Foliar applied zinc on different growth stages to improves the growth, yield, quality and kernel bio-fortification of fine rice

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Abstract

Zinc (Zn) is an essential nutrient required for plants growth and development, nonetheless, Zn deficiency is continuously increasing in our soils which is decreasing crop production. Further, the crops grown on Zn-deficient soils also contains a low amount of Zn which is also a major reason for Zn deficiency in humans. So, it is mandatory to supply the Zn to fulfill the crop needs with a corresponding increase in grain Zn. Thus, this study determined the impact of different rates of foliar applied Zn at different growth stages on the growth, yield, quality, and Zn bio-fortification of fine rice. The study comprised foliar application of distilled water (control), foliar applied Zn @ 0.5% at stem elongation stage + booting stage, foliar applied Zn @ 1.0% at stem elongation stage + booting stage, foliar applied Zn @ 0.5% at booting stage and milking stage, foliar applied Zn @ 1.0% at booting stage + milking stage, foliar applied Zn @ 0.5% at milking stage + dough stage and applied Zn @ 1.0% at milking stage + dough stage. The results indicated Zn applied different growth stages significantly improved productivity and Zn bio-fortification of rice crop. The maximum LAI, LAD, CGR, fertile tillers (363.17 and 372.17), 1000 KW (25.50 g and 25.61 g), kernel yield (5.45 t ha\(^{-1}\) and 5.44 t ha\(^{-1}\)), biomass yield (14.22 t ha\(^{-1}\) and 14.26 t ha\(^{-1}\)), HI, chlorophyll concentration (1.60 mg/g FW and 1.52 mg/g FW), relative water content (92.33% and 90.11%), and antioxidant activities were observed with foliar applied Zn (0.5%) at booting and milking stage and lowest values of all these traits were observed in control. Likewise, the maximum kernel protein (10.55% and 10.88%), amylose (27.85% and 26.18%), kernel length (6.54 mm and 6.68 mm) and width (2.37 mm and 2.77 mm), and grain Zn concentration (32.22 mg kg\(^{-1}\) and 30.21 mg kg\(^{-1}\)) was recorded with Zn (0.5%) at the booting and milking stage, and minimum kernel protein, amylose, kernel length, and width, and grain Zn concentration was noted in control. The current study findings
suggested that foliar-applied Zn (0.5%) at the booting and milking stage could be an important practice to get better productivity, quality, and grain Zn bio-fortification of rice in semi-arid conditions.

**Keywords:** antioxidant; bio-fortification; foliar spray; yield; zinc

**Abbreviations:** LAI: leaf area index; LAD: leaf area duration; CGR: crop growth rate; HI: harvest index; KW: kernel weight; OM: organic matter; Na: sodium; Ca: calcium; Mg: magnesium; DPTA: diethylenetriamine pentaacetae; RCBD: randomized complete block design; DAP: ammonium phosphate; SOP: sulfate of potash; N: nitrogen; K: potassium; RWC: relative water content; CAT: catalase; H$_2$O$_2$: hydrogen peroxide; APX: ascorbate peroxidase; ANOVA: analysis of variance; LSD: least significant difference; TDM: total dry matter; POD: peroxidase; @: at the rate of

**Introduction**

Zinc (Zn) is an important nutrient required for plants and humans. It is involved in cell-mediated immunity and it also protects against oxidative stress and chronic diseases (Prasad, 2020). The deficiency of Zn in humans is a major problem in developing countries which disturbs the sex hormones, and testicular development, and leads to hypogonadism, inflammation, and apoptosis (Chasapis et al., 2020; Beloucif et al., 2021). It has been found that deficiency of Zn can cause significant yield losses (Chattha et al., 2017; Hassan et al., 2022). Rice is a staple food for more than 50% of the world's population, however, the deficiency of Zn in our soils is continuously increasing which is significantly reducing the rice growth and yield (Nawaz et al., 2021; Sher et al., 2022). Besides this, the concentration of Zn is also reduced in edible grain parts which also leads to Zn deficiency in humans. Therefore, increasing grain Zn concentration is considered an important approach to increase Zn availability to humans (Ghoneim et al., 2016; Pradhan et al., 2020). Zn improves plant growth, chlorophyll synthesis and antioxidant activities (Zafar et al., 2017) and it also protects the membranes from oxidative damage by stabilizing the membrane stability and osmolytes accumulation which resulting in substantial increase in crop yield (Al-Zahrani et al., 2021).

Bio-fortification is an important way to enhance grain Zn-contents and Zn availability to plants and humans (Lividini et al., 2018). The increasing grain Zn concentration through the fertilizers approach has been noticed in different crops across the globe (Kumar and Pandey et al., 2015). Zn fertilizers are being used across the globe to provide Zn to plants and increase Zn bio-fortification (Hassan et al., 2022). The soil factors including pH, organic matter (OM), Na, Ca, and Mg, and soil phosphorus concentration strongly affect the Zn availability to plants (Chhabra and Kumar, 2018). The soil application is the most widely used method of Zn application across the globe, however, in this method, most of Zn is fixed in the soil which results in less availability of Zn to plants (Kachinski et al., 2022). In this context, the foliar spray of Zn is considered an effective strategy to quickly supply the Zn to plants to remove the Zn deficiency faced by plants (Wang et al., 2015; Hassan et al., 2019; Sher et al., 2022). Foliar applied Zn is more effective and beneficial as compared to soil application, particularly on calcareous and flooded soils where Zn availability is low (Suganya et al., 2020). Further, soil applied Zn at sowing has little impact on grain Zn concentration thus, the foliar spray is very effective to improve the grain Zn (Cakmak and Kutman, 2018). However, the impacts of exogenously applied on grain Zn content is also varied according to genotypes, rate of foliar spray, and climatic conditions (Kandil et al., 2022).

Foliar spray of Zn to leaves increase the Zn absorption and subsequent transformation into edible plant parts and this method is considered an effective and safe way to increase the grain Zn concentration. In foliar spray application substances directly penetrate the cuticle or stomata pathway to enter the lead and foliage feeding at the flowering stage can significantly increase the grain Zn concentration (Sher et al., 2022).
Nonetheless, the sustainability of Zn ions adhering to plant leaves is very challenging and sprayed Zn can easily drop from the leaf surface or they leached away by rain which affects the absorption of Zn by leaves (Wu et al., 2020). Further, foliar-applied Zn also significantly improved crop production and final quality (Yang et al., 2021). In literature rare studies are conducted on the impacts of foliar feeding of Zn on yield and quality of fine rice. Therefore, in the present study, we hypothesized that foliage feeding of Zn at diverse stages of plant life can affect the yield and quality of fine rice. Thus, we performed current study to determine the optimum rate of Zn application and stage of application to improve the growth, productivity, and quality of fine rice growing in semi-arid conditions.

**Materials and Methods**

**Experimental site**

The present research was performed for two years (2015 and 2016) at the University of Punjab Lahore, to determine the impact foliar applied Zn on productivity and quality of fine rice. The studied site has hot and semi-arid conditions and further weather conditions are given in Figure 1. Before the experiment, the soil samples were taken from the diverse location of the site and soil properties were determined (Homer and Pratt 1961). The soil was sandy loam with pH 7.72, organic matter (8.0 g kg\(^{-1}\)) Ec (1.02 dS m\(^{-1}\)), total N (0.032 g kg\(^{-1}\)) and available P and K (18 and 135 mg kg\(^{-1}\)) and DPTA Zn (30 mg kg\(^{-1}\)).

![Figure 1. Weather conditions during both years of study (2015 and 2016)](image)

**Experimental treatments**

The study was comprised of different treatments; foliar application of distilled water T\(_1\): (control), T\(_2\): foliar applied Zn @ 0.5% at stem elongation stage + booting stage, T\(_3\): foliar applied Zn @ 1.0% at stem elongation stage + booting stage, T\(_4\): foliar applied Zn @ 0.5% at the booting stage and milking stage, T\(_5\): foliar applied Zn @ 1.0% at the booting stage and milking stage, T\(_6\): foliar applied Zn @ 0.5% at milking stage + dough stage and T\(_7\): foliar applied Zn @ 1.0% at milking stage + dough stage. The study was executed in RCBD having three replications.
Crop husbandry

The soil was flooded and cultivated three times followed by planking to prepare the seed bed. Then rice nursery was transplanted and flooded conditions were maintained for 7 days afterward, the water was drained from the studied field and again flooding conditions were created until maturity. The fertilizers NPK was applied at the rate of 120:88:68 kg ha\(^{-1}\) in the forms of urea (46%), DAP (18% N and 46% P), and SOP (50% K). The full amount of P and K and half of N were applied at sowing whilst, rest of N was applied to crop at tillering stage. Moreover, other standard management practices were kept constant in order to get a good stand establishment.

Determination of growth and yield traits

The rows of rice plants having a 1-meter length were harvested from each plot and plants were separated into leaves and roots. After that 5 g leaves were taken to determine the leaf area and LAI, while LAD and CGR were calculated with methods of Watson (1947) and Hunt (1978) respectively. Moreover, ten plants from each pot were taken to determine the plant height, panicle per plant, and kernels per panicle. After that, the complete plots were hand harvested to determine biological and kernel yield and converted into the ton per hectare basis. Additionally, a sub-sample (1000 kernels) was taken for the determination of 1000 kernel weight.

Determination of quality traits

Rice kernel’s sub-sample was taken and sterile kernels were manually counted. On the other hand, 20 kernels from the harvested sample were taken and placed in light, to determine opaque kernels. Similarly, again 20% kernel was placed in light and differential into normal and abortive kernels based on size and light and not translucent to light. Kjeldhal method was for determined rice N concentration and kernel protein concentration was measured with (AOAC, 1990) method. On the other kernel, amylase concentration was determined with the procedures of Juliano (1971). For determining Zn concentration; rice kernels were over-dried and digested by the addition of a di-acid mixture (HClO\(_4\) : HNO\(_3\)) in and later on rice concentration was determined using an atomic absorption spectrophotometry (Prasad, 2006).

Determination of physiological and biochemical traits

To determine the relative water contents (RWC) leaf fresh samples (1 g) were taken and weight to determine the fresh weight (FW), after that samples were soaked in water for 24 hours to determine the turgid weight (TW), and then they were removed from the water and then oven dried and dry weight (DW) and RWC was following method: RWC = (FW-DW)/(TW-DW) ×100. For determination of leaf chlorophyll; 0.5 g rice leaf sample was taken and homogenized to get extract and absorbance was noted at 663 and 645 nm for determination of a and b concentration (Lichtenthaler, 1987). For CAT activity; 1 g of leaves were ground using 2.5 ml of 50 mM K-buffer and then centrifuged for 15 minutes and supernatant was taken and then 0.1 ml extract was added in 0.1 ml of H\(_2\)O\(_2\) (5.9 mM) and 2.5 ml of 5 % TCA buffer and absorbance recorded at 240 for determination of CAT activity (Aebi, 1984). In the case of POD activity; 0.5 g rice life samples were taken and homogenized by using the K-buffer (5 ml) and centrifuged for 15 minutes and the supernatant was taken and we noted the absorbance at 470 nm (Zhang, 1992). In the case of APX activity, again 0.5% g leaf samples were taken and 5 ml of KPB was added and centrifuged for 15 minutes, and then absorbance was noted for estimation of APX activity.

Data analysis

The collected data on different traits were analysed by one-way ANOVA and LSD test (p ≤ 0.05) was used to detect the significant levels among ANOVA sources (Steel et al., 1997). The figures were prepared by using Sigma-plot (8).
Results

**Growth traits**

The foliar applied Zn on different growth stages significantly affected the LAI and LAD of rice crops (Figure 1). The results indicated during both years maximum LAI and LAD were noted with foliar-applied Zn (1%) at the booting and milking stage followed by foliar-applied Zn (0.5%) at the same growth stages. Moreover, the minimum LAI and LAD during both years were recorded in the foliar application of distilled water (Figure 2). Similarly, foliar applied Zn also significantly affected the total dry matter and CGR (Figure 3). Again, maximum TDM and CGR were obtained with foliar applied Zn (1% and 5%) at the booting and milking stage followed by foliar applied Zn (1%) stem elongation and booting stages lowest TDM and CGR were recorded with foliar spray of water (control: Figure 3).

![Figure 2](image.png)

**Figure 2.** Effect of foliar applied Zn on different growth stages on LAI (A) and LAD (B) of rice during 2015 and 2016. The values in the above figure are means of three replicates with ±SE.
Yield traits

The study findings indicated that exogenous Zn spray at different stages of rice appreciably improved all yield traits in both study years (Table 1). The results indicates that taller plants with more tillers and kernels per panicle were recorded 0.5 foliar applied booting and milky stage followed by foliar applied Zn (1%) at the same growth stages and shorter plants with minimum tillers and kernels were obtained with foliar spray of water (Table 2). On the other hand, kernel weight, kernel yield, and HI were also significantly affected with foliar-applied Zn. Again, the maximum kernel weight (25.50 g and 25.61 g), kernel yield (5.45 t ha\(^{-1}\) and 5.44 t ha\(^{-1}\)), biological yield (14.22 t ha\(^{-1}\) and 14.26 t ha\(^{-1}\)) were noted with 0.5% Zn at booting and milking and minimum kernel weight, kernel and biomass yield and HI were recorded in control conditions with foliar spray of water (Table 2).

![Figure 3](image-url)
Table 1. Effect of foliar applied Zn on different growth stages on plant height, tillers and kernels/panicle of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Fertile tillers (m²)</th>
<th>Kernels per panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>94.68d</td>
<td>96.34d</td>
<td>309.90e</td>
</tr>
<tr>
<td>T2</td>
<td>100.32cd</td>
<td>98.32cd</td>
<td>328.23d</td>
</tr>
<tr>
<td>T3</td>
<td>106.11b</td>
<td>109.44b</td>
<td>338.20d</td>
</tr>
<tr>
<td>T4</td>
<td>104.32bc</td>
<td>100.99bc</td>
<td>363.17a</td>
</tr>
<tr>
<td>T5</td>
<td>118.15a</td>
<td>115.48a</td>
<td>346.57bc</td>
</tr>
<tr>
<td>T6</td>
<td>104.76bc</td>
<td>105.09bc</td>
<td>352.87d</td>
</tr>
<tr>
<td>T7</td>
<td>105.36bc</td>
<td>101.69bc</td>
<td>342.20bd</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.

Table 2. Effect of foliar applied Zn on different growth stages on 1000 KW, kernel and biological yield and harvest index of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1000 KW (g)</th>
<th>Kernel yield (t ha⁻¹)</th>
<th>Biological yield (t ha⁻¹)</th>
<th>Harvest index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>20.16c</td>
<td>20.49c</td>
<td>2.83d</td>
<td>3.16d</td>
</tr>
<tr>
<td>T2</td>
<td>23.45ab</td>
<td>22.12b</td>
<td>4.43bc</td>
<td>4.46bc</td>
</tr>
<tr>
<td>T3</td>
<td>23.26ab</td>
<td>23.92ab</td>
<td>4.94ab</td>
<td>4.91ab</td>
</tr>
<tr>
<td>T4</td>
<td>25.50a</td>
<td>25.61a</td>
<td>5.45a</td>
<td>5.44a</td>
</tr>
<tr>
<td>T5</td>
<td>23.01b</td>
<td>23.68b</td>
<td>4.95ab</td>
<td>4.96ab</td>
</tr>
<tr>
<td>T6</td>
<td>23.07b</td>
<td>23.73b</td>
<td>4.46bc</td>
<td>4.47bc</td>
</tr>
<tr>
<td>T7</td>
<td>22.06b</td>
<td>23.39b</td>
<td>4.52bc</td>
<td>4.43bc</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.

Photosynthetic pigments and antioxidant activities
Foliar applied Zn significantly affected the RWC, chlorophyll contents, and antioxidant activities of rice crop. The maximum RWC and chlorophyll contents were obtained with foliar applied Zn (0.5%) at the booting and milky stage followed by Zn (1%) applied at the elongation stage + booting stage and minimum RWC and chlorophyll contents were recorded with foliar spray of water (Table 3). In the case of antioxidant activities maximum APX, CAT, and POD activity during both study years was noted with foliar applied Zn (0.5%) at the booting stage and milking stage and minimum APX, CAT, and POD activity was recorded in control with foliar spray of water (Table 4).

Table 3. Effect of foliar applied Zn on different growth stages on RWC and chlorophyll concentration of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Relative water contents (%)</th>
<th>Chlorophyll contents (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>81.24e</td>
<td>79.21e</td>
</tr>
<tr>
<td>T2</td>
<td>85.20cd</td>
<td>82.13c</td>
</tr>
<tr>
<td>T3</td>
<td>90.22a</td>
<td>87.32b</td>
</tr>
<tr>
<td>T4</td>
<td>92.33a</td>
<td>90.11a</td>
</tr>
<tr>
<td>T5</td>
<td>88.91b</td>
<td>85.55b</td>
</tr>
<tr>
<td>T6</td>
<td>86.70c</td>
<td>83.44c</td>
</tr>
<tr>
<td>T7</td>
<td>84.50d</td>
<td>81.33d</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.
Table 4. Effect of foliar applied Zn on different growth stages on kernel protein, amylose, and kernel length and width of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>APX (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>POD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>32.44e</td>
<td>31.87f</td>
<td>5.00d</td>
</tr>
<tr>
<td>T2</td>
<td>36.11c</td>
<td>34.78d</td>
<td>5.34c</td>
</tr>
<tr>
<td>T3</td>
<td>38.51b</td>
<td>37.13b</td>
<td>6.00a</td>
</tr>
<tr>
<td>T4</td>
<td>40.32a</td>
<td>39.21a</td>
<td>6.14a</td>
</tr>
<tr>
<td>T5</td>
<td>37.33bc</td>
<td>36.55bc</td>
<td>5.89ab</td>
</tr>
<tr>
<td>T6</td>
<td>36.55c</td>
<td>35.19c</td>
<td>5.82b</td>
</tr>
<tr>
<td>T7</td>
<td>35.44d</td>
<td>33.18e</td>
<td>5.12d</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.

Quality traits

The study findings indicated that foliar applied remarkably improved the quality traits of rice crop (Table 5). The minimum spikelet sterility, opaque kernels, abortive kernels, and maximum normal kernels were obtained with foliar applied Zn (0.5%) at the booting stage and milky stage followed by foliar applied Zn (1%) at the elongation stage + booting stage and maximum spikelet sterility, opaque and abortive kernels, and normal kernels were obtained with foliar spray of water (Table 5). Likewise, maximum kernel protein (10.55% and 10.88%), kernel amylose (27.85% and 26.18%), kernel length (6.54 mm and 6.68 mm), and kernel width (2.37 mm and 2.27 mm) were obtained with 0.5% Zn applied at booting and milky stages followed by 1% foliar applied Zn at booting and milking stage and 1% at same stages and minimum kernel protein (9.03% and 8.73%), kernel amylose (20.95% and 16.92%), kernel length (5.06 mm and 5.23 mm) and kernel width (1.54 mm and 1.61 mm) was recorded in with a foliar spray of water (Table 6).

Table 5. Effect of foliar applied Zn on different growth stages on kernel quality traits of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spikelet sterility (%)</th>
<th>Opaque kernel (%)</th>
<th>Abortive kernels (%)</th>
<th>Normal kernels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>12.64a</td>
<td>12.98a</td>
<td>15.45a</td>
<td>14.45a</td>
</tr>
<tr>
<td>T2</td>
<td>10.24b</td>
<td>9.91b</td>
<td>14.75ab</td>
<td>14.41ab</td>
</tr>
<tr>
<td>T3</td>
<td>10.09b</td>
<td>9.75b</td>
<td>14.06abc</td>
<td>13.39abc</td>
</tr>
<tr>
<td>T4</td>
<td>6.47d</td>
<td>6.27d</td>
<td>11.95c</td>
<td>12.15c</td>
</tr>
<tr>
<td>T5</td>
<td>7.62c</td>
<td>7.28c</td>
<td>13.86ab</td>
<td>14.12ab</td>
</tr>
<tr>
<td>T6</td>
<td>7.23c</td>
<td>7.23c</td>
<td>13.03bc</td>
<td>12.77bc</td>
</tr>
<tr>
<td>T7</td>
<td>7.19c</td>
<td>7.43c</td>
<td>12.93bc</td>
<td>13.13bc</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.

Table 6. Effect of foliar applied Zn on different growth stages on kernel quality traits of rice during 2015 and 2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Kernel protein (%)</th>
<th>Kernel amylose (%)</th>
<th>Kernel length (mm)</th>
<th>Kernel width (mm)</th>
<th>Grain Zn (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>9.03c</td>
<td>8.73c</td>
<td>20.95c</td>
<td>19.62c</td>
<td>5.06c</td>
</tr>
<tr>
<td>T2</td>
<td>9.27b</td>
<td>9.80b</td>
<td>22.13d</td>
<td>21.20d</td>
<td>5.11e</td>
</tr>
<tr>
<td>T3</td>
<td>9.71b</td>
<td>9.45b</td>
<td>23.28c</td>
<td>22.95c</td>
<td>5.43d</td>
</tr>
<tr>
<td>T4</td>
<td>10.55a</td>
<td>10.88a</td>
<td>27.85a</td>
<td>26.18a</td>
<td>6.54a</td>
</tr>
<tr>
<td>T5</td>
<td>9.65b</td>
<td>9.98b</td>
<td>25.18b</td>
<td>24.84b</td>
<td>5.64b</td>
</tr>
<tr>
<td>T6</td>
<td>9.35b</td>
<td>9.58b</td>
<td>26.12b</td>
<td>25.35b</td>
<td>5.77b</td>
</tr>
<tr>
<td>T7</td>
<td>9.25b</td>
<td>9.58b</td>
<td>23.45c</td>
<td>24.39c</td>
<td>5.49c</td>
</tr>
</tbody>
</table>

The values given above are means of three replicates and diverse letters presenting the significance at p ≤ 0.05.
Grain Zn concentration

The study findings indicate that foliar-applied Zn markedly improved Zn concentration (Table 6). The results showed maximum grain Zn concentration during years was recorded from plants supplemented with foliar Zn (0.5%) used at booting and milky stages followed by 1% foliar applied Zn at stem elongation and booting stage and minimum grain Zn concentration was noted with foliar spray of water (Table 6).

Discussion

The results indicate that different rates of foliar applied Zn significantly increased the growth and yield traits of fine rice (Table 1). The foliar spray of Zn (0.5%) applied at booting and milky stages significantly enhanced rice yield. The foliar at later stages increase Zn availability in plant tissues which can substantially improve the final yield and grain Zn (Tuiwong et al., 2022a). The panicle formation and development need high amount of Zn and the application of Zn to rice plants produced higher panicles with more kernels owing to improved nutrient uptake (NPK) and enhanced assimilates production (Mu et al., 2020; Mu et al., 2020; Hamza et al., 2022). Zn applied at booting and milky stages effectively improved the overall, LAI, CGR, TDM, and yield traits (panicles/plant and kernel/panicle) owing to better Zn accumulation improved the chlorophyll synthesis (Table 3), RWC (Table 3) and antioxidant activities (Table 4) thus resulting in a marked increase in final yield. Further, Zn plays a key role in pollination, fertilization and kernel setting which consequently improved the final kernel yield (Hassan et al., 2019; Hassan et al., 2020).

We noted that foliar applied Zn (0.5%) at the booting and milking stages produced high yield (Table 2). The foliar spray at early reproductive stages results in better Zn transformation to plant reproductive parts and its accumulation in grains which ensures better production and better grain Zn concentration (Shukla et al., 2009). Further, Zn applied by foliar feeding is quickly absorbed by the epidermis of leaves and after re-mobilization it is trans-located to developing seeds by phloem thus resulting in the production of bold grains with higher grain yield (Hassan et al., 2019). The present increase in growth and yield is attributed to Zn mediated increase in chlorophyll synthesis (Table 3), RWC (Table 3) and antioxidant activities (Table 4: Rehman et al. 2018). These findings are the same with the outcomes of Patel et al. (2022) and Wang et al. (2017) they also found that Zn application improved the tillers, panicles and kernels/panicle owing to significant improvement in photosynthetic efficiency, chlorophyll synthesis and antioxidant activities. Likewise, other authors also found that the foliar sprays of Zn at reproductive stages improved the physiological activities which improved the overall growth and yield of plants (Kadam et al., 2018; Tuiwong et al., 2022a).

The foliar Zn appreciably increased LAI and CGR and decreased the LAI and CGR attenuation rates at the late stages. The increased LAI and CGR at grain filling stages allow more transformation of photosynthetic pigments from grains to leaves which results in the production of bold grains (Table 2) with more grain weight and final yield (Zhang et al., 2021). The results indicate that foliar applied Zn (0.5%) at booting and milking stages significantly improved kernel protein, and kernel amylose and reduced the spikelet sterility, opaque, and abortive kernels (Table 5). Zn activates different enzymes and protein synthesis and it also activates the decomposing sugar which improves the overall quality of rice kernels (Kheyri et al., 2019). Zn application (0.5%) at booting and milky stages improved kernel protein, and kernel amylose and reduced the spikelet sterility, opaque, and abortive kernels (Table 5). The present increase in quality traits can be due to Zn mediated increase in LAI (Figure 1), photosynthetic rates and TDM (Figure 2). Previous studies also indicate that Zn application promotes the transformation of assimilates to grains and increases protein synthase activity which improved the grain protein concentration (Dimkpa et al., 2020; Yang et al., 2021).

The results indicate that foliar Zn enhanced grain Zn, however, foliar application (0.5%) at booting and milking stages substantially enhanced grain Zn concentration as compared to other treatments (Table 6). The time of Zn application plays a crucial role to achieve a higher grain Zn concentration (Sher et al., 2022).
Likewise, Saha et al. (2017) noted Zn applied at flowering stages produced grains with dense Zn as compared to foliar applied Zn at tillering. While Sher et al. (2022) found that foliar applied at the heading stage effectively improved the grain Zn and utilization as compared to Zn applied at earlier growth stages. At earlier growth stages; plant leaves are less physiologically active, whilst matured leaves are fully active and they export assimilates from phloem to grains (Saha et al., 2017). Further, in foliar application Zn ions directly enter the leaf apoplast through the pores of stomata which also increases the Zn concentration in developing grains (Gupta et al., 2016). Moreover, foliar application at early grain filling stages induces re-translocation of Zn from vegetative tissues to grain and resulting in significant grain Zn contents (Wang et al., 2018). Therefore, the increase in grain Zn in the current study could be due to Zn re-translocation and direct absorption of Zn by plant leaves.

**Conclusions**

The present study finding indicated Zn applied at different growth stages appreciably improved rice growth and yield. The maximum improvement in growth, yield, and quality was seen with 0.5% Zn applied at the booting and milking stages. The application of Zn (0.5%) at the booting and milking stages also led to an appreciable improvement in grain Zn and proved the best to address the Zn nutrition problem in humans. Therefore, foliar-applied Zn (0.5%) at the booting and milky stage could be an imperative approach to improved rice yield, quality, and grain Zn. However, more research is required to explore the role of Zn applied at the booting and milking stage on rice crop under a wide range of soil and climate conditions.

**Authors’ Contributions**

Conceptualization: MBC, data collection; QA, writing original draft: MBC and MUH, writing, reviewing and editing: MNS, MA, SA, QA, ZI, MI, MAY, MRA, FMA, MH and MUH.

All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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