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Enhancing Wheat Growth and Yield through Foliar Application of Zinc in Combination with Methionine, Histidine and Citric Acid as Chelators

Sudershan Mishra [a++*], Km. Anjali [b++], Megha Panwar [c++], Prachi Saini [d#] and Sudhir K Guru [d†]

[a] *Department of Agricultural Botany, GMV PG College, Rampur Maniharan, Affiliated to Maa Shakumbhari University, Saharanpur, Uttar Pradesh, 247451, India.*
[b] *Department of Agriculture, College of Applied Sciences, J.B. Institute of Technology, Chakrata Road, Shankarpur, Dehradun, Uttarakhand, 248197, India.*
[c] *Department of Agriculture, Doon (P.G) College of Agriculture Science and Technology, Dehradun, Uttarakhand, 248197, India.*
[d] *Department of Plant Physiology, College of Basic Sciences and Humanities, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, 263145, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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[++] *Assistant Professor;*
[#] *Research Scholar;*
[†] *Professor;*
**Corresponding author: E-mail: drsudershanmishra@gmail.com;*

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a crucial global food source, necessitating enhanced production to meet increasing demand. This study, conducted at GBPUA&T, Pantnagar, US Nagar, India, explores the impact of foliar-applied zinc (Zn) combined with chelators—methionine, histidine, and citric acid—on the growth and yield of wheat cultivar PBW343. Eight treatment combinations involving two Zn levels (1135 ppm and 1702.5 ppm) and three chelators were tested at three growth stages (30, 45, and 60 days after emergence). Results indicated that Zn application significantly increased flag leaf dimensions, total aboveground dry matter, grain yield, biological yield, thousand seed weight, and harvest index. Higher Zn concentration (1702.5 ppm) with methionine chelator yielded the maximum increases: flag leaf length and width increased by 6.2% and 5.7%, respectively; total dry matter increased by 1.6% and 2.6% at maximum tillering and flowering stages, respectively; grain yield and biological yield rose by 11.3% and 10.3% over control, respectively. The study underscores the efficacy of combining Zn with methionine to enhance Zn uptake, boost enzymatic activity, and improve overall wheat productivity. These findings provide valuable insights for optimizing nutrient management practices to ensure sustainable wheat production.

Keywords: Foliar application; nutrient cycling; biostimulants; zinc chelators; sustainability.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cornerstone of global food security, serving as one of the most widely cultivated and consumed cereal grains. The versatility of wheat allows it to be processed into various forms, including flour, bread, pasta, and numerous other products. This adaptability makes it an indispensable component of the global food supply chain. However, with the increasing global population and changing dietary preferences, there is a pressing need to enhance wheat production to meet rising demands [1]. Achieving this requires optimizing agronomic practices and improving nutrient management strategies to boost wheat growth and yield. Among the various strategies to enhance wheat production, micronutrient management, particularly zinc (Zn) supplementation, has garnered considerable attention [2]. Zinc plays a pivotal role in numerous physiological processes, influencing plant growth, development, and yield. Zinc's role extends to enhancing the plant's resistance to abiotic stresses such as drought and heat by stabilizing the structure of proteins and membranes and by maintaining the integrity of biological membranes. Additionally, zinc is involved in the detoxification of reactive oxygen species (ROS), which can cause cellular damage under stress conditions [3-6]. Therefore, ensuring adequate zinc availability is essential for optimizing plant health and productivity [7]. Despite its importance, zinc deficiency is a prevalent issue that severely affects crop productivity and food security globally. Zinc deficiency manifests in plants as stunted growth, chlorosis (yellowing of leaves), and reduced leaf size, ultimately leading to lower yields. In wheat, zinc deficiency is particularly problematic as it directly impacts both grain quality and quantity. Factors such as high soil pH, low organic matter content, and imbalanced fertilizer use exacerbate zinc deficiency by reducing its bioavailability in the soil [8]. Moreover, the bioavailability of zinc in soils often limits its efficacy when applied through traditional soil fertilization methods. Foliar application of zinc is an effective technique to address zinc deficiency. This method involves applying zinc-containing solutions directly to the leaves, allowing for rapid absorption through the cuticle and stomata. Foliar zinc application bypasses soil-related issues that limit nutrient availability, ensuring that zinc is delivered directly to the site of action [9]. This approach leads to quicker and more efficient uptake compared to soil application, ultimately enhancing plant growth, photosynthetic efficiency, and yield [10]. Methionine, histidine, and citric acid are organic compounds that can act as chelators, enhancing the uptake and utilization of zinc in plants. Chelators form stable complexes with metal ions, improving their solubility and mobility within the plant [11]. This chelating property can enhance the efficiency of zinc uptake when applied in combination with foliar zinc sprays. Amino acids, acting as natural ligands, have the ability to create stable complexes with metals such as Zn through the use of carboxylic groups. Furthermore, amino acids exert numerous advantageous effects on plants [12]. Amino acids are fundamental components of proteins and are involved in the creation of glutamine, which serves as a precursor for nucleotides, hormones, and small nitrogen-containing molecules. Several plants have demonstrated an increase in protein content with the application of amino acids [13,14]. Additionally, the movement of amino acids is highly significant in plants. Unlike reduced carbon, which is only transported by phloem, amino acids are transported through both phloem and xylem. Consequently, the retranslocation of amino acids facilitates the recycling of nitrogen between the roots and shoots, as well as expedites the movement of immobile nutritional components, such as zinc, inside the plant [15-17]. The re-translocation of Zn is crucial for foliar spray treatments, especially when the goal is to transport Zn to the grain. Foliar application of zinc, combined with chelating agents such as methionine, histidine, and citric acid, presents a promising alternative to enhance zinc uptake and utilization by plants. This study investigates the impact of foliar-applied zinc in combination with these chelators on the growth and yield of wheat.

2. MATERIALS AND METHODS

The pot culture experiment was conducted at the pot culture facility of the Department of Plant Physiology, GBPUA&T, Pantnagar, US Nagar, India. Pantnagar, located at an elevation of 243.84 meters above sea level, is characterized by a humid subtropical climate. In this study, the wheat variety PBW343 was sown in plastic pots, each with a capacity of 10 kg, in early November and harvested in early April. Eight treatment combinations were tested, involving two zinc application levels (1135 ppm and 1702.5 ppm) and three different chelators (0.025% Methionine, 0.02% Histidine, and 0.1% Citric Acid). These treatments were administered at three specific growth stages: 30, 45, and 60 days after emergence (DAE). Standard wheat cultivation techniques were followed, and foliar applications were performed using a portable sprayer. Flag leaf length and width were measured using a scale. Total dry matter accumulation at maximum tillering and flowering stages was recorded using a weighing balance. At harvest, data were collected on grain yield, biological yield, thousand seed weight and harvest index. Statistical analyses were performed using the R statistical package (Version 4.1.2). The correlations among parameters were evaluated using linear regression models, and correlation matrices were generated to understand the relationships between different variables. The significance of the treatment effects and their interactions on the measured parameters was assessed using analysis of variance (ANOVA), and standard error of means (S.Em.±) and critical difference (CD) was evaluated at 5% level of significance ($p \leq 0.05$) by following standard statistical procedure.

3. RESULTS AND DISCUSSION

3.1 Flag Leaf Surface Area

The results demonstrate a significant increase in both the length and width of the flag leaf at various zinc concentrations when chelators are incorporated into the treatment (Fig. 1; Table 1). At the lower concentration of 1135 ppm Zn, the flag leaf dimensions increased moderately. However, at the higher concentration of 1702.5 ppm Zn, there was a more pronounced increase in both length and width. Specifically, the flag leaf length increased by 6.2%, and the width increased by 5.7% at 1702.5 ppm Zn compared to 1135 ppm Zn. This indicates that higher zinc availability significantly enhances the development of the flag leaf. The addition of chelators further amplified the positive effects of zinc. Methionine as a chelator resulted in the maximum increase in both flag leaf length and width, followed by histidine and citric acid. Furthermore, a significant interaction effect between zinc concentrations and the addition of chelators was observed for both the length and width of the flag leaf. The maximum increase in flag leaf length and width was recorded at the higher zinc concentration of 1702.5 ppm when methionine was used as the chelator.

3.2 Dry Matter Accumulation

Paired comparison of the effect of different concentrations of zinc (Zn) with or without chelators on the total aboveground dry matter (g/m^2) of the wheat cultivar PBW343 at the maximum tillering and flowering stages is outlined in Fig. 2. The application of zinc led to a significant increase in total aboveground dry matter at both maximum tillering and flowering stages. When the concentration of zinc was increased from 1135 ppm to 1702.5 ppm, there was a notable increase of 1.6% in dry matter at the maximum tillering stage and a 2.6% increase at the flowering stage. This suggests that higher zinc availability enhances the plant's biomass

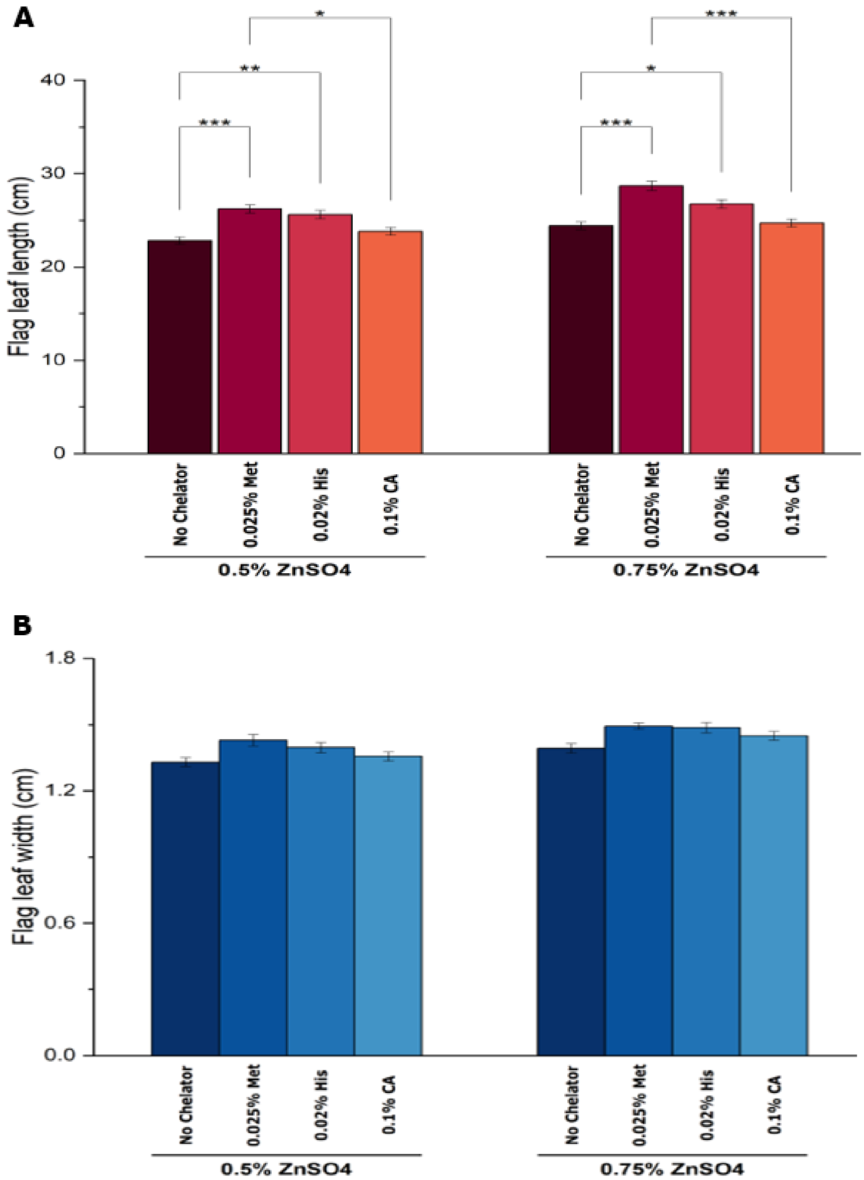

Fig. 1. Paired comparison plots - Effect of different concentrations of Zinc with or without chelators on flag leaf length (A) and flag leaf width (B) of wheat cultivar PBW343

*(The asterisks indicate the significance level ***- p<0.001, **- p<0.01, *- p<0.5.)*

production, likely due to improved enzyme activity and metabolic functions facilitated by zinc. he addition of chelators significantly amplified the effects of zinc on dry matter accumulation. Among the chelators tested, methionine was the most effective, leading to the highest increases in total aboveground dry matter at both growth stages. Histidine and citric acid also contributed to increased dry matter, but their effects were less pronounced compared to methionine. Interestingly, no significant interaction effects were observed between zinc concentrations and the addition of chelators for total aboveground dry matter at both maximum tillering and flowering stages (Table 2).

3.3 Yield and Other Related Attributes

The application of zinc led to significant increases in both grain yield and biological yield. When zinc concentration was increased from 1135 ppm to 1702.5 ppm, grain yield increased by 3.8% and biological yield increased by 1.9% (Fig. 3). This indicates that higher zinc availability enhances the plant's overall productivity and biomass accumulation, likely due to improved enzymatic functions and metabolic processes facilitated by zinc [10].

Similarly, thousand seed weight and harvest index also increased significantly with higher zinc concentration (Table 3). The increase in zinc concentration from 1135 ppm to 1702.5 ppm resulted in notable improvements in these parameters, indicating enhanced seed quality and overall crop efficiency. The addition of chelators significantly amplified the positive effects of zinc on grain yield, biological yield, thousand seed weight, and harvest index. Among the chelators tested, methionine was the most effective. The combination of 1702.5 ppm zinc with methionine resulted in the maximum grain yield, showing an 11.3% increase over the control, and the maximum biological yield, showing a 10.3% increase over the control. Significant interaction effects were also observed for thousand seed weight and harvest index. The optimal combination of 1702.5 ppm zinc with methionine produced the highest improvements in these parameters, demonstrating the combined effect of zinc and chelators in enhancing crop performance.

Table 1. Effect of different concentrations of Zinc with or without chelators on flag leaf length and width of wheat cultivar PBW343 (Sprays were done at 30, 45 and 60 DAE for all the treatments)

Chelators (A)	Flag Leaf Length (cm)			Flag Leaf Width (cm)		
	Zn Concentration (C, in ppm)			Zn Concentration (C, in ppm)		
	1135	1702.5		1135	1702.5	
Without Chelator	22.8 ± 0.67	24.4 ± 0.71		1.3 ± 0.04	1.4 ± 0.04	
0.025% Methionine	26.2 ± 0.76 (14.9%)	28.7 ± 0.84 (17.3%)		1.4 ± 0.05 (7.5%)	1.5 ± 0.03 (7.3%)	
0.02% Histidine	25.6 ± 0.75 (12.2%)	26.7 ± 0.78 (9.3%)		1.4 ± 0.04 (5.0%)	1.5 ± 0.04 (6.5%)	
0.1% Citric Acid	23.8 ± 0.70 (4.5%)	24.7 ± 0.72 (1.0%)		1.4 ± 0.04 (2.1%)	1.5 ± 0.03 (4.0%)	
	C	**A**	**C x A**	**C**	**A**	**C x A**
S.Em ±	0.019	0.027	0.038	0.013	0.018	0.026
C.D(P=0.05)	0.047	0.066	0.094	0.032	0.046	ns

Figures in parenthesis indicate per cent change with respect to control (Zn spray without chelator)

Table 2. Effect of different concentrations of Zinc with or without chelators on total aboveground dry matter at maximum tillering and flowering stages of wheat cultivar PBW343 (Sprays were done at 30, 45 and 60 DAE for all the treatments)

Concentrations of Chelators Used (A)	Total Dry Matter at Maximum Tillering (g/m2)			Total Dry Matter at Flowering (g/m2)		
	Zn Concentration (C, in ppm)			Zn Concentration (C, in ppm)		
	1135	1702.5		1135	1702.5	
Without Chelator	454 ± 4	459 ± 2		845 ± 28	878 ± 10	
0.025% Methionine	535 ± 5 (18.0%)	543 ± 9 (18.3%)		922 ± 12 (9.1%)	951 ± 32 (8.3%)	
0.02% Histidine	521 ± 2 (14.9%)	527 ± 9 (14.7%)		899 ± 29 (6.3%)	915 ± 15 (4.2%)	
0.1% Citric Acid	482 ± 11 (6.3%)	496 ± 2 (8.0%)		872 ± 28 (3.1%)	891 ± 34 (1.4%)	
	C	**A**	**C x A**	**C**	**A**	**C x A**
S.Em ±	1.692	2.393	3.384	3.005	4.250	6.010
C.D(P=0.05)	5.13	7.25	ns	9.11	12.89	ns

Figures in parenthesis indicate per cent change with respect to control (Zn spray without chelator)

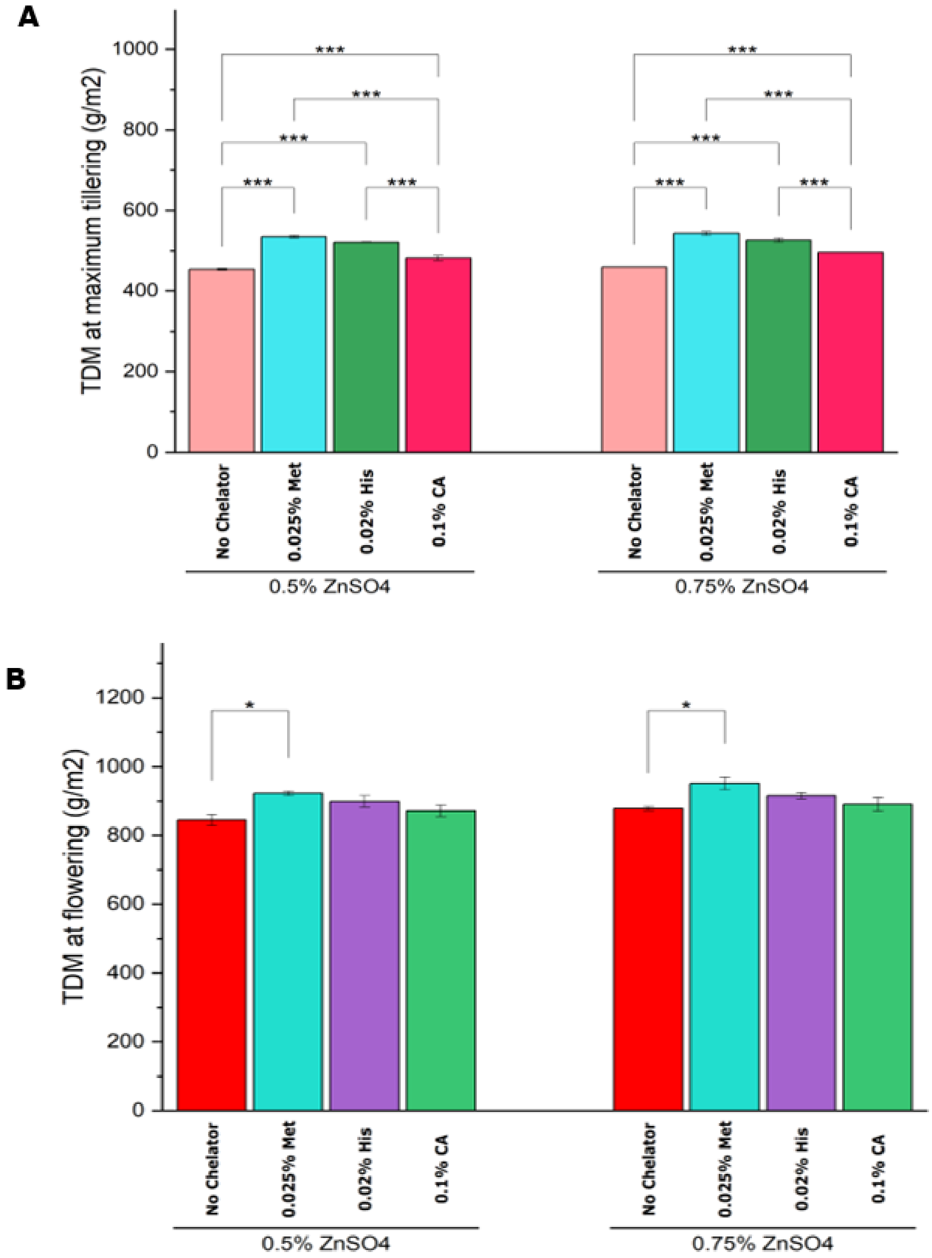

Fig. 2. Paired comparison plots - Effect of different concentrations of Zinc with or without chelators on total aboveground dry matter at maximum tillering (A) and flowering (B) stages of wheat cultivar PBW343

*(The asterisks indicate the significance level: ***- p<0.001, **- p<0.01, *- p<0.5.)*

3.4 Discussion

This study highlights the significant positive effects of combining zinc with chelators on the growth, yield and yield related traits of wheat cultivar PBW343. The scatterplot matrix depicting the associations between the different variables under study is presented in Fig. 4. Spraying zinc with chelators like methionine, histidine, and citric acid enhances zinc uptake and mobility within the plant. Increased zinc availability boosts enzymatic activity and chlorophyll synthesis, leading to larger flag leaf dimensions (length and width). The combination of 1702.5 ppm Zn with methionine produced the maximum flag leaf length and width. This synergy highlights the importance of selecting appropriate chelators to maximize the benefits of zinc fertilization in wheat. The flag leaf is a major photosynthetic organ in wheat, playing a crucial role in the

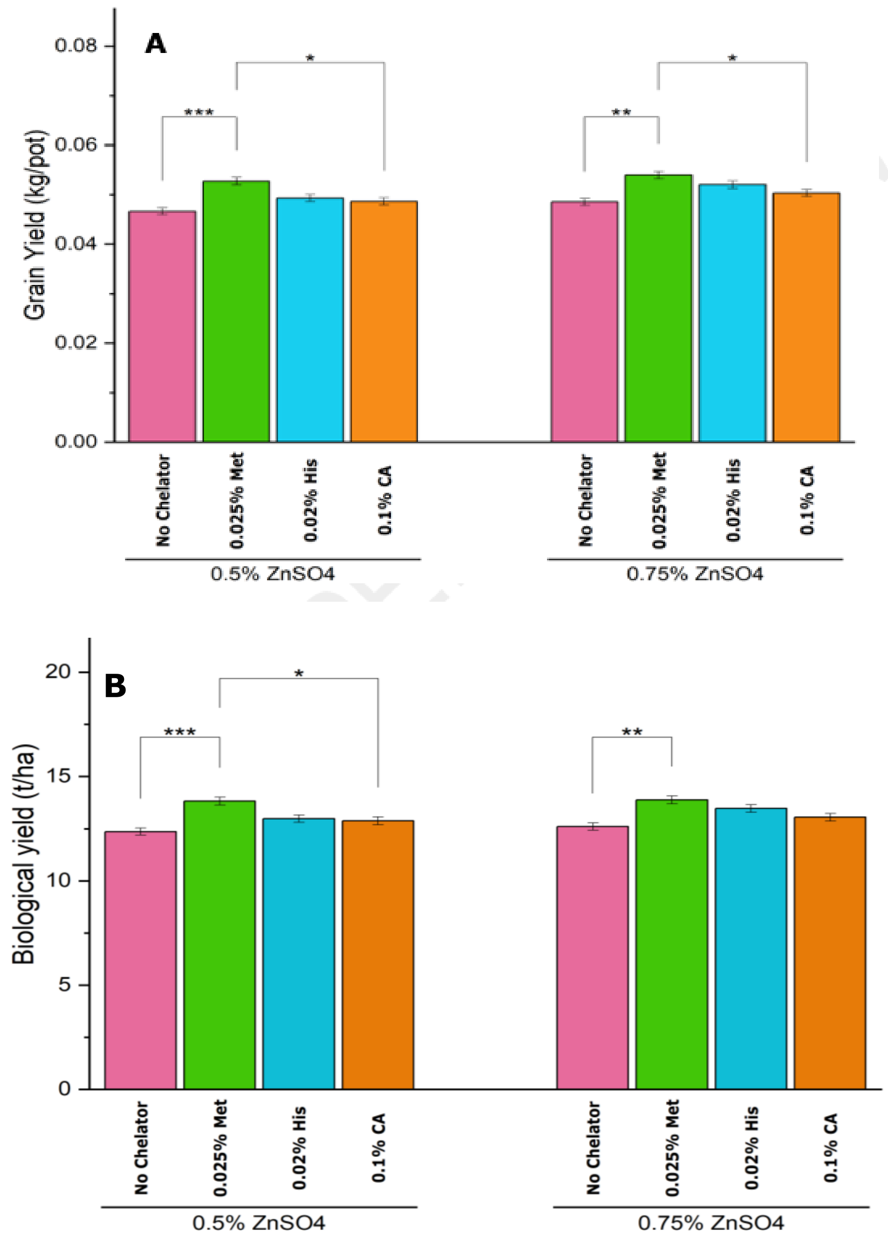

Fig. 3. Paired comparison plots - Effect of different concentrations of Zinc with or without chelators on grain yield (A) and biological yield (B) of wheat cultivar PBW343
*(The asterisks indicate the significance level: ***- p<0.001, **- p<0.01, *- p<0.5.)*

assimilation of carbon and the production of biomass. An increase in flag leaf area enhances the plant's photosynthetic capacity by increasing light interception. The increased photosynthetic activity leads to greater carbon assimilation, which is then converted into biomass, contributing to higher total aboveground dry matter. Similar increase in leaf area upon foliar application of amino acids have been reported by Ahmad et al. [18]. Increased total aboveground dry matter reflects better overall plant growth and vigor. This higher biomass is crucial for supporting reproductive structures and ensuring adequate grain production. The harvest index, which is the ratio of grain yield to total biological yield, improves with better resource allocation and higher grain yield. Similar enhancements in yield in wheat have been reported when foliar zinc was applied along with other amino acid chelates (Ghasemi et al., [14] and Biostimulants [8]. Efficient nutrient and carbohydrate utilization lead to a greater proportion of the plant's biomass being directed towards grain production. Among the chelators

Table 3. Effect of different concentrations of Zinc (with or without chelators) on Thousand Seed Weight (g) and Harvest Index (%) of wheat cultivar PBW343 (Sprays were done at 30, 45 and 60 DAE for all the treatments)

Chelators (A)	Thousand Seed Weight (g)			Harvest Index (%)		
	Zn Concentration (C, in ppm)			Zn Concentration (C, in ppm)		
	1135	**1702.5**		**1135**	**1702.5**	
Without Chelator	40.63 ± 0.33	41.20 ± 0.04		47.12 ± 0.07	48.05 ± 0.07	
0.025% Methionine	41.17 ± 0.10 (1.32%)	42.27 ± 0.08 (2.61%)		47.63 ± 0.07 (1.07%)	48.56 ± 0.07 (1.07%)	
0.02% Histidine	41.16 ± 0.05 (1.30%)	42.15 ± 0.05 (2.31%)		47.47 ± 0.07 (0.74%)	48.25 ± 0.07 (0.43%)	
0.1% Citric Acid	41.09 ± 0.09 (1.13%)	41.74 ± 0.27 (1.31%)		47.17 ± 0.07 (0.09%)	48.19 ± 0.07 (0.30%)	
	C	**A**	**C x A**	**C**	**A**	**C x A**
S.Em ±	0.043	0.060	0.085	0.004	0.006	0.008
C.D(P=0.05)	0.13	0.18	0.26	0.0006	0.0009	0.0013

Figures in parenthesis indicate per cent change with respect to control (Zn spray without chelator)

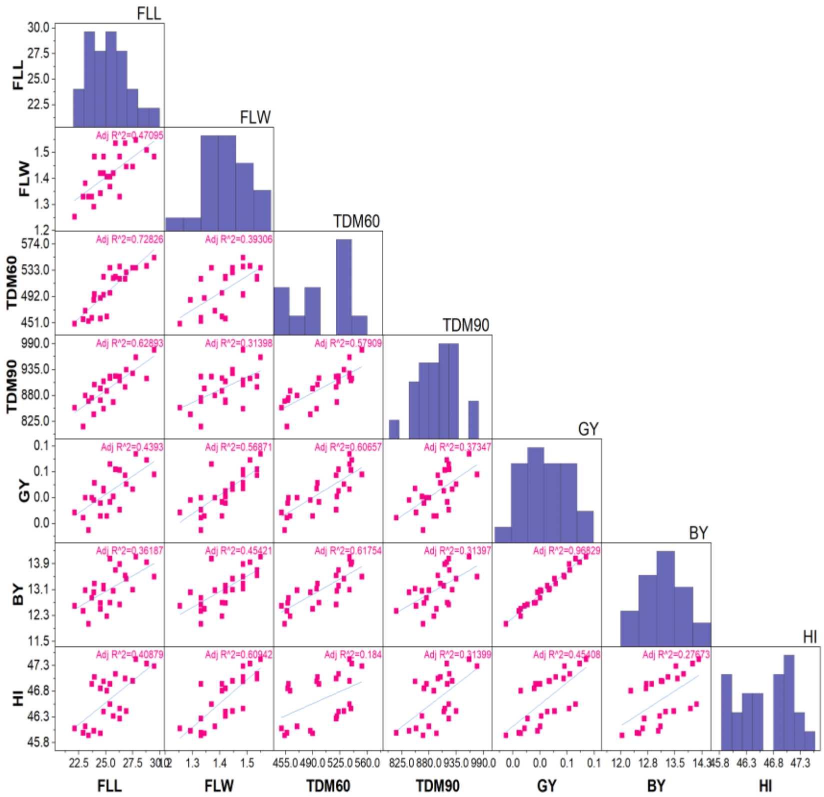

Fig. 4. Scatterplot matrix between different growth, yield and yield contributing factors in wheat cultivar PBW343

Different parameters are represented in their abbreviated notations viz. Flag Leaf Length (FLL), Flag Leaf Width (FLW), Total aboveground dry matter at maximum tillering (TDM60), Total aboveground dry matter at flowering (TDM90), Grain Yield (GY), Biological Yield (BY) and Harvest Index (HI) tested, methionine was the most effective. The combination of 1702.5 ppm zinc with methionine resulted in the maximum grain yield, showing an 11.3% increase over the control, and the maximum biological yield, showing a 10.3% increase over the control. Histidine and citric acid also contributed to increased dry matter and yield, but their effects were less pronounced compared to methionine. Methionine's superior chelating ability likely enhances zinc solubility and uptake, making it more available for the plant's physiological needs. Methionine, being an amino acid, also contributes to protein synthesis and other metabolic functions that support plant growth. Its superior performance as a chelator in this study may be due to its dual role in enhancing zinc bioavailability and participating in growth-promoting metabolic pathways.

4. CONCLUSION

The study revealed significant interaction effects between zinc concentrations and the addition of chelators for various growth parameters, highlighting the synergistic benefits of these combined treatments. Chelators increase the solubility and mobility of zinc, ensuring a steady supply of this micronutrient to the plant. Higher zinc concentrations, combined with effective chelators, optimize zinc availability and utilization, leading to significant improvements in yield parameters, including grain yield, biological yield, thousand seed weight, and harvest index. Methionine, in particular, demonstrated superior performance in enhancing the effectiveness of zinc applications. The synergistic effect of 1702.5 ppm Zn with methionine underscores the importance of selecting suitable chelators for optimizing zinc fertilization in wheat. It follows from the study that tailored nutrient management strategies that optimize zinc concentrations and chelator use can lead to more efficient nutrient utilization, reduced fertilizer wastage, and better environmental sustainability. Future investigations should be carried out to find out more specific chelators of zinc as well as to optimize the spray concentration of those chelators for specific agro-ecological and agro-pedological conditions. Moreover some specific Zn transporters particularly loading zinc in chelated forms need to be identified to increase the specificity of the retranslocation process.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that 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 manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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