Original Research Article

Enhancing Growth and Zinc bioavailability in Rice (*Oryza sativa* L.) Cultivars through Agronomic Biofortification Strategies

ABSTRACT

A field experiment was conducted using four rice varieties *viz.,* Uma, Pournami, Gouri and DRR Dhan 45 under varying levels of $ZnSO_4$ foliar application (control, 0.1 per cent, 0.5 per cent and 1.0 per cent) in a randomized complete block design. $ZnSO_4$ @ 1.0 per cent recorded taller plants at panicle initiation (80.5 cm) and harvest stages (113 cm) and was comparable with $ZnSO_4$ @ 0.5 per cent. Higher tillers per hill and dry matter production were also recorded at panicle initiation and harvest stages with $ZnSO_4$ @ 1.0 per cent which was statistically similar to $ZnSO_4$ @ 0.5 per cent. Zinc application at 0.5 per cent and 1.0 per cent enhanced Zn concentration in rough rice, brown rice, white rice, and rice bran. The highest Zn accumulation in white rice (21.2 mg kg^{-1}) was achieved with 1.0 per cent $ZnSO_4$ foliar spray, which was comparable to 0.5 per cent $ZnSO_4$ spray. Application of 1 per cent $ZnSO_4$ during maximum tillering and milk stage led to substantial reductions in the phytate: Zn molar ratio in rough rice (28.3), brown rice (32.7), white rice (6.31), and rice bran (25.9). Dhan 45 treated with 1.0 per cent $ZnSO_4$ achieved the lowest ratios in rough rice (18.9) and brown rice (21.8). Although the 1.0 per cent treatment yielded greater reductions in the phytate: Zn molar ratio, the 0.5 per cent $ZnSO_4$ treatment produced notable decrease in rough rice (30.2), brown rice (34.8), white rice (6.72), and rice bran (27.6) making it a viable option for lower Zn input. Overall, foliar application of Zn improved Zn bioavailability in both whole grains and milled rice, aligning phytate: Zn molar ratios closer to optimal levels for human nutrition.

Keywords: Bioavailability, Foliar application, $ZnSO_4$, Phytate: Zn molar ratio, Rice grain fractions

1. INTRODUCTION

The global challenge of micronutrient deficiencies, particularly Zn deficiency continues to affect millions of people worldwide. It is an essential nutrient with a crucial role in structural, regulatory and catalytic functions of human body (Li *et al*., 2020). According to the National Institute of Health (NIH) dietary guidelines, adult women are advised to consume 8 mg Zn per day, whereas men should aim for 11 mg daily (NIH, 2022). Agronomic biofortification involves the use of micronutrient enriched fertilizer and is an easy and rapid way to enhance the nutritional content of crops. Consuming these fortified crops helps to improve human nutrition (Cakmak and Kutman, 2018). The effectiveness of applying Zn based fertilizers to crops varies depending on factors such as the method of application (e.g., soil, foliar, seed priming, or combinations), the type of Zn used, timing of the application, as well as the crop's genetic makeup and the environmental conditions in which it is grown (Yaseen and Hussain, 2021 & Prasad *et al*., 2014). Most rice genotypes can mobilize foliar applied Zn from the leaves to the grains; however, this ability may differ depending on the plant genetic composition and soil Zn availability (Mabesa *et al*., 2013). The enrichment of Zn in grains should be evaluated in relation to changes in other key nutritional traits of the grain, such as concentrations of iron, phytic acid, the phytic acid to zinc molar ratio, and protein levels (Cakmak *et al*., 2010 & Hussain *et al*., 2012). These compositional changes in grains can influence Zn bioavailability, as it is well recognized that the two main factors affecting Zn absorption in adults are the levels of Zn and phytate in the diet (Miller *et al*., 2007). Bioavailability conveys the fact that not all of the consumed Zn is really absorbed by human body. Zinc bioavailability refers to the portion of absorbed Zn in the blood stream that is available for use in regular physiological functions (La Frano *et al*., 2014). The bioavailability of Zn in rice grains is affected by the Zn content in the grain, and enriching rice grains with Zn has significantly increased the amount of bioavailable Zn. However, certain antinutrients like phytic acid can lower Zn bioavailability by binding to Zn and forming indigestible complexes in the human body. Recently several approaches have been proposed to increase the Zn content in grains for enhanced nutritional value. The present study was conducted to assess the impact of Zn foliar application on growth, Zn concentration and bioavailability in grains of selected rice cultivars.

2. MATERIALS AND METHODS

2.1 Field Parameters

A field trail of rice was conducted on a farmer's field located at 8° 43' N latitude and 76° 45' E longitude, at an elevation of 52 meters above sea level in the southern coastal plains of Kerala during the kharif (rainy) season of 2020-21. The area received 935.8 mm of seasonal rainfall over 50 rainy days, which proved beneficial for crop growth and grain development. The average seasonal temperature ranged from a maximum of 30.3° to 32.7°C and a minimum of 24.5° to 25.9°C.

2.2 Experiment Layout

The field experiment was designed using a two factor factorial randomized complete block layout, with three replications and 16 treatment combinations.

2.3 Treatments

2.3.1 Varieties

The treatment included four medium duration rice varieties as factor (V):

V_1: Uma, V_2: Pournami, V_3: Gouri and V_4: DRR Dhan 45

2.3.2 Zinc foliar application

Four levels of foliar Zn application as factor (F)

F_1: control, F_2: $ZnSO_4$ @ 0.1 per cent, F_3: $ZnSO_4$ @ 0.5 per cent and F_4: $ZnSO_4$ @ 1 per cent.

2.3.3 Treatment combinations

v_1f_1	v_2f_1	v_3f_1	v_4f_1
v_1f_2	v_2f_2	v_3f_2	v_4f_2
v_1f_3	v_2f_3	v_3f_3	v_4f_3
v_1f_4	v_2f_4	v_3f_4	v_4f_4

Note: The study involved both biofortified varieties and conventional rice varieties. The key characteristics of the rice varieties used in the study were given in Table 1. Foliar Zn application of the rice crop was applied in two stages with a uniform spray concentration. The initial spray was given during the maximum tillering and the second spray on the milk stage.

Table 1. Characteristics of the rice cultivars tested in the experiment

Cultivars	Station	Parentage	Duration in days	Zn in grains (mg kg^{-1})	Phytic acid (g kg^{-1})
Uma (MO-16)	Rice Research Station, Monkompu	Cul.12814/ Mo.6	120-135	13.8	6.12
Pournami (MO-23)	Rice Research Station, Monkompu	Mo.4/ Cul. 25331	115-120	15.4	5.99
Gouri (MO-20)	Rice Research Station, Monkompu	KAUM 109-1-2-1/ IET 23739	120	21.5	6.06
DRR-Dhan 45	Indian Institute of Rice Research, Hyderabad	IR 73707-45-3-2-3/ IR 77080-B-34-3	125	28.6	6.28

2.4 Soil Testing

Before the field experiment, a composite soil sample was collected from a depth of 0-15 cm and analysed for its physio chemical properties. The experimental soil was clay loam in texture, very strongly acidic (pH 5.4), high in organic carbon (1.32 per cent), sufficient in Zn (1.05 mg kg^{-1}), medium in available nitrogen (282 kg ha^{-1}), phosphorus (12.7 kg ha^{-1}) and potassium (187 kg ha^{-1}).

2.5 Application of foliar spray

In accordance with treatments, the spray solution for foliar Zn fertilization was applied to the crop in the late afternoon, continuing until the solution just started to drip off the leaves, following the recommendations of Cakmak *et al.* (2010).

2.6 Growth Parameters

2.6.1 Plant Height

Plant height was recorded at maximum tillering, panicle initiation, and at harvest stages using the method described by Gomez (1972). Height was measured from the base of the plant to the tip of the longest leaf or tip of the longest panicle, which ever was longer and the average was recorded in centimetres (cm).

2.6.2 Tillers per Hill

At maximum tillering, panicle initiation, and harvest stages, tiller counts were obtained from six tagged hills, and the mean value was expressed as number of tillers per hill.

2.6.3 Dry Matter Production

Dry matter production was recorded at maximum tillering and panicle initiation stages, six sample hills were randomly selected and uprooted from the area defined for destructive sampling outside the net plot area leaving the border rows. The samples were washed, air dried in shade and then oven dried till constant weight was attained. The total dry matter production was computed and was expressed in gram per hill. At harvest stage, six sample hills were uprooted, separated into grain and straw, air dried under shade and later oven dried to a constant weight. The dry weight of each sample plant was recorded separately as grain, straw and total dry matter and expressed in gram per hill.

2.7 Collection and analysis of rice grains

The rough rice from each plot was cleaned to eliminate foreign matter, washed to remove dust, air dried, and then oven dried to a constant weight. A representative sample of rough rice obtained from various Zn fertilization and cultivars was then dehulled to produce brown rice and husk. The entire amount of husk was collected, weighed and set aside for analysis. The total quantity of brown rice produced was milled into white rice and bran, and the white rice was used to prepare cooked rice. During milling both the white rice and bran were collected, weighed and stored for analysis. The dehulling and milling processes were conducted using a compact mill, resulting in husk, bran and white rice. These along with rough rice and brown rice were later analysed for Zn and phytate using the standard procedures.

2.8 Cooking of the processed grains

A 100 g portion of white rice was washed twice with 250 ml of water and then soaked in 250 ml of distilled water for 30 minutes before cooking. After rinsing water soaked grains were cooked on a hot plate at 380^0 C with 600 ml of water. Cooking was stopped when a few cooked kernels showed no white kernel left behind when pressed between two glass slides (opaque core of cooked rice just disappeared). After cooking, rice and decanted water were separated. Both were dried in a hot air oven at 60 ^{0}C until they reached a constant weight. The samples were then ground using a pestle and mortar, passed through a 0.5 mm sieve, and stored in airtight polyethylene bags at room temperature prior to digestion (Suman, 2011). Zinc content in the samples were analysed as per the standard procedures.

2.9 Zinc and phytate analysis and Zinc bioavailability

Zinc can be analysed by nitric-perchloric acid (9:4) digestion and atomic absorption spectrometry (Jackson, 1973). Phytate was extracted with trichloroacetic acid and subsequently precipitated as ferric salt. The iron concentration of the precipitate was measured calorimetrically using a spectrophotometer at 480 nm. This value was used to compute the phytate concentration, assuming a constant 4Fe: 6P molecular ratio in the precipitate (Sadasivam and Manickam, 2016).

Phytate: Zn molar ratio was calculated using the following formula (Murphy *et al*., 1992; Gibson, 2005).

$$\text{Phytate: Zinc molar ratio} = \frac{Phytic\ acid\ concentration\ (mg\ kg^{-1})/\ 660}{Zn\ content\ in\ (\ mg\ kg^{-1})/65.4}$$

where, 660 is molecular weight of phytic acid and 65.4 is atomic weight of Zn. The inhibitory effect of phytate on Zn bioavailability in humans i.e., the absorbability of dietary Zn in humans, can be predicted from phytate: Zn molar ratio in human diet (Gibson, 2005). Algorithm of Murphy and co-workers to estimate bioavailability of Zn in humans, based on work of Murphy *et al*. (1992) is given as follows

Zn bioavailability estimate (per cent)	Phytate: Zn molar ratio
55	0-5: 1
35	5-15: 1
15	15-30: 1
10	>30: 1

2.10 Statistical Analysis

The data were subjected to statistical analysis, and critical difference at 5 per cent significance level was calculated for each parameter. The data generated were analysed by using the software GRAPES (Gopinath *et al*., 2020).

3. Result and Discussion

3.1 Plant height

The plant height was affected significantly with foliar application of Zn during panicle initiation and harvest stage (Table 2.) and was not significantly influenced by the effect of varieties and their interaction with fertilization at panicle initiation and harvest stages. Higher plant height was recorded with 1.0 per cent $ZnSO_4$ and was comparable to 0.5 per cent $ZnSO_4$ at both the panicle initiation stage (80.5 cm, 79.1 cm) and harvest stage (113 cm,110 cm). The lower plant heights across all the growth stages were observed in the control. The enhancement in height can be attributed to the sufficient supply of Zn, which likely improved the availability and uptake of other essential nutrients leading to better crop growth. Sudha and Stalin (2015) reported that foliar application of micronutrients significantly increased plant height, attributing to the enhanced enzymatic activity and auxin metabolism in the plants. Shivay *et al*. (2016) noted that the application of $ZnSO_4$ at 0.5 per cent resulted in taller plants.

3.2 Tillers per hill

The variation in tillers per hill due to foliar Zn fertilization was also found to be significant. Data related to tillers per hill under the influence of different levels of foliar Zn fertilization and varieties has been shown in Table 2. Foliar fertilization of Zn had significant effect on the tiller count per hill. At the panicle initiation stage higher number of tillers per hill (20.2) were registered with $ZnSO_4$ at 1.0 per cent, which was at par with 0.5 per cent $ZnSO_4$ treatment (19.0). Similarly, at harvest the higher tiller count was recorded with 1 per cent $ZnSO_4$ application (15.7) and was on par with 0.5 per cent (15.4). The absence of Zn foliar fertilization resulted in lower tiller number across all the growth stages. Mustafa *et al*. (2013) stated that optimum quantity of Zn enhanced number of tillers at all growth stages. Increase in number of tillers due to Zn application was also reported by Slaton *et al*. (2005).

3.3 Dry Matter Production

The data presented in Table 2. revealed that at panicle initiation and harvest stages dry matter production was significantly influenced by Zn fertilization. It is clear from the data that higher dry matter production was recorded with $ZnSO_4$ @ 1.0 per cent at both the panicle initiation (12.0 g hill^{-1}) and harvest stage (28.9 g hill^{-1}) and was on par with $ZnSO_4$ @ 0.5 per cent. This was owing to the reason that dry matter generation in plant depends on potential photosynthetic capacity which in turn depends on leaf area, nutrient consumption and favourable environmental circumstances (De Datta, 1981). Higher dry matter production with varied Zn treatments could be ascribed to increased plant height and leaf area index, as Zn is essential for auxin and enzyme synthesis. Increase in dry matter production due to Zn application was also observed by Tetarwal *et al*. (2011), Kumar *et al*. (2011) and Ravi *et al*. (2012).

Table 2. Effect of Zn foliar application on Growth parameters

Treatments	**Growth Parameters**					
	Plant Height (cm)		**Tillers Per Hill (nos)**		**Dry matter Production (g hill^{-1})**	
	Panicle Initiation	**Harvest**	**Panicle Initiation**	**Harvest**	**Panicle Initiation**	**Harvest**
Varieties (V)						
Uma	74.4	100	17.3	15.0	10.7	25.4
Pournami	70.5	97	16.3	14.4	10.3	24.8
Gouri	71.6	96	15.7	14.8	10.1	24.1
DRR Dhan-45	73.1	99	16.8	14.9	10.5	25.1
Sem ((±)	2.0	4	0.8	0.3	0.3	0.9
CD (0.05)	NS	NS	NS	NS	NS	NS
Zinc Foliar application (F)						
No $ZnSO_4$	63.6	82	12.9	13.9	8.7	20.8
$ZnSO_4$ @ 0.1%	66.4	86	14.0	14.1	9.5	22.4
$ZnSO_4$ @ 0.5	79.1	110	19.0	15.4	11.4	27.3
$ZnSO_4$ @ 1 %	80.5	113	20.2	15.7	12.0	28.9
Sem ((±)	2.0	4	0.8	0.3	0.3	0.9

CD (0.05)	5.85	10.9	2.16	0.71	1.06	2.53

Note: Interaction (V × F) non-significant

3.4 Effect of Zn application on Estimated Zn bioavailability in rice grain fractions

3.4.1 Zinc

The results of the study illustrated that the accumulation of Zn in white rice increased with the application of $ZnSO_4$ at 0.5 per cent (19.5 mg kg^{-1}), which was statistically similar to the level obtained with the application of $ZnSO_4$ at 1 per cent (21.2 mg kg^{-1}). Additionally, $ZnSO_4$ at 0.5 per cent enhanced the Zn content in harvested rough rice, brown rice, rice bran and cooked rice 22, 26,75.3, and 12.8 mg kg^{-1} respectively, reaching levels comparable to those achieved with $ZnSO_4$ at 1 per cent (Fig.1.). The interaction between rice varieties and Zn foliar application was not significant. However, foliar application of $ZnSO_4$ consistently increased Zn content across all rice cultivars (Fig.2.). Notably, Dhan 45 showed higher Zn content when treated with 1 per cent $ZnSO_4$ followed by 0.5 per cent application as the next most effective treatment. Zinc applied through foliar spray is effectively absorbed by the leaves and can be translocated to the grains (Boonchuay *et al*., 2013). The timing of foliar spray is also a key factor for enhancing Zn content in grains. Phuphong *et al*. (2018) reported that applying Zn foliar sprays after flowering significantly increases the Zn concentration in both polished and brown rice.

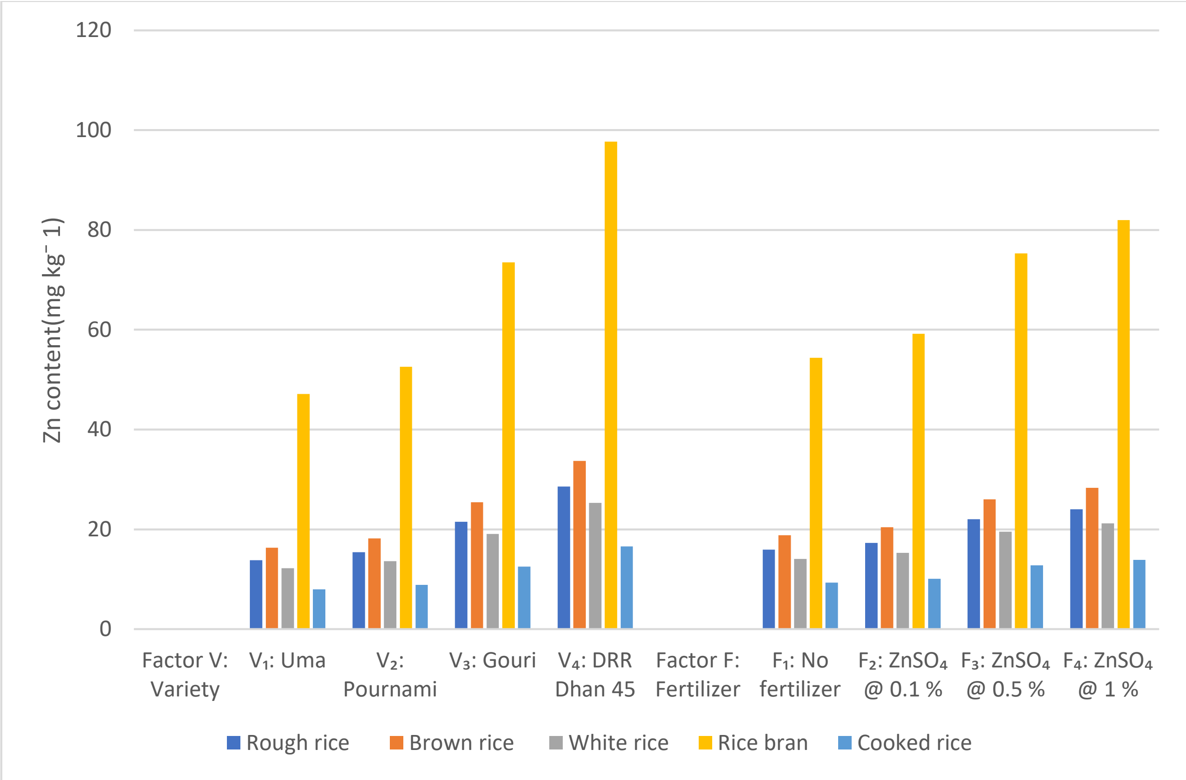

Fig.1. Effect of varying levels of Zn foliar fertilization on Zn content in grain fractions of various rice varieties

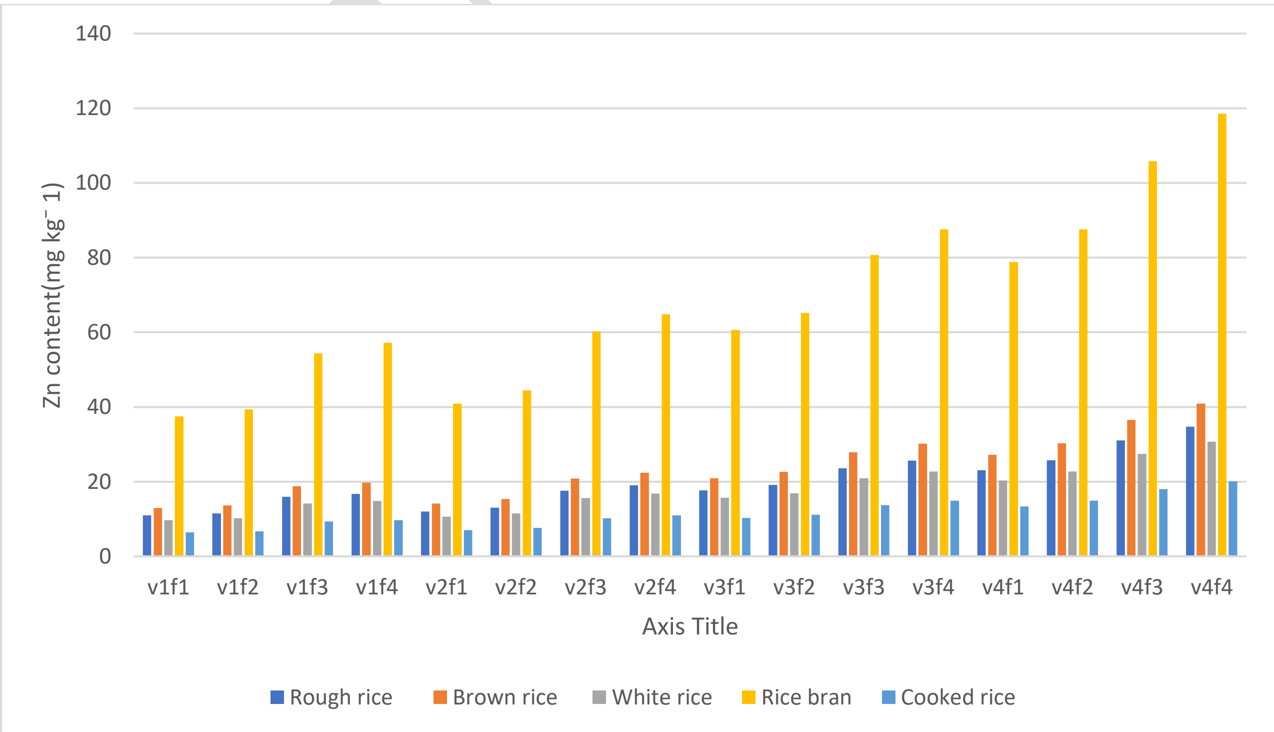

Fig.2. Interaction between rice varieties and Zn foliar fertilisation on Zn content of rice varieties

3.4.2 Phytate

The study found that varieties, Zn fertilization and their interactions had no significant effect on phytate concentrations in whole grain and milled fractions, in contrast to prior findings that showed that soil or foliar Zn application reduced phytate concentrations in rice grain considerably (Mabesa *et al*., 2013 & Imran *et al*., 2015). Possible explanation could be that foliar Zn application inhibits the conversion of inorganic phosphorus to phytate in rice grain.

3.4.3 Phytate: Zn molar ratio

The results of the study illustrated that Zn application significantly reduced the phytate: Zn molar ratio in polished and brown rice for all rice cultivars over control, the lowest phytate: Zn molar ratio was noted in foliar application with $ZnSO_4$ @ 1.0 per cent which was followed by foliar application with $ZnSO_4$ @ 0.5 per cent (Table 3.). These findings align with the established recommendation that a phytate: Zn molar ratio below 20 is optimal for Zn nutrition in human diets (Weaver and Kannan, 2002). Irrespective of the varieties and Zn fertilization levels, the phytate: Zn molar ratio in polished white rice consistently remained below 20, indicating optimum Zn nutrition in human diets. Among the rice varieties evaluated, the genetically biofortified Dhan 45 recorded significantly lower phytate: Zn molar ratios in rough rice (22.1), brown rice (25.5), white rice (5.12), and rice bran (21.3). Fertilization with $ZnSO_4$ @ 1.0 per cent at maximum tillering and milk stage resulted in significantly lower phytate: Zn molar ratios in rough rice (28.3), brown rice (32.7), white rice (6.31), and rice bran (25.9) respectively. In the case of rough rice (18.9) and brown rice (21.8), foliar application of $ZnSO_4$ @ 1.0 per cent in Dhan 45 recorded significantly lower phytate: Zn molar ratio. This suggests that varieties, in combination with Zn fertilization can further enhance Zn bioavailability. While the 1 per cent concentration achieved the greatest reduction in the phytate: Zn molar ratio, the 0.5 per cent treatment provided a substantial improvement, for instance the phytate: Zn molar ratio decreased to 30.2 in rough rice, 34.8 in brown rice, 6.72 in white rice, and 27.6 in rice bran with the 0.5 per cent $ZnSO_4$ treatment. This makes it a viable alternative where lower Zn input is preferred. Fertilization with higher Zn concentrations in whole grain and milled fractions resulted in decreased phytate: Zn molar ratios, bringing phytate: Zn molar ratios closer to desirable reference levels for improved Zn bioavailability. The result is in consonance with the observation of Hussain *et al*. (2012) & Hama-Salih *et al*. (2021) who reported that foliar Zn fertilization increased the estimated Zn bioavailability and decreased the molar ratio of phytate: Zn in rice. Saha *et al*. (2017) also reported a notable decrease in the phytate: Zn molar ratio with Zn foliar application at the maximum tillering and flowering stages in rice. The distribution of phytic acid within the rice kernel further explains the variations observed in the phytate: Zn molar ratio across different rice fractions. Brown rice is richer than milled rice in terms of phytic acid that may inhibit absorption of minerals. Major amount of phytic acid present in the aleurone layer will be removed by milling process (Itani *et al*., 2002).

Table 3. Effect different levels of Zn foliar fertilization on Phytate: Zn molar ratio of rough rice, brown rice, rice bran and white rice

Treatment	Rough rice	Brown rice	Rice Bran	White rice
Factor V: Variety				
V_1	45.5	52.6	42.3	10.21
V_2	39.6	45.7	37.5	9.19
V_3	28.3	32.7	27.3	6.56
V_4	22.1	25.5	21.3	5.12
SEm(±)	0.1	0.1	0.3	0.08
CD (0.05)	0.26	0.30	0.90	0.247
Factor F: Fertilizer				
F_1	38.0	43.9	38.6	9.29
F_2	39.1	45.1	36.3	8.77
F_3	30.2	34.8	27.6	6.72
F_4	28.3	32.7	25.9	6.31
Sem (±)	0.1	0.1	0.3	0.08
CD (0.05)	0.26	0.30	0.90	0.247
Interaction (VxF)				
v_1f_1	54.9	63.3	51.0	12.31
v_1f_2	52.2	60.3	48.5	11.82
v_1f_3	38.0	43.9	35.3	8.46
v_1f_4	37.1	42.8	34.4	8.27
v_2f_1	43.7	50.5	46.5	11.19

v_2f_2	46.5	53.7	43.2	10.39
v_2f_3	35.3	40.7	31.2	7.88
v_2f_4	32.8	37.8	29.1	7.31
v_3f_1	29.7	34.3	31.8	7.65
v_3f_2	32.5	37.6	30.3	7.26
v_3f_3	26.4	30.5	24.6	5.89
v_3f_4	24.5	28.2	22.7	5.44
v_4f_1	23.7	27.4	25.1	6.03
v_4f_2	25.1	29.0	23.3	5.61
v_4f_3	20.8	24.0	19.3	4.64
v_4f_4	18.9	21.8	17.5	4.20
Sem (±)	0.2	0.2	0.62	0.171
CD (0.05)	0.53	0.61	1.80	0.495

4. CONCLUSION

Growth parameters were significantly affected by Zn foliar fertilization in various rice varieties and was comparable between 0.5 per cent and 1.0 per cent $ZnSO_4$ concentrations. Foliar application with 1.0 per cent and 0.5 per cent $ZnSO_4$ reduced the phytate: Zn molar ratios. Although the 1.0 per cent $ZnSO_4$ concentration resulted in greater reductions, 0.5 per cent $ZnSO_4$ treatment proved to be the optimal, achieving significant zinc enrichment with minimal phytate interference. The results suggest that brown rice can be processed into white rice to produce desired phytate: Zn ratio for optimum Zn nutrition in humans.

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Details of the AI usage are given below:

1.ChatGpT

COMPETING INTERESTS

Authors have declared no competing interests exists.

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