

INTERACTIVE EFFECTS OF ZINC AND COPPER ON WHEAT YIELD AND QUALITY UNDER MANGANESE-DEFICIENT AND SUFFICIENT SOILS

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(Received : 15 March 2024; Accepted : 30 March 2024)

SUMMARY

Wheat stands as a cornerstone of global food security. To increase its productivity as well as quality, nutrient management is one of the most important factors. To evaluate the potential contribution of micronutrients to enhancing wheat yield, an experiment was conducted to investigate the interactive effect of zinc and copper on yield and quality indicators (protein, sedimentation value) of wheat under manganese-deficient and sufficient soils in potted plants during the rabi season 2020-21. The experiment was laid out in CRD with three replications and treatments comprised of Zn and Cu levels each @ 0, 2.5, 5.0, 7.5, and 10.0 mg kg⁻¹ applied through ZnSO₄ & CuSO₄. The results revealed a significant increase in both grain and straw yield, as well as the quality of wheat over their respective control with the application of Zn and Cu. The highest wheat grain yield (6.24 g per pot) was achieved in soils with sufficient Mn when treated with Zn at 10.0 mg kg⁻¹ and Cu at 10.0 mg kg⁻¹. It was further observed that the significant increase in protein content was noticed only up to Cu_{7.5} when compared with Cu_{2.5}, and a further increase in Cu level from Cu_{7.5} to Cu_{10.0} did not affect the protein content significantly in Mn-sufficient soil. The treatments with the best economic aspect were found 5.0 mg kg⁻¹ of zinc sulfate x 7.5 mg kg⁻¹ of copper sulfate, in terms of using less zinc and copper fertilizers and having a favorable impact on the attributes. In conclusion, the application of micronutrients like Zn and Cu should be considered an integral part of a holistic nutritional approach to support optimal wheat growth and its quality. This approach enables farmers to achieve the highest potential yield and quality in their wheat crops.

Key words: Micronutrients, wheat yield, protein, sedimentation value, Mn deficient and sufficient soil

India is the world's second-largest wheat producer, with wheat under cultivation on approximately 31.4 million hectares producing 110.55 mt of wheat at a productivity rate of 3520 kg/ha (USAD, 2024). Wheat (*Triticum aestivum* L.) is the major crop in India, which is mainly cultivated for grain production (Panwar and Arya, 2016). It is an important staple food crop of our country, commonly cultivated and eaten worldwide, and also one of the most important cereal crops in mid Himalayan region (Katoch *et al.*, 2020).

Micronutrients play integral roles in numerous essential physiological processes within plants. Their importance increases due to their role in plant nutrition and increasing soil productivity (Zain *et al.*, 2015).

Zinc is a vital micronutrient essential for both plant and animal/human systems. It plays a central role in physiological processes such as enzyme

synthesis, metabolic functions, protein production, and chlorophyll synthesis in plants. Additionally, zinc acts as a critical coordinator of immune responses in animals and humans (Singh *et al.* 2018). Magnesium is an important micronutrient; it is required for biological redox system, enzyme activation, and carrying oxygen in nitrogen fixation, (Staiger *et al.*, 2006). According to Li *et al.* (2007), both of zinc and magnesium are highly effective in increasing the quantity and quality of wheat grain yield.

Copper as an essential micronutrient for normal growth and metabolism of plants is well documented (Ghani and Naheed, 2011), and as a micronutrient, plays a critical role in various physiological functions, particularly as a component of numerous enzymes involved in electron transport and catalyzing redox reactions within the mitochondria and chloroplasts (Hansch and Mendel, 2009). These

micronutrients help in chlorophyll formation, nucleic acid, protein synthesis and play an active role in several enzymatic activities of photosynthesis as well as respiration (Reddy, 2004).

Micronutrient deficiencies can lead to a wide range of alterations in normal plant growth and development, (Langridge, 2022). Micronutrient deficiency is prevalent across various Asian countries, primarily stemming from factors such as the calcareous nature of soils, high pH, low organic matter content, salt stress, continuous drought, high bicarbonate content in irrigation water, and imbalanced application of NPK fertilizers (Narimani *et al.* 2010). A recent analysis of Indian soils has revealed that there is, on average, 51.2% deficiency in Zinc (Zn), 11.4% deficiency in Copper (Cu), and 17.4% in Manganese (Mn), (Shukla *et al.* 2021). Consequently, there is a pressing need for a systematic study of the interaction and association of Zinc and Copper to improve nutrition, enhance crop yields, and improve their quality.

MATERIALS AND METHODS

An experiment was conducted in a controlled environment (screen house) at CCS Haryana Agricultural University to investigate the interactive effect of zinc and copper on yield and quality indicators (protein, sedimentation value) of wheat under manganese-deficient and sufficient soil during the Rabi 2020-21. Soil samples from a depth of 0-15 cm were collected from various locations at the Soil Research Farm of CCS HAU and Balsamand Village in Hisar and were subsequently analyzed in a laboratory to determine their properties. This experiment was set up using a Completely Randomized Design (CRD) and involved the application of five levels of Zn (0, 2.5, 5.0, 7.5, and 10.0 mg kg⁻¹) through ZnSO₄ 7H₂O and five levels of Cu (0, 2.5, 5.0, 7.5, and 10.0 mg kg⁻¹) through CuSO₄ 5H₂O. Each pot was planted with ten seeds of the wheat variety WH 1105 and replicated three times. A total of 30 pots were filled with 4 kg of thoroughly mixed, air-dried soil. Thinning of the wheat plants was conducted on the 15th day after seeding, reducing the number to four plants per pot, and these were allowed to grow until maturity. The pots were irrigated with de-ionized water as needed. After the wheat harvest, soil and plant samples were collected for further analysis.

The collected soil samples were air-dried, ground, and sieved through a 2 mm sieve before being thoroughly mixed and stored in polythene bags. These

processed soil samples were used for both the screen house and laboratory studies. The physico-chemical characteristics of the soil, including texture, pH, electrical conductivity (EC), organic carbon, calcium carbonate, DTPA-extractable micronutrients, namely zinc (Zn), copper (Cu) and manganese (Mn), were analyzed. Plant samples were ground and stored separately for subsequent chemical analysis.

To determine yield, after harvesting, the crop from each pot was threshed separately. Grain yield from each pot was recorded and computed as g pot⁻¹. Straw yield for each pot was worked out by subtracting the grain yield from the total biological yield of individual pot & computed as g/pot.

The protein content in grain was computed by multiplying total N with a factor of 6.25.

The sedimentation test is based on the fact that gluten protein absorbs water and swells considerably when treated with Sodium dodecyl sulphate (SDS) lactic acid reagent. The extent of swelling depends upon quality of gluten protein. It was determined by the method described by Axford *et al.* (1979). The required mixture of SDS and lactic acid was prepared by dissolving 20 g of SDS in one litre of distilled water. Later, 20 ml of diluted lactic acid (prepared by diluting one part by volume of lactic acid with eight parts by volume of distilled water) was added. Contents were shaken until homogeneous mixture was obtained. Whole meal (atta) was prepared by passing wheat grains through a Tecator Cyclotec mill fitted with 0.5 mm screen.

RESULTS AND DISCUSSION

Initial soil physio-chemical characteristics

The data presented in (Table 1) highlights the contrasting soil textures in the manganese (Mn) deficient and Mn sufficient soils. In the Mn deficient soil, the predominant texture is sandy, with a composition of 94% sand, 4% silt, and 2% clay. Conversely, the Mn sufficient soil exhibits a sandy loamy texture, composed of 67% sand, 19% silt, and 14% clay. In terms of soil properties, the pH of the Mn deficient soil was measured at 8.2, with an electrical conductivity (EC) of 0.10 ds/m, whereas the Mn sufficient soil showed a pH of 8.4 and an EC of 0.30 ds/m. The organic carbon content in the Mn deficient soil was recorded at 0.18%, while the Mn sufficient soil displayed a higher organic carbon content of 0.47%. Regarding DTPA-extractable

TABLE 1
Initial physico-chemical characteristics of experimental soils with their methods

Characteristic	Soils		Method
	Mn deficient	Mn sufficient	
Sand (%)	94	67	International pipette method (Piper, 1966)
Silt (%)	04	19	
Clay (%)	02	14	
Soil texture	Sand	Sandy loam	
pH (1:2)	8.2	8.4	Jackson (1973)
EC (dS/m)	0.10	0.30	Richard (1954)
Organic carbon (%)	0.18	0.47	Walkley and Black (1934)
Calcium carbonate (%)	Tr	Tr	Puri (1949)
DTPA-extractable micronutrient (mg/kg)			
Zn	0.40	0.85	Lindsay and Norvell (1978)
Cu	0.16	0.44	
Mn	1.50	2.98	

micronutrient levels, the Mn deficient soil exhibited concentrations of 0.40 mg/kg for Zn (zinc), 0.16 mg/kg for Cu (copper), and 1.50 mg/kg for Mn (manganese). In contrast, the Mn sufficient soil showed higher concentrations, with 0.85 mg/kg for Zn, 0.44 mg/kg for Cu, and 2.98 mg/kg for Mn.

Effect of zinc and copper on wheat yield and quality indicators

Grain and straw yield of wheat

The data regarding to grain and Straw yield (Fig. 1 and 2) indicated that different levels of Zn and Cu exerted a significant effect on grain yield and straw yield in both Mn deficient and sufficient soils over their respective controls. In Mn deficient soil (Fig. 1), the grain yield of wheat increased significantly (3.93-4.80 g/pot) with increasing levels of zinc ($Zn_{2.5}$ to $Zn_{10.0}$) over control (2.83 g/pot). Amongst the treatment of Zn levels, grain yield was influenced significantly when Zn level increased from $Zn_{2.5}$ to $Zn_{5.0}$ and $Zn_{5.0}$ to $Zn_{7.5}$. Further increase in Zn level from $Zn_{7.5}$ to $Zn_{10.0}$ did not influence the yield significantly and they were statistically at par. Regarding Cu treatment, more or less same result, the grain yield was also significantly increased (4.07-4.33 g/pot) with increased level of Cu over control (3.89 g/pot). Amongst the treatment of Cu levels, grain yield was influenced significantly when Cu level increased from $Cu_{2.5}$ to $Cu_{5.0}$ and further increase in Cu level either from $Cu_{5.0}$ to $Cu_{7.5}$ or $Cu_{7.5}$ to $Cu_{10.0}$ did not result in significant increase in grain yield. The interaction between zinc and copper was found to be significant; the lowest yield (2.74 g/pot)

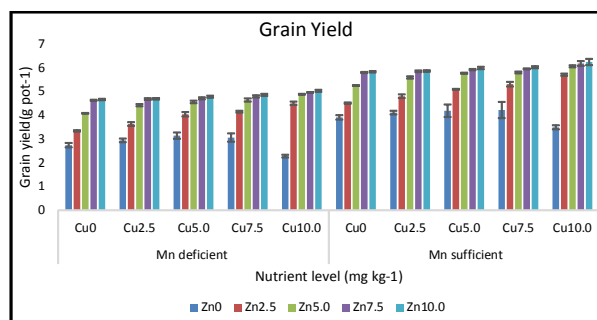


Fig. 1. Effect of Zn and Cu application on grain yield (g/pot) of wheat in Mn deficient and sufficient soils.

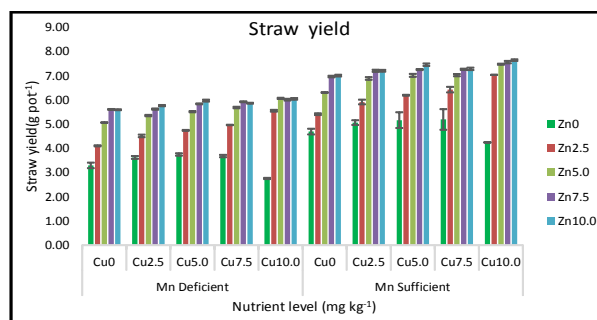


Fig. 2. Effect of Zn and Cu application on straw yield (g/pot) of wheat in Mn deficient and sufficient soil.

was recorded under the treatment Zn_0Cu_0 whereas the highest yield 5.03 g/pot was noticed with the treatment combination of $Zn_{10.0}Cu_{10.0}$. In case of Mn sufficient soil (Fig. 1), grain yield of wheat was significantly increased (5.08-5.99 g/pot) with increasing level of zinc over control (3.99 g/pot). Amongst the treatments, significant increase in yield was recorded between $Zn_{2.5}$ to $Zn_{5.0}$ and $Zn_{5.0}$ to $Zn_{7.5}$. The grain yield was also recorded to be significant (5.24-5.54 g pot⁻¹) with increased level of Cu over control (5.06 g/pot). Amongst Cu treatments, the significant increase was

observed between Cu_{2.5} to Cu_{5.0}, further wasn't found significant outcome and best economic result was with Cu_{5.0}. More or less same trend was observed in case of straw yield of wheat in the experimental soil. Straw yield varied significantly in both Mn deficient and sufficient soils (Fig. 2) with increasing levels of Zn and Cu over their respective controls. In Mn deficient soil, straw yield varied from 3.42 to 5.85 and 4.73 to 5.28 g pot⁻¹ under the treatment of Zn and Cu application, respectively. Among different levels of Zn, the highest straw yield (5.85 g pot⁻¹) was recorded at Zn_{10.0}, but the significant increase was only up to Zn_{7.5} over control (3.42 g pot⁻¹). The interaction between zinc and copper was also found to be significant. The response concerning Cu treatments in case of straw was found to be significant up to Cu_{5.0} and thereafter the values were statistically at par. In Mn sufficient soil (Fig. 2) also showed that straw yield increased significantly with Zn and Cu levels of application over their respective controls, however amongst various levels of Zn and Cu, it increased with increasing levels but significant increase in case of Zn treatment was up to 7.5 mg kg⁻¹ while in case of Cu treatments, it was recorded only up to 5.0 mg kg⁻¹ of its application. Significant Zn x Cu interaction was also observed for Mn sufficient soil. The result showed that the treatments with the best economic aspect were found 7.5 mg kg⁻¹ of zinc sulfate x 5.0 mg kg⁻¹ of copper sulfate, in terms of using less zinc and copper fertilizers and having a favorable impact on the attributes (grain and straw). The observations attest to earlier findings by Nadim *et al.* (2011) who reported that use of micronutrients significantly affected wheat yield. The

findings of our study align closely with those reported by Kumar *et al.* (2009), who investigated the impact of copper on the growth, yield, and concentration of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in wheat plants (*Triticum aestivum* L.) through pot experiments, and applied copper at various levels ranging from 0 to 2.5 mg/kg. Notably, our results corroborate theirs, indicating that the grain yield reached its peak at a copper application level of 1.5 mg/kg. The results are strongly supported by Asad and Rafique (2000), also Zeidan *et al.* (2010) who indicated that wheat grain yield and straw yield were significantly increased by application of Zn over control. This might be due to their critical role in crop growth, involving in photosynthesis processes, respiration and other biochemical and physiological activates and thus their importance in achieving higher yields. Keram *et al.* (2013) also reported that the straw yield of wheat, was significantly increased with the application of zinc. This was perhaps due to an abundant supply of Zn nutrition and balanced NPK, which increased the protoplasmic constituents, accelerates the process of cell division and elongation, photosynthesis processes, respiration, nitrogen metabolism-protein synthesis, other biochemical and physiological activates. Firdous *et al.* (2018) also found that Grain and straw yield of wheat significantly increased with application of Zinc. The increase in the grain yield is attributable to the improved physiology of plants with the added Zn consequently correcting the efficiency of different enzymes, chlorophyll content, IAA hormone and improvement in nitrate conversion to ammonia in plant leading to higher yield (Abbas *et al.* 2010).

TABLE 2
Effect of Zn and Cu application on protein content (%) of wheat in Mn-deficient and Mn-sufficient soils

Nutrient level (mg/kg)	Cu ₀	Cu _{2.5}	Cu _{5.0}	Cu _{7.5}	Cu _{10.0}	Mean
Mn deficient						
Zn ₀	10.08	10.08	10.24	10.30	10.56	10.25
Zn _{2.5}	10.25	10.45	10.56	10.63	10.75	10.56
Zn _{5.0}	10.65	10.71	10.75	10.63	10.69	10.69
Zn _{7.5}	10.50	10.69	10.69	10.81	10.88	10.69
Zn _{10.0}	10.50	10.75	10.75	10.94	11.06	10.81
Mean	10.38	10.56	10.63	10.69	10.81	-
C. D. (P=0.05)	Zn: 0.08		Cu: 0.08		Zn x Cu: 0.18	-
Mn sufficient						
Zn ₀	10.31	10.56	10.81	10.94	10.94	10.13
Zn _{2.5}	10.38	10.81	10.94	11.00	11.13	10.38
Zn _{5.0}	10.50	11.00	11.06	11.19	11.31	10.63
Zn _{7.5}	10.88	10.88	11.19	11.38	11.56	10.38
Zn _{10.0}	11.00	11.25	11.56	11.81	11.88	11.63
Mean	10.13	10.44	10.75	10.88	10.94	-
C. D. (P=0.05)	Zn: 0.10		Cu: 0.10		Zn x Cu: 0.23	-

Protein and sedimentation value by wheat

The protein content of wheat grain in Mn deficient soil (Table 2) increased significantly with increasing levels of Zn and Cu over their respective controls. Amongst the levels of Zn and Cu, protein content differed significantly with increased levels of Zn and Cu only up to Zn_{5.0} and Cu_{5.0} and further increase in both elements did not affect the protein content significantly. There was significant interaction between Zn and Cu for protein content. The lowest protein content (10.25%) was observed with Zn₀ (no zinc). The magnitude of increase in protein content was 3.0, 4.3, 4.3, and 5.5% over control with 2.5, 5.0, 7.5 & 10.0 mg Zn kg⁻¹ application, respectively. The magnitude of increase in protein content was 1.7, 2.4, 3.0, and 4.1% over control with 2.5, 5.0, 7.5 & 10.0 mg Cu kg⁻¹, respectively. It was further observed that there was not much variation in the content of protein as a result of Zn or Cu application in Mn deficient soil.

The protein content of wheat grain in Mn-sufficient soil (Table 2) increased significantly (10.38-11.63% and 10.44 to 10.94%) with increasing levels of Zn and Cu application up to its highest level over controls (10.13%). For Zn application, a significant increase in protein content was noticed at Zn_{5.0} and Zn_{10.0} levels when compared with Zn_{2.5} and Zn_{7.5}, respectively. There was significant interaction between Zn and Cu for protein content. The percent increase in protein content was 2.5, 4.9, 2.5, and 14.8 at 2.5,

5.0, 7.5, and 10 mg Zn/kg, respectively over control. It was further observed that the significant increase in protein content was noticed only up to Cu_{7.5} when compared with Cu_{2.5} and further increase in Cu level from Cu_{7.5} to Cu_{10.0} did not affect the protein content significantly. The lowest protein content (10.13%) was observed with control (no copper). The same was true for Zn application. However, the protein content of wheat grain was more pronounced with Cu application as compared to Zn application. The percent increase in protein content was to the tune of 3.1, 6.1, 7.4 and 8.0 at 2.5, 5.0, 7.5 and 10.0 mg Cu kg⁻¹, respectively over control. The value for protein content was observed to be slightly higher with Zn (10.13 to 11.63%) and Cu (10.13 to 10.94) application in Mn sufficient soil as compared to Mn deficient soil (10.25 to 10.81 and 10.38 to 10.81% with Zn and Cu application, respectively). A positive impact of Zn and Cu application on protein content has been reported by (Niyigaba *et al.* 2019; Chaure *et al.* 2019; Bhatt *et al.* 2020; Sharafi *et al.* 2021; Ziaieian and Malakouti 2001; Habib, 2009; Afzal *et al.* 2017). In another investigation, Pandey and Kumar (2017) observed that protein percentage in wheat grain and straw increased from 11.9 to 12.8 and 3.3 to 3.6%, respectively with 5 kg Zn ha⁻¹. The rise in protein content resulting from zinc addition can be ascribed to its role in the nitrogen metabolism of plants, (Mishra *et al.* 2017). Similarly, Ramzan *et al.* (2020) reported that bio-fortification with zinc and iron improves the grain quality and yield of wheat crop. The improvement in components of

TABLE 3
Effect of Zn and Cu application on sedimentation value (mL) of wheat in Mn deficient and sufficient soils

Nutrient level (mg/kg)	Cu ₀	Cu _{2.5}	Cu _{5.0}	Cu _{7.5}	Cu _{10.0}	Mean
Mn deficient						
Zn ₀	36.2	38.1	40.6	43.2	44.3	40.5
Zn _{2.5}	38.6	40.4	43.9	44.6	46.2	42.7
Zn _{5.0}	40.1	44.1	45.5	46.4	47.2	44.7
Zn _{7.5}	41.3	46.3	47.6	50.2	51.4	47.4
Zn _{10.0}	41.6	48.4	51.1	51.6	52.2	49.0
Mean	39.6	43.5	45.7	47.2	48.3	-
C. D. (P=0.05)	Zn: 1.8		Cu: 1.8		Zn x Cu: 6.6	-
Mn Sufficient						
Zn ₀	43.1	44.3	45.1	47.3	48.2	45.6
Zn _{2.5}	45.2	47.2	48.6	49.3	50.2	48.1
Zn _{5.0}	46.3	48.4	51.3	51.8	52.2	50.0
Zn _{7.5}	48.3	51.1	52.6	53.2	53.6	51.8
Zn _{10.0}	50.1	52.4	54.0	54.3	54.7	53.1
Mean	46.6	48.7	50.3	51.2	51.8	-
C. D. (P=0.05)	Zn: 1.5		Cu: 1.5		Zn x Cu: 3.4	-

yield of wheat crop might be due to involvement of Zn and Fe and their critical role in biochemical process including photosynthesis. Dhaliwal *et al.* (2023) also found that the categorization of existing genotypes in wheat toward Zn and Mn application is extremely important to select the most efficient Zn as well as Mn genotype with higher yield and quality. In another study, Tao *et al.* (2018) found that zinc fertilizer significantly increased grain protein content and yield in both wheat cultivars ($P < 0.05$). Compared with all other Zn treatments, the highest grain protein content and yield achieved was observed in the Zn₁₅ treatment in both wheat cultivars.

The sedimentation value of wheat in Mn deficient soil (Table 3) increased significantly (42.7-49.0 mL) with increasing level of Zn over control (40.5 mL). Amongst the treatments, the significant increased trend was also recorded between each successive levels of Zn. Similarly, the sedimentation value also increased significantly (43.5-48.3 mL) with increasing level of Cu over control (39.6 mL), but among treatments significant increase in sedimentation value was recorded between Cu_{2.5} to Cu_{5.0} and further increase in Cu level from Cu_{5.0} to Cu_{7.5} and Cu_{7.5} to Cu_{10.0} did not influence the sedimentation value. There was significant interaction between Zn and Cu to sedimentation value. The percent increase in sedimentation value was 5.4, 10.4, 17.0 and 21.0 at 2.5, 5.0, 7.5 and 10.0 mg Cu kg⁻¹, respectively over control. More or less similar trend for sedimentation value with respect to level of significance was noticed with Zn and Cu application in Mn sufficient soil as it was observed in Mn deficient soil. However, as in case of protein content higher sedimentation values were also recorded in Mn sufficient soil with Zn (45.6 to 53.1 mL) and Cu application (46.6 to 51.8 mL) as compared to Mn deficient soil (40.5 to 49.0 and 39.6 to 48.3 mL, respectively). The percent increase for sedimentation value was 5.4, 9.6, 13.5 and 16.4 at 2.5, 5.0, 7.5 and 10 mg Zn/kg, respectively over control. The corresponding values for Cu were 4.5, 7.9, 9.9 and 11.1, respectively. These results are in line with those obtained by (Sobolewska *et al.* 2020; Forrã *et al.* 2017). Peck *et al.* (2008), who observed that Zn nutrition influences the protein composition of flour in bread wheat (*Triticum aestivum* L.) and used two varieties of bread wheat at six rates of Zn, including foliar sprays of Zn. The results demonstrate that Zn nutrition can alter protein composition and the effects of Zn may interact with grain filling temperatures. Stepien and Wojtkowiak. (2016) who

reported that extra Mn fertilization significantly increased protein and gluten content, Zeleny sedimentation index, and grain hardness. As a result of foliar fertilization with Cu, Mn, and a combination of micronutrients, the content of ω , α/β , and γ gliadins increased. Fertilization with Cu increased the content of low molecular weight glutenins, and fertilization with Zn and Mn increased high molecular weight and low molecular weight glutenin fractions. Applying a combination of micronutrients increased the content of high molecular weight glutenins, but in contrast, Jankowski *et al.* (2016) studied the yield and quality of winter wheat (*Triticum aestivum* L.) in response to different systems of foliar fertilization and concluded that intensified foliar fertilization significantly lowered total protein content, gluten content and protein quality (lower sedimentation value) in winter wheat grain.

CONCLUSION

The grain and straw yield of wheat increased significantly with increasing levels of either Zn or Cu over their respective controls and also the yield was more pronounced with the application of Zn as compared to Cu in both soils. The highest grain yield and straw yield (6.24 g/pot & 7.64 g/pot) were recorded under Zn₁₀Cu₁₀ in Mn-sufficient soil. Increasing copper from Cu_{2.5} to Cu_{5.0} significantly boosted grain yield, but further increases to Cu_{7.5} or Cu_{10.0} didn't enhance it in Mn-deficient soil. Similarly, the effects of Cu or Zn application hinted a significant gain in protein percentage and sedimentation value of wheat grain under both soil Mn conditions over their respective controls. The sedimentation values obtained in Mn sufficient soil at various levels of Zn or Cu were higher as compared to Mn deficient soil. The treatments with the best economic aspect were found 5.0 mg/kg of zinc sulfate x 7.5 mg/kg of copper sulfate, in terms of using less zinc and copper fertilizers and having a favorable impact on the attributes. It was concluded that micronutrient applications such as Zn and Cu need to be seen as part of a balanced nutrition approach to support plant growth so farmers can achieve the highest yield and quality potential in wheat crops.

ACKNOWLEDGEMENT

First author Ms. Khatera Qane gratefully acknowledge the Grain Project, funded by USAID and implemented by Michigan State University, for

providing fellowship support during her M.Sc. degree program.

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