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ORIGINAL RESEARCH ARTICLE

Enhancing rice yields through foliar application of essential micronutrients: A study on zinc, copper, and boron nutrition in context of Nepal

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ABSTRACT

Quantitative data on the effects of essential micronutrients—boron, zinc, and copper—on rice (*Oryza sativa* L.) are limited, which hampers optimized crop management strategies. This study aimed to enhance rice yields through the foliar application of these micronutrients. Conducted in 2022 at Gokuleshwor Agriculture and Animal Science College using a randomized complete block design with three replications, the research tested different combinations of these elements on the local rice variety Kaljade. The treatments included: T_1 (control), T_2 (zinc), T_3 (copper), T_4 (boron), T_5 (Zn + Cu + B), T_6 (Zn + Cu), and T_7 (Zn + B), with doses of 5 kg/ha for zinc and 2 kg/ha each for boron and copper. The results demonstrated significant effects of the micronutrient treatments on all parameters studied. Notably, the combination of zinc, copper, and boron (Zn + Cu + B) led to the most favorable outcomes. This treatment resulted in the shortest maturity duration (116.3 days after transplanting), the highest number of tillers (22.5), and the tallest plant height (107.7 cm). Additionally, Zn + Cu + B produced the greatest leaf area index (0.0724), the highest number of grains per panicle (22.6), and the heaviest thousand-grain weight (21.83 g). Furthermore, Zn + Cu + B achieved the highest biological yield (12.35 t/ha) and grain yield (5.9 t/ha), markedly higher than the control treatment (4.12 t/ha). These findings highlight the significant role of zinc, copper, and boron in rice cultivation. The study underscores the potential of foliar application techniques to optimize micronutrient availability, thereby enhancing rice yields. For future agricultural practices in the study area, focusing on zinc, copper, and boron nutrition is crucial to further augment crop productivity and ensure food security.

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INTRODUCTION

Rice (*Oryza sativa* L.), an essential agricultural commodity, sustains over 3.5 billion individuals globally by serving as a primary staple cereal crop (IRRI, 2017). Rice cultivation in Nepal covers an extensive area of 1.48 million hectares, boasting an average productivity of 3.47 tons per hectare and a remarkable total annual production of 5.13 million tons in 2021/22 (MOALD, 2022). Micronutrients are as important as macronutrients and involved in vital metabolic events in plants. The absence of a single vital micronutrient can disrupt developmental cascades, leading to a significant decrease in crop yield (Tripathi *et al*., 2015). Zinc is identified as a key limiting nutrient for rice production, the application of zinc in the soil assumes paramount importance to realize the full potential of rice yield (Rahman *et al*., 2011). The multifaceted significance of zinc as a crucial micronutrient for rice is evident through its pivotal role in governing diverse aspects of plant growth and metabolism, encompassing protein synthesis, enzyme activation, as well as carbohydrate, lipid, auxin, and nucleic acid metabolism (Chang *et al*., 2005). The prominent role of zinc in auxin synthesis makes its deficiency directly responsible for manifesting distinct symptoms in rice, including leaf distortion and a noticeable shortening of internodes (Irshad *et al*., 2004). The incorporation of zinc-containing fertilizer exerts a substantial influence on both rice grain yield and zinc content. Thus, adopting an appropriate application method at the right time and in a suitable manner becomes critical to ensure the efficient utilization of zinc by rice plants (Guo *et al*., 2016). Khaira disease is the most common zinc deficiency symptom in rice (Dubey *et al*., 2016). The deficiency symptoms of zinc become apparent approximately 2-3 weeks after transplanting, characterized by the appearance of brownish blotches and streaks on the leaves, causing stunted growth, and, in severe instances, leading to plant mortality (Wissuwa *et al*., 2006). Copper plays a vital role in facilitating the utilization of iron during the process of chlorophyll synthesis. Insufficient copper levels lead to the accumulation of iron within plant nodes. It has a unique involvement in enzyme systems of plants like oxidase enzymes, terminal oxidation by cytochrome oxidase, photosynthetic electron transport mediated by plastocyanin, etc. Furthermore, it serves as an "electron carrier" within enzymes, facilitating oxidation-reduction reactions essential for plant metabolic processes. Copper plays a pivotal role as an essential component of metalloenzymes, actively regulating enzyme activity and facilitating oxidative reactions. Additionally, copper is involved in crucial processes such as nitrogen exchange, protein, and hormone metabolism, as well as respiration and photosynthesis, further underscoring its significance in rice plant physiology. Copper deficiency in rice led to the restricted emergence of new leaves, resulting in reduced tillering and increased pollen sterility, while boron deficiency caused stunted growth and decreased the number of panicles (Dobermann & Fairhust 2000).

Boron (B) is reported to be involved in keeping cell wall structure and maintaining membrane function (Marschner, 1995). It is believed to improve the strength of the membrane and cell wall with the cross-linked polymer and strengthen the plant's vascular bundles which hold back the invasion of pathogens (Stangoulis, & Graham, 2007). Dobermann and Fairhurst (2000) also noted the function of B in enhancing lignin formation in rice. Different methods are used for micronutrient application such as seed priming, soil application, and fortification, but foliar application stands out as the most beneficial approach because it allows for direct absorption of micronutrients by the plants, resulting in improved nutrient uptake and overall plant health (Rehm, & Albert, 2006). Foliar application of nutrients seems helpful compared to soil amendments for efficient use of nutrients and to cure the visual deficiency problem quickly, providing rapid relief to the affected plants (Fageria, 2009). The micronutrient application minimizes Soil deficiency problems through foliar spray as compared to soil application (Modaihsh, 1997). Foliar fertilization with micronutrients is proved to be an effective strategy to remove the deficiency when soil application is not beneficial or fails to adequately address the nutrient requirements of the plants. Under field conditions, the application of zinc through the foliar method improves Zn concentration in edible parts (Cakmak, 2008). B can also be applied as foliage, particularly under water-deficient conditions, as a successful approach to overcome B deficiency and promote healthy plant growth (Mortvedt, 2000). The indispensable role of Zn, B, and Mn in plant metabolism is undeniable, as plants depend predominantly on these micronutrients, and their presence profoundly impacts various physiological and biochemical processes in plants, including maintaining cell wall integrity, synthesizing chlorophyll, proteins, and facilitating photosynthesis (Nadeem & Farooq, 2019).

The underutilization of micronutrients like zinc, copper, and boron in Nepalese rice cultivation has likely contributed to suboptimal yields and quality. While Nepalese farmers frequently apply macronutrients (N, P, and K), the application of micronutrients is not a common practice. Addressing this gap, the aim of this study was to investigate the effects of foliar application of zinc, copper, and boron on the growth and yield components of rice.

MATERIALS AND METHODS

Description of the experimental site

The field experiment was conducted in the Gokuleshwor Agriculture and Animal Science College's experimental farm in Dilasaini, Baitadi. It is located in the Baitadi district of Nepal, which is 811 meters above sea level. Geographically, it is positioned at 29˚40' North latitude and 80˚34' East longitude. This region experiences an average annual temperature of 22.87˚C and an average annual rainfall of 1037mm. The soil type present in the study area was sandy loam soil. Characteristics of soil in the experimental field are presented in Table 1.

Cultural operation

Following the meticulous seed-sowing process, which took place on June 30, the local rice variety (Kaljade) was subjected to pre-sowing treatment with Bavistin @2gm/kg, 24 hours before sowing (Chandrasekaran *et al*., 2010). The field underwent a comprehensive preparation regimen, including ploughing with mini-tiller ploughs, subsequent harrowing, and meticulous planking. This was succeeded by field puddling and the strategic application of chemical fertilizer. The seedlings were then methodically transplanted into the field, maintaining a precise

Table 1. The characteristics of the soil in Gokuleshwor.

Location	pH	OM (%)	Nitrogen (%)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Zn (ppm)	Cu (ppm)	B (ppm)	Texture
Experimental field, Gokuleshwor	6.2	1.86	0.09	11.13	72.22	0.38	0.73	0.24	Sandy loam

Source: Soil and fertilizer testing laboratory, Sundarpur, Kanchanpur.

spacing of 15cm*15cm, a practice endorsed by Krishi Diary in 2079, where chemical fertilizers were applied at a ratio of 100:30:30 kg/NPK. The nitrogen-phosphorous-potassium trifecta was administered with precision - half of the nitrogen dose and the complete phosphorous and potassium doses were administered as a basal dose during land preparation, while the remaining half of the nitrogen was judiciously divided into two split doses, expertly timed for 15 days after transplanting (DAT) and 30 DAT. Rigorous manual weeding was performed at two distinct intervals - 30 DAT and 45 DAT - to ensure the unfettered growth of the crop. At the booting stage in August 2022, a critical phase for crop development, a foliar spray comprising Zn, B, and Cu was diligently applied. The uniformity of all other agronomic practices was maintained across all experimental plots, while irrigation was provided in alignment with the crop's exacting demands. The culmination of this intricate cultivation journey materialized with the harvesting of the crop during the month of November, yielding valuable insights and results.

Description of the treatment

T_1: control
T_2: Zinc (Zn)
T_3: Copper (Cu)
T_4: Boron (B)
T_5: Zn + Cu + B
T_6: Zn + Cu
T_7: Zn + B

Experimental design

Throughout the duration spanning from the final week of June in 2022 to the initial week of November in 2023, the field experiment was meticulously executed at Gokuleshwor Agriculture and Animal Science College situated in Baitadi, Nepal. Employing a rigorous experimental design, a randomized complete block design (RCBD) was adopted, encompassing 7 distinct treatments, each replicated 3 times. The precise dimensions of each net plot were established at 2x2 square meters, while maintaining a plot-to-plot separation of 0.5 meters and a replication-to-replication distance of 1 meter, ensuring a systematic arrangement across the experimental area. An impressive total of 144 rice plants were meticulously cultivated within each plot, reflecting the comprehensive and detail-oriented nature of the study.

Data collection

A comprehensive dataset was compiled, encompassing 20 rice plant samples from each distinct plot, meticulously collected at various stages of the rice plant's growth cycle. The recorded observations encompassed an array of critical parameters, including plant height, tiller count, measurements of leaf length and breadth used to calculate the leaf area index (LAI = leaf area m^2/ ground area m^2), panicle length, time to reach physiological maturity, panicle grain count, weight of 1000 grains, as well as both grain and biological yields. Moreover, the calculation of the harvest index (H. I.) % was also a pivotal aspect of the study, derived as the ratio of grain yield to biological yield, multiplied by 100.

Statistical analysis

Following data collection, the accumulated information was meticulously input into MS-Excel Professional Plus 2019, subsequently undergoing rigorous Analysis of Variance (ANOVA) through the utilization of GenStat software. To discern statistically significant variations among mean values, Fisher's Least Significance Difference (LSD) was employed for multiple comparisons, while maintaining a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Days to physiological maturity

Examining the statistical analysis of the provided data, it becomes evident that the days required for rice to reach physiological maturity were notably impacted by the foliar administration of Zinc, copper, and Boron. The control group exhibited the lengthiest period to maturity at 127 days after transplanting (DAT), whereas the synergistic foliar treatment involving zinc, copper, and boron led to the shortest maturity duration of 116.3 DAT. These findings suggest that the application of zinc, copper, and boron also has implications for the maturation process in rice crops (El-Nahhal *et al*., 2013).

Number of tillers

Applying zinc, copper, and boron via foliar treatment has a noticeable impact on the tiller count in rice plants. Statistical analysis of the data underscores the highly significant influence of zinc, copper, and boron foliar application on the days to maturity of rice. The combination of zinc, copper, and boron (Zn + Cu + B) resulted in the highest tiller count recorded at 22.50, while the control group exhibited the lowest tiller count at 15.67. Notably, boron, along with several other micronutrients, exhibits limited mobility within the soil matrix, making leaf spraying a corrective approach to enhance plant metabolic processes compared to soil-based applications (Marschner, 1995). Furthermore, copper plays a vital role in various enzymatic reactions, including those involving ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase, and plastocyanin. Similarly, iron is also involved in redox reactions, undergoing reversible oxidation from Cu^+ to Cu^{2+} (Taiz & Zeiger, 2011).

Plant height

Analyzing the data statistically reveals that the impact of foliar application of zinc, copper, and boron on plant height is highly significant. The combination of zinc, copper, and boron (Zn + Cu + B) resulted in the tallest plants, reaching a height of 107.7 cm, while the control group exhibited the lowest plant height at 97.4 cm. This increase in plant height may be attributed to the involvement of these micronutrients in various physiological processes such as enzyme activation, electron transport, chlorophyll formation, and stomatal regulation, ultimately leading to enhanced dry matter accumulation (Asad *et al*., 2000).

Table 2. Effect of micronutrient foliar application on days of maturity, number of tillers, plant height, leaf area index.

Treatment	Days of maturity (DAT)	Number of tillers	Plant height (cm)	Leaf area index
Control	127.0[e]	15.67[a]	97.4[a]	0.0627[a]
Zinc	118.7[b]	18.53[b]	104.6[bcd]	0.066[b]
Copper	123.7[d]	16.93[b]	103.1[bc]	0.065[b]
Boron	122.0[cd]	17.77[b]	103.3[b]	0.0645[ab]
Zn + Cu + B	116.3[a]	22.50[c]	107.7[e]	0.0724[d]
Zn + Cu	120.7[c]	21.27[c]	106.0[de]	0.0707[cd]
Zn + B	118.3[b]	21.03[c]	104.3[cd]	0.0701[c]
Grand mean	120.95	19.10	103.66	0.0647
LSD	1.722	1.559	2.194	0.002
SEM ±	0.559	0.506	0.712	0.0007
CV%	0.8	4.6	1.2	1.7
F test	***	***	***	***

Significant traits are denoted *** for p<0.001.

Table 3. Effect of micronutrient foliar application on length of panicle, 1000 grain weight, grain per panicle, and biological yield.

Treatment	Length of panicle	1000 grain weight	Grain per panicle	Biological yield
Control	18.19[a]	19.26[a]	97.4[a]	10.06[a]
Zinc	20.13[b]	20.47[b]	103.6[b]	11.22[b]
Copper	20.07[b]	20.33[b]	103[b]	11.02[b]
Boron	20.62[c]	20.30[b]	103.3[b]	11.01[b]
Zn + Cu + B	22.6[e]	21.83[d]	107.7[c]	12.35[d]
Zn + Cu	21.86[d]	21.49[cd]	105.3[bc]	11.99[c]
Zn + B	21.76[d]	21.33[c]	105.3[bc]	11.91[c]
Grand mean	20.747	20.717	103.66	11.366
LSD	0.481	0.4	2.609	0.5958
SEM ±	0.156	0.13	0.847	0.1934
CV%	1.3	1.1	1.4	2.9
F test	***	***	***	***

Significant traits are denoted *** for p<0.001.

Leaf area index

Analysis of the data highlights a substantial influence of foliar application of zinc, copper, and boron on leaf area index. The statistical examination underscores the high significance of zinc, copper, and boron foliar application on the leaf area index of rice. The combination treatment of zinc, copper, and boron (Zn + Cu + B) yielded the highest leaf area index recorded at 0.0724, while the control group displayed the lowest leaf area index at 0.0627. The synergistic foliar application of zinc, copper, and boron demonstrated a notable enhancement in leaf area index (Zayed *et al.*, 2011).

Length of panicle

Upon conducting a thorough statistical analysis, it became evident that the length of rice panicles underwent significant alterations due to the foliar application of Zinc, Copper, and Boron. The simultaneous application of Zinc, Copper, and Boron yielded the highest panicle length, reaching 22.6 cm, while the control plots exhibited the lowest panicle length at 18.19 cm. This increase in panicle length could potentially be attributed to the participation of micronutrients in various physiological processes such as enzyme activation, electron transport, chlorophyll formation, and stomatal regulation, ultimately contributing to a higher accumulation of dry matter as documented by references (Najafi *et al.*, 2016).

1000 grain weight

Analysis of the data through statistical methods demonstrates a significant impact resulting from the foliar application of Zn, Cu, and B on the 1000-grain weight of rice, as detailed in Table 3. Notably, the application of Zn + Cu + B via foliar treatment yielded the highest 1000-grain weight, measuring 21.83 grams, while the control plots exhibited the lowest 1000-grain weight at 19.26 grams. The outcomes of this study are consistent with the research carried out by (Manal *et al.*, 2010). The potential augmentation in this characteristic through foliar spraying could be attributed to the participation of zinc and boron from the spray in activating enzymes, maintaining membrane integrity, facilitating chlorophyll synthesis, regulating stomatal equilibrium, and enhancing starch utilization during the initial growth phases. These processes contribute to heightened assimilate accumulation in the grains, ultimately leading to higher grain weight in mature rice (Soylu *et al.*, 2005; Guenis *et al.*, 2003).

Grain per panicle

The impact of the foliar application of zinc, copper, and boron on the grains per panicle of rice is presented in detail in Table 3. Data analysis reveals that the treatments exert a significant influence on the grains per panicle in the crop. The highest number of grains per panicle (107.7) was observed in plots treated with a combination of zinc, copper, and boron, while the lowest grains per panicle (97.4) were noted in plots that did not receive any spray. This increase in the number of grains per spike could potentially be attributed to the foliar application, facilitated by the role of boron (B) in pollen tube formation, which may lead to enhanced seed settlement. The deficiency of boron during the reproductive stage could potentially result in male sterility in rice (Jamjod & Rerkasem, 1999), characterized by shortened anthers and reduced fertility in numerous florets, ultimately contributing to inadequate grain set per ear (Huang *et al.*, 2000; Chaudry *et al.*, 2007).

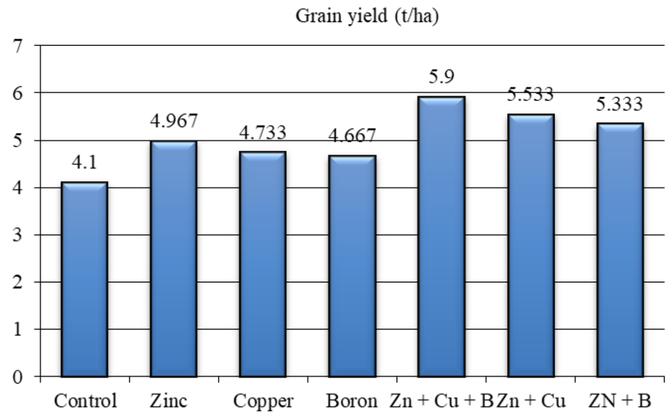

Figure 1. *Effect of micronutrient foliar application on grain yield t/ha.*

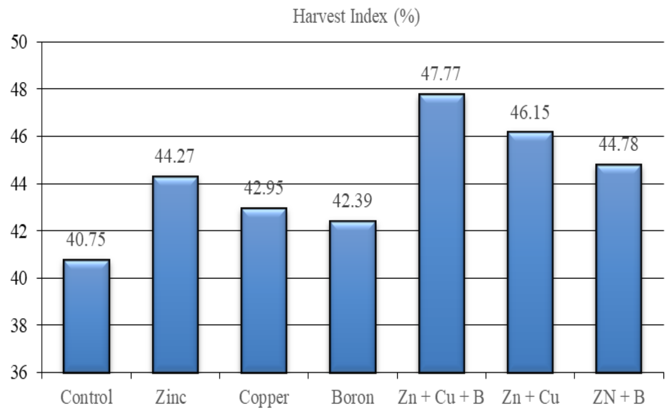

Figure 2. *Effect of micronutrient foliar application on harvest index %.*

Biological yield

The data about biological yield is comprehensively displayed in Table 3. It is evident from the table that the foliar application of zinc (Zn), copper (Cu), and boron (B) had a significant impact on the biological yield. The highest biological yield (12.35 tons per hectare) was achieved with the foliar application of Zn, Cu, and B, whereas the lowest biological yield (10.06 tons per hectare) was observed in plots without any spray. The application of micronutrients plays a pivotal role in augmenting various physiological processes within plants, leading to heightened growth and increased production of dry matter (Hussain *et al*., 2002). As previously indicated in Table 2, the application of zinc, copper, and boron contributed to increased plant heights, which in turn contributed to a higher biological yield of the crop.

Grain yield

The influence of foliar application of Zn, Cu, and B on the grain yield of rice is visually presented in Figure 1, showcasing a significant impact. Through statistical analysis of the data, it was evident that the simultaneous application of Zn, Cu, and B resulted in the highest grain yield (5.9 tons per hectare), while the control group exhibited the lowest grain yield (4.1 tons per hectare). The foliar applications of Cu and B have been shown to significantly boost rice yields by 27% in field trials, primarily attributed to increased grain filling rates and a greater 1,000-grain weight (Liew *et al*., 2012). Interestingly, the synergy of zinc and boron in combined use demonstrated a notably greater increase in crop yield compared to their application. These findings are consistent with the results (Arif *et al*., 2012; Quddus *et al*., 2011 & Sharma *et al*., 2013). Zinc, copper, and boron's crucial roles in numerous physiological processes of plants, including chlorophyll formation, stomatal regulation, and starch utilization, contribute to the enhancement of rice grain yield.

Harvest index

Figure 2 displays the impact of foliar application of zinc, copper, and boron on the harvest index of rice. A meticulous analysis of the data highlights notable variations in the harvest index (HI) across different treatments. The foliar application of zinc, copper, and boron exerts a significant influence on the harvest index of rice. The plots subjected to the combined foliar treatment of zinc, copper, and boron recorded the maximum harvest index at 47.77%, while the control group displayed the lowest harvest index at 40.75%. The observed increase in harvest index compared to the control group can be attributed to the roles of zinc, copper, and boron in plant metabolism and growth. Although this difference was not statistically significant, it aligns with previous reports indicating that leaf spraying of zinc, boron, and copper significantly impacted various aspects of rice, including the number of panicles per plant, grain per panicle, harvest index, and grain yield (Jafari *et al*., 2012). Additionally, similar investigations (Singh *et al*., 2018) have indicated that higher harvest index may result from improved carbohydrate translocation from source to sink, contributing to increased grain size through enhanced starch utilization and seed.

Conclusion

In conclusion, the findings of this study underscore the significant impact of micronutrients, particularly Zinc, Copper, and Boron, on enhancing various growth parameters and productivity in rice cultivation. The synergistic effects of these micronutrients, observed through foliar application, resulted in notable improvements across multiple aspects of rice growth and yield. This emphasizes the importance of considering and managing these essential micronutrients to optimize rice production and ensure sustainable agriculture practices. The management recommendations derived from this study, particularly regarding foliar application of Zinc, Copper, and Boron, hold promise for enhancing nutrient availability and maximizing yields, even under well-watered conditions. Additionally, addressing deficiencies of these micronutrients in soil is crucial for promoting optimal physiological processes within rice plants and ultimately improving crop quality and yield. Moving forward, further research efforts should continue to refine nutrient management strategies and explore additional avenues for maximizing rice production while ensuring food security in the region and beyond. By prioritizing the management of essential micronutrients and implementing effective foliar application techniques, agricultural practices can be optimized to support sustainable rice cultivation and meet the growing demands for food globally.

DECLARATION

Authors contribution

Conceptualization, LNY, and SB; methodology, LNY; software, LNY; validation, SB, MB, TT, AU, BK and LNY; formal analysis, MRB; investigation, TT; resources, SB, TT, AU, MRB; data curation, LNY; writing—original draft preparation, LNY; writing—review and editing, LNY; visualization, AU, BK and LNY. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare no conflict of interest.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors gave their consent to publish.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

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