

3. RESULTS

3.1 Physical and Chemical Properties of the Soil of the Experimental Sites

Analytical laboratory results of selected physicochemical properties of soil for the study sites are presented in Table 1. The result of the soil analysis before sowing and after harvest indicated that the soil texture in the experimental sites was sandy clay loam in *Mikial abyi* and clay loam in *the K/weyane* sites. The texture indicates the high degree of weathering that occurred in geological times and the high nutrient and water-holding capacity of the soil. The pH of the experimental site was 7.44 and 7.00 before sowing and after harvest, respectively, *in Mikial abyi*, while in *the K/weyane* sites the pH was 7.36 and 7.66 for the respective times. The soil reaction at the experimental sites ranges from neutral to moderately alkaline [31]. FAO [38] reported that the preferred pH ranges for most crops and productive soils were 4 to 8. Soils with inherent pH values between 6 and 7.5 are ideal for the availability of P, while pH values below 5.5 and between 7.5 and 8.5 limit the availability of P to plants due to calcium fixation often associated with the parent soil materials. Soil P cycles take many different forms; some are readily available and some are not.

Metal nutrients (e.g. copper, iron, manganese, zinc) are small molecules that dissolve in water with 2 to 3 positive charges, thus creating a high charge-to-size ratio. They bind strongly to the surface of soil particles. At high pH (i.e., basic, low concentration of H+), these metal ions stick so tightly that they are not readily found in soil solution, and thus are less available for plant uptake. At low pH (i.e., acidic, high concentration of H +), fewer can stick to the soil surface, making them more available for plant uptake [39].

In general, nitrogen (N), potassium, calcium, magnesium and sulfur are more available within soil pH 6.5 to 8, while boron (B), copper, iron, manganese, nickel (Ni) and zinc are more available within soil pH 5 to 7. At pH less than 5.5, high concentrations of H +, aluminum and manganese in soil solution can reach toxic levels and limit crop production (3, 4). Phosphorus is most readily available within soil pHs 5.5 to 7.5 [39].

But the pH of the soil hindered the uptake of iron and zinc micronutrients. The availability and solubility of micronutrients in the soil are influenced by the pH of the soil and the influence of organic matter. According to Essington [40], the availability of Fe, Zn, Cu, Mn, and B tends to decrease drastically under the influence of elevated pH.

The soil of the study sites had an organic carbon (OC) of 1.30-1.44 before sowing and after harvest (Table 1), which is low according to the Tekalign [41] and MoA and ATA [31], indicating the low potential of the soil to supply nitrogen to plants by mineralization of organic carbon. The low amount of soil OC could be due to the low addition of crop residues and less aeration due to the soil compaction of subsoils. Thus, a regular application of organic manure, such as crop residues and farmyard manure, is required for the study area. The low organic carbon content of the soil is also an indicator of low soil nitrogen, as it is a major source of native mineral nitrogen for plant growth [42].

The cation exchange capacity (CEC) is an important parameter of soil because it indicates the type of clay mineral present in the soil and its ability to retain nutrients against leaching. Cation exchange capacity (CEC) describes the potential fertility of soils and indicates soil texture, organic matter content, and the dominant types of clay minerals present. The soil analysis revealed that the cation exchange capacity (CEC) was 32.70 and 43.97 cmol (+) kg-1 in the *Mikial abyi* site before sowing and after harvest, respectively. In the case of *the K/weyane* site, a CEC of 50.80, and 29.88 cmol (+) kg^{-1} was recorded before sowing and after harvest, respectively. According to Landon [43], the soil result of the study sites was classified as high and very high capacity to retain cations before sowing and after harvest, respectively, at the *Mikial abyi* site and very high and high capacity to retain cations at *the K/weyane* site before sowing and after harvest, respectively (Table 1). In general, soils high in CEC content are considered agriculturally fertile.

The analysis of the soil revealed that the total nitrogen content was 0.003% and 0.07% in *Mikial abyi* before sowing and after harvest, respectively. At the *K/weyane* site, the total N content was 0.06% and 0.06% before sowing and after harvest, respectively. According to the soil classification made by Tekalign (30), the total soil N of the soil samples was found to have very low and low levels of total N content in *Mikial abyi* before sowing and after harvest, respectively, and low in *the K/weyane* site at both times. According to MoA and ATA [31], the soil samples classification of the result is very low (Table 1). The results of the soil samples indicated that the nutrient is a limiting factor for optimum crop growth. Therefore, the level of N in the soil showed the need to apply N to the wheat crop to obtain optimal yield and quality.

Soil analysis revealed that, according to Olsen classification, the results of soil samples were considered as high at the *Mikial abyi* site before sowing and after harvest, and high and low at *the K/weyane* site before sowing and after harvest, respectively, in available P. The reason for the low phosphorus at *the K/weyane* site after harvest could be the moderately alkaline soil pH value (7.66). According to the classification made by MoA and ATA [31], the soil samples results were considered optimal at the Mikial abyi site before sowing and after harvest and optimum and very low at *the K/weyane* site before sowing and after harvest, respectively (Table 1).

According to MoA and ATA [31], the Fe content of the experimental sites was considered very high (Table 1). Regarding Zn, the soil of the experimental sites was considered optimal in total zinc of the soil (Table 1). On calcareous soils, soils that have very high or optimum total iron and zinc, but it is low in available Fe and Zn due to the high soil reaction (pH). According to Essington [40], the availability of Fe, Zn, Cu, Mn, and B tends to decrease drastically under the influence of elevated pH. Due to soil pH, the availability of iron and Zn to the crop was low.

3.2 Combined Effect of Late Application rate of N and Foliar Application of Fe and/or Zn on Grain Protein Content, Iron and Zinc Grain Concentration of Bread Wheat

3.2.1 Grain protein content

The main effect of the late application rate of nitrogen fertilizer and the effect of the interaction on the grain protein content (GPC) was significant at ($p<0.01$) and the main effect of the foliar application of Fe and/or Zn was significant at ($p<0.05$) (Appendix Table 1).

Regardless of the application of micronutrients, the increase in the nitrogen application rate has revealed an increase in the grain protein content of wheat bread. However, the application of Fe and Zn independently has an increase in the grain protein content at both sites. The results presented in Table 2 showed that the foliar application of Fe and Zn has a positive response with the application of nitrogen fertilizer at a rate that does not exceed 25 kg of urea / ha. The combined treatment of 50 kg of urea / ha in the boot stage and Fe at 12 kg $FeSO_4.7H_2O$ /ha+ Zn at 10 kg of $ZnSO_4.7H_2O$ /ha increased the grain protein content by 19.03% over the absence of the application of nutrients (Table 2).

3.2.2 Concentration of grain iron

The effect of the foliar application of Fe and/or Zn had a significant effect ($p<0.01$) on grain iron concentration. The combined interaction of both factors had a significant effect ($p<0.05$) on the iron concentration of wheat bread. However, the late application of nitrogen fertilizer showed a non-significant effect ($p<0.05$) on the iron concentration of bread wheat grain (Appendix Table 1).

Table 1. Some physicochemical properties of the study sites

Soil parameters		Before harvesting		After harvesting	
		Mikial abyi	***K/weyane***	***Mikial abyi***	***K/weyane***
pH		7.44	7.00	7.36	7.66
OC (%)		1.44	1.32	1.38	1.30
CEC (cmol(+)/kg)		32.70	50.80	43.97	29.88
Total N (%)		0.003	0.06	0.07	0.06
Available P (mg/kg)		58.78	71.36	66.70	6.21
Total Fe (mg/kg)		470	561	476	483
Total Zn (mg/kg)		3.07	6.7	3.06	2.7
Soil texture	Sand	50	40.0	51	34
	Silt	24	28.0	26	31
	Clay	26	32.0	23	35
Textural class		Sandy clay loam	clay loam	Sandy clay loam	Clay loam

Table 2. Effect of the interaction of the rate of late application of nitrogen fertilizer and the foliar application of Fe and Zn fertilizer on the grain protein content (%)

Treatments			
Foliar application Fe/Zn	**Rate of late application of nitrogen fertilizer**		
	N1	**N2**	**N3**
MI1	9.57^{g}	9.72^{gf}	10.89^{b}
MI2	10.15^{c-g}	10.74^{bc}	10.54^{b-e}
MI3	9.98^{e-g}	10.65^{b-d}	10.76^{bc}
MI4	10.24^{c-f}	10.04^{d-g}	11.82^{a}
Grand mean	10.42		
LSD (0.05)	0.638**		
P-Value	0.004		
CV (%)	3.5		

According to LSD, treatments followed by the same letter are non-significant differences at P<0.01
*LSD=Least Significance Difference; CV=Coefficient of variation, ** = significant at 1%*

Table 3. Effect of the interaction of the late application rate of nitrogen fertilizer and the foliar application of Fe and Zn fertilizer on the grain Fe concentration (mg/kg)

Treatments			
Foliar application Fe/Zn	**Rate of late application of nitrogen fertilizer**		
	N1	**N2**	**N3**
MI1	22.92^{e}	25.48^{d}	26.13^{d}
MI2	29.65^{ab}	29.32^{b}	28.30^{bc}
MI3	26.10^{d}	28.22^{bc}	26.92^{cd}
MI4	29.20^{b}	31.08^{ab}	31.12^{a}
Grand mean	27.87		
LSD (0.05)	1.735*		
P-Value	0.028		
CV (%)	3.6		

According to LSD, treatments followed by the same letter are non-significant differences at P<0.05
*LSD=Least Significance Difference; CV=Coefficient of variation * = significant at 5%*

Table 4. Effect of the interaction of the late application rate of nitrogen fertilizer and the foliar application of Fe and Zn fertilizer on the grain Zn concentration (mg/kg)

Treatments			
Foliar application Fe/Zn	**Rate of late application of nitrogen fertilizer**		
	N1	**N2**	**N3**
MI1	17.23^{h}	18.84^{fg}	19.49^{ef}
MI2	18.10^{gh}	19.42^{ef}	19.89^{de}
MI3	20.47^{d}	21.59^{c}	24.58^{a}
MI4	21.79^{c}	22.33^{c}	23.62^{b}
Grand mean	20.61		
LSD (0.05)	0.890**		
P-Value	0.005		
CV (%)	2.6		

According to LSD, treatments followed by the same letter are non-significant differences at P<0.01
*LSD=Least Significance Difference; CV=Coefficient of variation ** = significant at 1%*

Regardless of the application of micronutrients, increasing the rate of nitrogen application has revealed an increase in the grain Fe concentration of wheat bread. However, the application of Fe and Zn independently increases the grain Fe concentration. The results presented in Table 3 showed that the foliar application of Fe and Zn has a positive response with increasing application of nitrogen fertilizer. However, when combined with the independent application of Fe and Zn, the combined application of these micronutrients shows an increased response to the grain Fe concentration of wheat bread. Table 3 shows the highest iron concentration 31.12 mg/kg that was obtained from plots treated with N3 (50 kg urea / ha in booting) and MI4 (Fe at 12 kg $FeSO_4.7H_2O$ /ha+ Zn at 10 kg $ZnSO_4.7H_2O$ /ha), while the lowest grain iron concentration 22.92 mg/kg was obtained from the control. The combined treatment of 50 kg of urea / ha in the boot stage and Fe at 12 kg $FeSO_4.7H_2O$ /ha+ Zn at 10 kg $ZnSO_4.7H_2O$ /ha increased the grain Fe concentration by 26.35 % over the control.

3.3.3 Concentration of grain zinc

The main effect of late nitrogen fertilizer application, foliar application of Fe and / or Zn, and their interaction with grain Zn content were highly significant ($p<0.01$) (Appendix Table 1).

Regardless of the application of micronutrients, increasing the rate of nitrogen application has revealed an increase in the grain Zn concentration of the wheat bread. However, the application of Fe and Zn independently increases the grain Zn concentration. The results presented in Table 4 revealed that the foliar application of Fe and Zn has a positive response with increasing application of nitrogen fertilizer on the grain Zn concentration of bread wheat. Table 4 shows the highest zinc concentration 24.58 mg/kg that was obtained from plots treated with N3 (50 kg urea / ha in booting) and MI3 (Zn at 10 kg $ZnSO_4.7H_2O$ /ha), while the lowest grain zinc concentration 17.23 mg/kg was obtained from the control. Combining the treatment with N3 and MI 3 and N3 and MI 4 increased the grain zinc concentration by 29.9 % and 27.05 % over the control, respectively (Table 4).

4. DISCUSSION

Nitrogen has greater influence on growth and yield of crop plants than any other essential plant nutrient. It plays a pivotal role in many physiological and biochemical processes in plants. Nitrogen is a component of many important organic compounds ranging from proteins to nucleic acids. It is a constituent of the chlorophyll molecule, which plays an important role in plant photosynthesis. Many enzymes are proteinaceous; hence, N plays a key role in many metabolic reactions. Nitrogen is also a structural constituent of cell walls.

Nitrogen-deficient plants grow slowly, and their leaves are small. Nitrogen deficiency also decreases leaf area index (LAI), lowers radiation use efficiency, and lowers photosynthesis activity in plants [44]. Seeds are small and yields are reduced in cereals and legume crops under N-deficient conditions. The reduction in yield and quality are directly related to the severity of the N deficiency. Nitrogen crucial factor of grain quality in the bread wheat. Grain quality is a complex trait resulting from the interactions between numerous protein components [45]. Several study indicates that, wheat grains contain 8–20% proteins, which are divided into two major categories: prolamins including gliadins and glutenins and non-prolamins consisting of water-soluble albumins and salt-soluble globulins. The protein composition of wheat seeds is important in determining bread-making quality [46]. Grain protein increases have been found with late season N applied anytime between boot and early-milk stages. The average protein increase for the same amount of applied N was about two times higher when N was applied before- or during flowering than after flowering in dryland production [10,47].

As shown in Table 2; late application nitrogen fertilizer combined with foliar application of Fe and Zn has a positive response on grain protein content. The increase in grain protein may be due to the application of micronutrients such as Fe, Zn, Cu, Mn, and Cl, which are involved in different aspects of plant photosynthesis, as cofactors for different metabolic processes. Depending on the enzyme, Fe, Mn, Zn, Cu, Ni, Mo, and Cl all participate in the functioning of different enzymes, including DNA/RNA polymerases, N-metabolizing enzymes, superoxide dismutases, catalases, dehydrogenases, oxidases, ATPases, and numerous other enzymes involved in redox processes [48]. This cofactor role of micronutrients is crucial for enzyme and non-enzyme activities in plant metabolism under different environmental conditions. The increased rate of late N application and micronutrients are considered a primary means of increasing the concentration of wheat flour protein in relation to improving human nutrition and food security, in which cereal grains are the main source of protein for human consumption in the study areas.

The result of Table 2 is in line with the report by Singh [49] that indicated a higher protein content in grain when N at 160 kg ha^{-1} was applied in combination with the soil and foliar application of Zn and Fe. Yadav [50] also reported that the maximum protein yield was obtained from 120 kg

N, 60 kg P_2O_5 and 60 kg K_2O with 5 kg Zn through $ZnSO_4$ and 10 tons of farm yard manure/ha and foliar application of 2% $FeSO_4$. Narimani [51] also reported that the foliar application of iron enhanced the protein and yield of wheat. Niyigaba [52] also reported a similar finding that grain protein was significantly improved by foliar application of Zn and Fe fertilizers ($ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$). El-Dahshouri [53] reported a significant increase in plant nutritional status and grain protein with Zn foliar application treatments. Similarly, optimized N management is also required to realize the yield potential, which is critical from an economic point of view, and to maximize grain protein concentration [54, 55]. Similarly, Haile [56] reported the highest grain protein content (13.09%) applying the highest N rate, which was 1/4 at anthesis (65 kg urea ha^{-1}). Zemikial [57] also reported the same result, where applying 38 kg urea ha^{-1} at anthesis resulted in optimum grain protein (11.82%) and the economic return of the crop around the study area (*K/weyane)*.

As shown in Table 3; Late application nitrogen fertilizer combined with foliar application of Fe and Zn has a positive response on grain iron concentration. The foliar spray of Fe and Zn in the wheat boot stage significantly increased the total protein of the grain, and the total uptake and concentration of Fe and Zn in the grain could be due to the efficient absorption of these micronutrient applications. The result of Table 3 is in line with Singh [49], who reported a good agricultural practice to improve the concentration of Fe and Zn in grain in bread wheat with the split application of N at 160 kg ha^{-1} in combination with soil and the foliar application of Fe and Zn. According to Zeidan [58], increase in Fe and Zn content in wheat grains over control when Fe and Zn were applied foliar at the rate of 1, and 0.5%, respectively, at tillering & heading stage. Abdoli [59] also reported that foliar application of (Fe + Zn) in the tilling and heading stage increased the Fe concentration (from 84.93 to 139.6 mg / kg) compared to the control. Similarly, Cakmak [60] reported that foliar application of Fe increased the concentration of Fe in bread wheat grain from 80.7 to 116.2 ppm.

A greater supply of N promoted better Zn uptake, translocation of Zn to the shoot, and accumulation of Zn in the grain of wheat plants. Therefore, the management of the nutritional status of N is a promising route for the biofortification of wheat grains with Zn [61]. The enrichments of the grain with Fe and Zn occurred because of the effective uptake of the nutrients, which indicates a strong correlation between the uptake of Fe and Zn by the crop and the concentration of wheat grain. The appropriate distribution of Zn to the grain is defined from the point of view of Zn biofortification and refers to the situation in which all mobilizable Zn in the vegetative parts of the shoot was transported or remobilized to the grain [62]. Optimal or adequate N supply significantly improved the rate of root Zn uptake and, more evidently, root-to-shoot transport and re-translocation of radioisotope Zn from leaves to [63]. Maintaining large amounts of Zn in leaves by spraying with foliar Zn would undoubtedly contribute to an increase in the grain Zn concentration [64].

The result of Table 4 is in line with [65] who reported enhanced grain Zn by applying soil and foliar Zn fertilizers and adding an adequate N fertilizer to wheat bread. The same author also reported that N nutrition is a critical factor in both the acquisition and grain allocation of Zn and Fe in durum wheat. [49] also reported that the split application of N in combination with the soil and foliar application of Fe and Zn can be a good agricultural practice to enhance Fe and Zn concentration in grain in bread wheat. El-Dahshouri [53] also reported that the application of Zn foliar significantly increased the nutritional status of the plant and the content of grain protein, Zn, and Fe. Nitrogen supply limited root uptake and root-to-shoot translocation, as well as Zn remobilization, which reduced Zn accumulation in the grain [66]. The partition of the entire plant and the Zn studies in wheat revealed that the suboptimal nitrogen supply limited root uptake and root-to-shoot translocation, as well as Zn remobilization, which reduced the accumulation of Zn Zn in the grain [63, 66]. Similarly, Wang [67] observed that in winter wheat three foliar applications of 0.3% $ZnSO_4$ alone and with 1.7% urea during the early milking stage significantly increased grain protein along with the concentration of P, K and Zn in the grain. The supply of N is beneficial for improving the nutrition of grain Zn and the quality of human dietary [64].

Improving plant N nutrition can contribute to grain Zn and Fe concentrations by affecting the levels of Zn or Fe chelating nitrogenous compounds required for the transport of Zn and Fe within plants and/or the abundance of Zn or Fe transporters needed for the uptake of the roots and the loading of Zn and Fe phloem. Kutman [66] reported that N nutrition is critical in both the uptake and translocation of Zn and Fe to wheat grain and showed that at a high rate, nearly 80% and 60% of the total Zn and Fe shoots, respectively. Cakmak [68] reported that nitrogen management represents an effective agronomic tool to contribute to grain Zn and Fe concentrations. Increased foliage of plants due to nitrogen application can improve the uptake of micronutrients. Facilitated uptake and transport of Fe and Zn is attributed to the presence of metal chelating compounds such as 2-deoxymuginic acid, which is responsible for the translocation of Fe and Zn from wheat flag leaves to grains [69].

5. CONCLUSIONS

The interaction between the late application rate of nitrogen fertilizer and the foliar application of Fe and/or Zn had a better effect on grain protein content and grain iron and zinc concentration than the late application of nitrogen fertilizer or the foliar application of Fe and/or Zn alone. The result showed that the late application 50 kg of urea / ha in booting combined with the foliar application of Fe at 12 kg $FeSO_4.7H_2O$ /ha+ Zn at 10 kg $ZnSO_4.7H_2O$ /ha had the highest grain protein content (GPC) and grain iron (Fe) concentration of bread wheat. However, the late application of 50 kg of urea / ha in booting combined with the foliar application of Zn at 10 kg of $ZnSO_4.7H_2O$ /ha had the highest grain zinc concentration (Zn).

Therefore, it can be concluded that:

- Late application of nitrogen fertilizer at 50 kg of urea / ha, in combination with the foliar application of Fe and Zn in the booting, considerably improved the grain protein content and grain Fe.
- Late application of nitrogen fertilizer at 50 kg urea / ha in the booting in combination with the foliar application of Zn in the booting significantly improved the grain Zn.

DISCLAIMER (ARTIFICIAL INTELLIGENCE

No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Appendix Table 1. Analysis of variance for the effect of nitrogen, Zn and Fe fertilizer application on grain protein content, Fe and Zn grain concentration of bread wheat

		Mean square		
S.O. V	**Df**	**GPC**	**Grain concentration**	
			Fe	**Zn**
Replication	2	1.314	7.556	0.220
Location	1	1.461ns	47.808**	140.504**
Rate of nitrogen	2	6.509**	15.651ns	37.534**
Error (a)	4	0.0.319	2.328	0.420
Foliar of Fe/Zn	3	1.277*	108.008**	78.000**
Rate of nitrogen*Foliar of Fe/Zn	6	1.297**	6.238*	2.736**
Error (b)	18	0.268	2.003	0.596
CV % (a)		1.9	1.9	1.1
CV % (b)		3.5	3.6	2.6.

S.O. V=Source of Variation, Df =Degree of freedom, Cv= Coefficient of Variation
GPC= Grain protein content, Fe= Iron and Zn= Zinc
***=Significant at 1%, *=Significant at 5% and ns=Non-significant*

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