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ROLE OF ZINC AND BORON IN ENHANCING PHYSIOLOGICAL ATTRIBUTES OF RICE IN SALINE ENVIRONMENT

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ABSTRACT

A field experiment was conducted during Kharif 2022-23 at the College of Agriculture, V.C. Farm, Mandya, using factorial RCBD. The study involved two factors: soil application (SA) of zinc sulfate at three levels (0, 20 and 40 kg ha^{-1} - Zn_0, Zn_{20} and Zn_{40}) and three times foliar sprays (FS) of boric acid at four levels (0, 0.25, 0.5 and 0.75 % - B_0, $B_{0.25}$, $B_{0.5}$ and $B_{0.75}$). The objective was to assess the impact on salt stress in rice. The experimental design aimed to combine the effects of soil application zinc and foliar spray of boron on to evaluate rice growth the under-salt stress conditions. The salt stress tolerance indices in rice include relative water content, SPAD values, chlorophyll a, chlorophyll b and total chlorophyll were in higher amounts and proline, Na^+/K^+ and Na^+/Ca^{2+} in index leaves and roots of rice were in lower amounts with $Zn_{40}+B_{0.75}$ and $Zn_{40}+B_{0.5}$, after a week of first and second spray. However, after third FS of B, $Zn_{40}+B_{0.25}$ recorded higher relative water content (90.23 %), SPAD values (42.50), chlorophyll a (2.24 mg g^{-1}), chlorophyll b (2.00 mg g^{-1}) and total chlorophyll contents (4.58 mg g^{-1}) and lower amounts of proline (1.30 mg g^{-1}). The same treatment combination was found to be most effective in reducing the Na^+ (0.32) concentration only in index leaves and enhancing the K^+ (1.86) and Ca^{2+} (0.37), resulting in the lowest Na^+/K^+ ratio (0.17) and Na^+/Ca^{2+} ratio (0.86), where as in rice roots $Zn_{40}+B_{0.75}$ even week after 3rd spray recorded lower Na^+ (0.74) enhanced K^+ (1.50) and Ca^{2+} (0.98) resulting in lowest Na^+/K^+ ratio (0.50) and Na^+/Ca^{2+} ratio (0.76), indicating SA of Zn at 40 and three FS of B at 0.25 per cent is better strategies for rice cultivation to overcome salt stress.
***Keywords* :** Proline, chlorophyll content, SPAD values, Relative water content, Na^+/Ca^{2+}, Na^+/K^+

Introduction

Rice (*Oryza sativa* L.) is a vital cereal crop which is staple food for 70 per cent of world population. Globally rice is cultivated in an area of 164.19 m ha with production of 514.80 m t (FAO, 2022; SRD, 2022). In India, rice is cultivated in an area of 46.27 m ha with a production and productivity of 129.47 m t and 2798 kg ha^{-1}, respectively. In Karnataka, it is being grown in 1.39 m ha area with annual production and productivity of 431.83 m t and 3089 kg ha^{-1}, respectively (INDIASTAT, 2022).

Salt stress is a major abiotic factor influencing crop productivity, impacting over 833 million hectares of arable land globally (FAO, 2022). It leads to reduced photosynthesis, stomatal conductance, transpiration Cattivelli *et al.* (2008) and water use efficiency (WUE) in rice plants (Ramezani *et al.*, 2012), negatively influencing biomass and seed yield (Gholipoor *et al.*, 2002). Salt stress has been shown to have molecular and biological impacts, affecting rice physiology (Munns, 2002; Tester and Davenport, 2003). These physiological disruptions result in abnormal growth and development, potentially causing plant death (Prida and Das, 2005).

Zinc improves resistance to salt stress in rice by enhancing biological and physiological activities (Latef *et al.*, 2017) including biosynthesis of photosynthetic pigments, modifying stomata and trichome morphology. It boosts K^+ absorption and lowering Na^+

ion levels and enhances the activity of antioxidant enzymes such as glutathione, ascorbate peroxidase, superoxide dismutase and catalases, which aid in scavenging reactive oxygen species (Faizan *et al*., 2021; Singh *et al*., 2021). Additionally, zinc binds to phospholipids and sulfhydryl groups to maintain cell membranes, especially in stressful situations (Hafeez *et al*., 2013).

Boron is a crucial micronutrient for plants, playing vital roles in growth, development and stress protection. It regulates physiological processes, enhances antioxidant defense components and improves cytoskeletal integrity especially under salt stress. B supplementation reduces malondialdehyde levels and electrolyte leakage while stimulating the methylglyoxal detoxification through upregulation of glyoxalase system's activity. There by it supports water relations, nitrogen metabolism, chlorophyll synthesis (Alharby *et al*., 2021) and cell wall development. Additionally, B is essential for the formation of pollen tubes, seed development, floret fertility (Wang *et al*., 2003; Oosterhuis, 2001) and the synthesis of phenolic compounds.

Materials and Methods

The experiment was carried out at College of Agriculture, Vishweshwaraiah Canal Farm, Mandya, India during *Kharif* 2022. The experimental site was classified as *Alfisols* with sandy clay loam with saline soil reaction (8.47), electrical conductivity (0.72 dSm^{-1}) was low and organic carbon content (5.81 g kg^{-1}) was found to be medium. The available nitrogen (237.08 kg ha^{-1}), phosphorus (28.93 kg P_2O_5 ha^{-1}) and potassium (198.09 kg K_2O ha^{-1}) was medium. The experiment was laid out in 3 × 4 Factorial Randomized Complete Block Design (Factorial-RCBD) with twelve treatment combinations and three replications (Table)

Table 1: Treatment combinations:

Zn_0 B_0	No Zinc sulfate + No Boric acid
$Zn_0B_{0.25}$	No Zinc sulfate + FA of Boric acid @0.25 %
Zn_0 $B_{0.5}$	No Zinc sulfate + FA of Boric acid @0.5 %
$Zn_0B_{0.75}$	No Zinc sulfate + FA of Boric acid @0.75 %
Zn_{20} B_0	SA of Zinc sulfate @ 20 kg + No Boric acid
$Zn_{20}B_{0.25}$	SA of Zinc sulfate @ 20 kg + FA of Boric acid @0.25 %
Zn_{20} $B_{0.5}$	SA of Zinc sulfate @ 20 kg + FA of Boric acid @0.5 %
$Zn_{20}B_{0.75}$	SA of Zinc sulfate @ 20 kg + FA of Boric acid @0.75 %
Zn_{40} B_0	SA of Zinc sulfate @ 40 kg + No Boric acid
$Zn_{40}B_{0.25}$	SA of Zinc sulfate @ 40 kg + FA of Boric acid @0.25 %
Zn_{40} $B_{0.5}$	SA of Zinc sulfate @ 40 kg + FA of Boric acid @0.5 %
$Zn_{40}B_{0.75}$	SA of Zinc sulfate @ 40 kg + FA of Boric acid @0.75 %

Note: RDF - Recommended dose of fertilizer (125:62.5:62.5 N: P_2O_5: K_2O kg ha^{-1}) and Farm yard manure @12.5 t ha^{-1} are common for all the treatments

FA- Foliar application of Boric acid at required concentration was sprayed at 15, 30 and 45 days after transplanting

SA- Soil application of Zinc sulfate at required rate was applied on the day of transplanting

In the present experiment, rice (*Oryza sativa* L.) variety MSN 99 was grown as the test crop. Two raised nursery beds of size 10 m length and 1.5 m breadth were prepared. Well decomposed 85 kg of farm yard manure and 0.60 kg urea, 0.85 kg SSP and 0.25 kg MOP fertilizers were applied uniformly to both the beds. The main plots were then prepared after 15 days with gross plot and net plot size of 8.2 m × 2.8 m and 7.4 m × 2.4 m, respectively. The recommended FYM (12.5 t ha^{-1}) and N: P_2O_5: K_2O for salt affected soil *i.e.*, 125: 62.5: 62.5 kg ha^{-1} was supplied using Urea, Di Ammonium Phosphate (DAP) and Muriate of potash (MOP) respectively.

Application of zinc and boron

Soil application zinc sulfate

Soil application of zinc in the form of $ZnSO_4$. $7H_2O$ at the rate of 20 and 40 kg ha^{-1} was applied at the time of transplanting, which amounts to 2 and 4 g per m^2 respectively.

Foliar application of boric acid

Foliar spray of boron was sprayed in three intervals as per the treatment requirements. The first foliar spray was carried out after 15 days of transplanting (DAT), and the second and third spray at 30 DAT and 45 DAT and the pH of the boric acid was maintained around 6 to 6.5 by potassium hydroxide. The boric acid (17.5 % B) dissolved at the rate of 2.50, 5.00 and 7.50 g L^{-1} for obtaining 0.25, 0.50 and 0.75 per cent concentrations. The total spray volume of 250, 300 and 500 litres per ha^{-1} was used for 1^{st}, 2^{nd} and 3^{rd} foliar spray respective of total amount of 2.65 and 5.25 and 7.88 kg ha^{-1} of boric acid for 0.25, 0.50 and 0.75 per cent foliar sprays (cumulative of 3 sprays).

Recording Physiological attributing parameters

Estimation of Na^+, K^+ and Ca^{2+} Concentrations in roots and Index leaves

Potassium and Sodim in the plant sample was analysed by flame photometer (Piper, 1966). Calcium was analysed using standard EDTA after adding necessary reagents required for calcium as described by Piper, (1966).

SPAD readings

SPAD (SPAD-502, MINLOTA Japan) chlorophyll was used to measure the amount of chlorophyll in random five leaves of each treatment at tillering, grand growth and panicle initiation stages respectively.

Estimation of Relative water content (%)

Leaf relative water content (RWC) was estimated by recording fresh weight and leaf sample immersed in water overnight and turgid weight was recorded. Samples were kept in hot air oven and the dry weight was recorded until a constant weight was reached. The relative content was then determined using the formula provided by (Weatherley, 1950)

$$\text{RWC}(\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Estimation of Photosynthetic pigments (mg g^{-1})

Total chlorophyll, chlorophyll a and chlorophyll b and contents were determined by spectrophotometrically (Agilent - carry 60 UV-Vis) at 645, 663 and 480 nm following the method of Arnon, (1949).

Chlorophyll a (mg/ml) = 0.0127 × A663 – 0.0027 × A645

Chlorophyll b (mg/ml) = 0.0229 × A645 – 0.0046 × A663

Total chlorophyll (mg/ml) = 0.0202 × A645 + 0.00802×A663

Estimation of Proline content (mg g^{-1})

According to Bates *et al*. (1973), the proline content was calculated using the absorbance measured in an Agilent spectrophotometer (Agilent - carry 60 UV-Vis).
A standard curve was used to calculate the proline concentration at 520 nm using a fresh weight basis.

$$\text{Proline}(\text{mg/g}) = \frac{X}{2} \times \frac{10}{500} \times 1000$$

Results

Effect of zinc and boron on sodium, potassium and calcium concentration in rice

Higher concentration of Na^+ both in roots (0.80 %) and index leaves (0.45 %) and lowest K^+ and Ca^{2+} content correspondingly resulting in higher Na^+/K^+ (0.79 and 0.61) and Na^+/Ca^{2+} (1.28 and 1.88) was noticed in the treatment with no zinc and boron application (Zn_0+B_0). Soil application of zinc and foliar spray of boron at different levels reduced the Na^+ content. Soil application of zinc at 40 kg ha^{-1} and spray of boron at 0.75 % was found to be more effective in reducing Na^+ concentration both in root and index leaves week after 1st spray and enhanced the accumulation of K^+ and Ca^{2+}, resulting in the lowest Na^+/K^+ (0.56 and 0.32) and Na^+/Ca^{2+} (0.75 and 0.94) in root and index leaves, respectively (Table 2).

The data on relative concentrations of ions in rice roots recorded after 1 week of second foliar spray of boron (WA-SS) showed (Table 3) similar trend indicating maximum concentration of K^+ (1.25 %), Ca^{2+} (0.88 %) and lowest Na^+ (0.70 %), Na^+/K^+ (0.55) and Na^+/Ca^{2+} (0.76) with $Zn_{40}+B_{0.75}$. However, effect B spray as such did not show significant variation with respect to K^+ and Na^+. The relative concentration of Na^+ in index leaves decreased with increased amount of Zn but foliar application of B recorded decreasing trend up to 0.5 % (0.36 %) while, 0.75 % accumulated higher Na^+ (0.40 %) as compared to 0.25% (0.39 %) however, was lesser than B_0 (0.51%). The higher amount of K^+ and Ca^{2+} was recorded with application of 40 kg ha^{-1} Zn and 0.5 per cent foliar spray of B. Lowest Na^+/K^+ and Na^+/Ca^{2+} was noted in the same treatment and further increase in B to 0.75 per cent enhanced these parameters in index leaves.

The Na^+, Na^+/K^+ and Na/Ca^{2+} content in rice roots after one week 3rd spray of boron (WA-TS) revealed a decreasing trend when the rate of Zn and B application increased, recording lowest amount of 0.74 per cent, 0.50 per cent and 0.76 per cent, respectively in $Zn_{40}+B_{0.75}$. The same treatment recorded highest K^+ (1.5 %) and Ca^{+2} (0.98 %) in rice roots (Table 4).The concentration of these ions in index leaves showed similar variation with increasing rate of soil application of Zn *i.e.*, Na^+, Na^+/K^+ and Na^+/Ca^{2+} and increased K^+ and Ca^{2+} but the foliar spray of B was effective only up to 0.25 % that recorded lowest Na^+ (0.32 %), Na^+/K^+ (0.17) Na^+/Ca^{2+} (0.86) and further increasing its concentration to 0.5 and 0.75 % increased these parameters.

Relative water content

The interactive effects of zinc and boron levels recorded significant variation. The highest amount of 77.86 % was recorded with $Zn_{40}+B_{0.75}$ followed by 76.34 and 75.21 % in $Zn_{40}+B_{0.5}$ and $Zn_{40}+B_{0.25}$ as compared to amounts of 65.34 % in Zn_0+B_0 at WA-FS. However, the RWC after second and third spray showed variation recording decreased trend with $B_{0.75}$ as compared to $B_{0.5}$ and $B_{0.25}$ with or without Zn. The highest RWC of 88.23 % and 90.23 % was noticed in $Zn_{40}+B_{0.5}$ and $Zn_{40}+B_{0.25}$ after WA-SS and WA-TS, respectively.

Effect of zinc and boron on proline content in rice

In the interactive effect of Zn and B the proline was significant at all stages and lowest content of 0.74 mg g^{-1} was recorded with $Zn_{40}+B_{0.75}$ at WA-FS. However, after successive 2nd and 3rd B spray the proline content has enhanced to 1.23 and 1.55 mg g^{-1}, respectively in the same treatment. The treatment $Zn_{40}+B_{0.25}$ was found better in overcoming the salinity oxidative stress by accumulating the lowest proline content of 1.30 mg g^{-1} at WA-TS as compared to other treatments.

Effect of zinc and boron on photosynthetic pigments and SPAD values in rice

The interactive effect of Zn and B showed significant protection to photosynthetic pigments by overcoming the salinity stress. Single spray of $Zn_{40+}B_{0.75}$ was found most effective at early stages (WA-FS) that recorded highest SPAD readings of 36.40 and 1.36, 1.28 and 3.51 mg g^{-1} of chlorophyll a, b and total content, respectively. But, successive two sprays decreased these contents indicating oxidative stress induced with higher B accumulation in index leaves. Foliar sprays of B at the rate of 0.25 per cent accumulated relatively highest photosynthetic pigments (42.50, 2.24 mg g^{-1}, 2.00 mg g^{-1} and 4.58 mg g^{-1}, respectively) after three successive sprays (WA-TS).

Discussion

From the present investigation, it was interesting to note that soil application of zinc may be attributed to its ability to maintain homeostasis of these ions which helps exclusion of Na^+ resulting in higher concentration of K^+ and Ca^{2+} in stems and roots. Potassium is helpful in preserving the turgidity and controlling vital enzyme activity of the cell. At the same time, plant growth and development are also hampered by shortage of K^+ ions and high Na^+/K^+ ratio. Therefore, applying zinc enhanced the better adaptation toward salt stress in rice by lowering the Na^+, Na^+/K^+ and Na^+/Ca^{2+} ratios in them. Lowering Na^+/K^+ and Na^+/Ca^{2+} balance is a key trait of salinity tolerance which helps plants better adjust to salinity stress (Dey and Somaiah, 2022).

The foliar application of B had differential response to ion accumulation in rice roots and index leaves. Increasing rate of B from 0.25 to 0.75 % decreased the Na^+, Na^+/K^+ and Na^+/Ca^{2+} while, K^+ and Ca^{2+} content increased in rice roots at all stages of crop growth indicating no toxic effect on root growth and ionic homoeostasis. The inability of B to translocate rapidly from shoot to root owing to its relative slow mobility through phloem tissues (Brown and shelp, 1997). However, B present in the phloem retranslocate to sink regions through restricted mobility to satisfy the demands plants. Thus, might have helped rice roots to overcome salinity stress through its critical role in strengthening the plant cell wall and also to maintain ionic homeostasis. The foliar application of B in index leaves showed positive responses with increasing concentration after the 1st spray. However, after subsequent spray higher concentration of B *i.e.*, at 0.75 and 0.5 % affected the ionic homeostasis after second and third spray, respectively. The narrow range of B critical concentration make it either deficient or toxic, studies indicate that optimum range of B in rice (4 - 6 ppm) grain below and above levels either deficient or toxic, affecting ionic balance and metabolic activity (Milka, 2020).

Reactive oxygen species (ROS), such as $^1O_2$, H_2O_2 and $OH^•$ are produced by oxidative stress caused by an excessive build-up of Na^+ and Cl^- (Mehta *et al.*, 2010). In addition to harming the chloroplast and damages the photosynthetic pigment-protein machinery of photosynthesis, ROS is detrimental to the cell and its structure (Parihar *et al.*, 2015). Furthermore, salinity stress alters the water flux that affects function of stomata. To overcome stress under saline environment zinc and boron application helps in triggering the activation of antioxidant enzymes (CAT, APX, SOD and GR) that detoxify ROS radicals (Mogazy and Hanafy, 2022). With the reduction in ROS species proline also decrease, Proline is the most common organic solute that functions as an osmolyte and preserves the cytosolic pH and osmotic state of cells during salinity stress. This would help in, respectively cell turgidity via continuous influx of water (Singh *et al.*, 2022; Alharby *et al.*, 2021; Al-zehrani *et al.*, 2021) and optimum levels of zinc and boron helps in maintaining the ionic homeostasis under saline conditions that protects photosynthetic pigments against oxidative stress (Massange-Sanchez *et al.*, 2021). The upregulation of ROS-scavenging enzymes that quickly break down the ROS radicals found in the thylakoid membranes of chloroplasts is the primary cause of this increase in photosynthetic pigment content (Singh *et al.*, 2022; Alhraby *et al.*, 2021).

Conclusion

The present study highlighted the soil application of zinc and foliar spray of boron evaluating their effect on physiological attributes of rice. The best results were observed with soil application of zinc at 40 kg ha^{-1} and foliar spray of boron at 0.25 %, which enhanced ionic concentrations, proline content, relative water content, SPAD values and chlorophyll contents. The combination of 40 kg ha^{-1} zinc and 0.5% boron foliar spray was the second most effective treatment for improving these physiological attributes.

Table 2: Effect of Zn and B on ions concentration in rice roots and index leaves at tillering stage (WA-FS)

Treatments	Roots					Index leaves				
	Na^+ (%)	K^+ (%)	Ca^{2+} (%)	Na^+/K^+	Na^+/Ca^{2+}	Na^+ (%)	K^+ (%)	Ca^{2+} (%)	Na^+/K^+	Na^+/Ca^{2+}
Soil application of zinc										
Zn_0	0.79	1.07	0.66	0.75	1.19	0.42	0.79	0.25	0.54	1.67
Zn_{20}	0.74	1.14	0.75	0.66	0.99	0.34	0.84	0.27	0.40	1.25
Zn_{40}	0.68	1.20	0.85	0.59	0.83	0.31	0.90	0.30	0.35	1.04
S.Em±	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.01**	**0.02**	**0.01**	**0.01**	**0.04**
C.D @ 5 %	**0.05**	**0.06**	**0.06**	**0.05**	**0.08**	**0.03**	**0.06**	**0.02**	**0.04**	**0.11**
Foliar spray of boron										
B_0	0.76	1.11	0.72	0.70	1.07	0.38	0.83	0.26	0.47	1.46
$B_{0.25}$	0.75	1.12	0.74	0.68	1.04	0.36	0.84	0.27	0.44	1.35
$B_{0.5}$	0.73	1.16	0.77	0.65	0.97	0.35	0.85	0.28	0.41	1.27
$B_{0.75}$	0.71	1.16	0.78	0.63	0.93	0.33	0.86	0.28	0.39	1.20
S.Em±	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.01**	**0.02**	**0.01**	**0.01**	**0.04**
C.D @ 5 %	**NS**	**NS**	**0.07**	**0.06**	**0.09**	**0.03**	**NS**	**0.02**	**0.04**	**0.12**
Interaction (Zn × B)										
Zn_0B_0	0.80	1.05	0.64	0.79	1.28	0.45	0.77	0.24	0.61	1.88
$Zn_0B_{0.25}$	0.79	1.06	0.66	0.76	1.22	0.42	0.79	0.25	0.56	1.69
$Zn_0B_{0.5}$	0.78	1.09	0.68	0.73	1.15	0.40	0.79	0.25	0.51	1.60
$Zn_0B_{0.75}$	0.77	1.10	0.68	0.71	1.13	0.39	0.82	0.26	0.48	1.51
$Zn_{20}B_0$	0.76	1.10	0.72	0.69	1.06	0.36	0.84	0.26	0.43	1.37
$Zn_{20}B_{0.25}$	0.75	1.11	0.73	0.68	1.03	0.35	0.84	0.27	0.41	1.28
$Zn_{20}B_{0.5}$	0.73	1.16	0.76	0.64	0.97	0.33	0.85	0.28	0.39	1.18
$Zn_{20}B_{0.75}$	0.71	1.18	0.78	0.62	0.90	0.32	0.85	0.28	0.38	1.15
$Zn_{40}B_0$	0.70	1.18	0.80	0.61	0.88	0.32	0.88	0.29	0.37	1.12
$Zn_{40}B_{0.25}$	0.70	1.20	0.83	0.60	0.87	0.32	0.90	0.30	0.35	1.09
$Zn_{40}B_{0.5}$	0.68	1.21	0.87	0.58	0.80	0.31	0.90	0.30	0.34	1.03
$Zn_{40}B_{0.75}$	0.66	1.21	0.88	0.56	0.75	0.29	0.90	0.31	0.32	0.94
S.Em±	**0.03**	**0.04**	**0.04**	**0.04**	**0.05**	**0.02**	**0.04**	**0.01**	**0.02**	**0.07**
C.D @ 5 %	**0.10**	**0.11**	**0.11**	**0.11**	**0.16**	**0.06**	**0.12**	**0.03**	**0.07**	**0.21**

Factor 1 **: Soil application of $ZnSO_4$**
Zn_0 : No Zinc sulphate
Zn_{20} : Zinc sulphate @ 20 kg ha^{-1}
Zn_{40} : Zinc sulphate @ 40 kg ha^{-1}

Factor 2 **: Foliar spray of boric acid**
B_0 : No Boric acid
$B_{0.25}$: Boric acid @ 0.25 %
$B_{0.5}$: Boric acid @ 0.50 %
$B_{0.75}$: Boric acid @ 0.75 %

Table 3: Effect of Zn and B on ions concentration in rice roots and index leaves at grand growth stage (WA-SS)

Treatments	Roots					Index leaves				
	Na^+ (%)	K^+ (%)	Ca^{2+} (%)	Na^+/K^+	Na^+/Ca^{2+}	Na^+ (%)	K^+ (%)	Ca (%)	Na^+/K^+	Na^+/Ca^{2+}
Soil application of zinc										
Zn_0	0.86	1.09	0.66	0.79	1.21	0.48	1.04	0.29	0.46	1.64
Zn_{20}	0.79	1.13	0.75	0.70	0.98	0.36	1.19	0.32	0.31	1.14
Zn_{40}	0.73	1.21	0.85	0.61	0.83	0.39	1.40	0.35	0.28	1.13
S.Em±	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.01**	**0.02**	**0.00**	**0.01**	**0.04**
C.D @ 5 %	**0.05**	**0.06**	**0.06**	**0.05**	**0.08**	**0.03**	**0.07**	**0.01**	**0.04**	**0.10**
Foliar spray of boron										
B_0	0.82	1.12	0.72	0.74	1.09	0.45	1.15	0.31	0.40	1.46
$B_{0.25}$	0.80	1.13	0.74	0.70	1.03	0.40	1.22	0.32	0.34	1.27
$B_{0.5}$	0.80	1.15	0.77	0.69	1.00	0.37	1.27	0.33	0.30	1.15
$B_{0.75}$	0.77	1.17	0.78	0.65	0.93	0.42	1.21	0.31	0.36	1.34
S.Em±	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.01**	**0.03**	**0.01**	**0.01**	**0.04**
C.D @ 5 %	**NS**	**NS**	**0.07**	**0.06**	**0.09**	**0.04**	**0.08**	**0.02**	**0.04**	**0.12**

Interaction (Zn × B)										
Zn_0B_0	0.89	1.07	0.64	0.82	1.31	0.51	1.01	0.28	0.50	1.79
$Zn_0B_{0.25}$	0.86	1.07	0.66	0.79	1.25	0.48	1.04	0.29	0.46	1.64
$Zn_0B_{0.5}$	0.86	1.10	0.68	0.78	1.20	0.43	1.10	0.30	0.39	1.44
$Zn_0B_{0.75}$	0.84	1.12	0.68	0.75	1.10	0.49	1.02	0.29	0.48	1.69
$Zn_{20}B_0$	0.82	1.12	0.72	0.73	1.05	0.41	1.13	0.31	0.37	1.34
$Zn_{20}B_{0.25}$	0.80	1.13	0.73	0.71	1.00	0.34	1.20	0.32	0.29	1.07
$Zn_{20}B_{0.5}$	0.80	1.14	0.76	0.70	0.97	0.33	1.24	0.33	0.27	1.00
$Zn_{20}B_{0.75}$	0.76	1.14	0.78	0.66	0.92	0.36	1.19	0.31	0.30	1.14
$Zn_{40}B_0$	0.76	1.16	0.80	0.65	0.90	0.41	1.30	0.33	0.32	1.25
$Zn_{40}B_{0.25}$	0.74	1.20	0.83	0.61	0.84	0.39	1.42	0.35	0.27	1.10
$Zn_{40}B_{0.5}$	0.73	1.22	0.87	0.60	0.82	0.36	1.47	0.36	0.24	0.99
$Zn_{40}B_{0.75}$	0.70	1.25	0.88	0.55	0.76	0.40	1.41	0.34	0.29	1.19
S.Em±	**0.03**	**0.04**	**0.04**	**0.03**	**0.06**	**0.02**	**0.05**	**0.01**	**0.02**	**0.07**
C.D @ 5 %	**0.10**	**0.13**	**0.11**	**0.10**	**0.16**	**0.06**	**0.13**	**0.03**	**0.07**	**0.21**

Factor 1 **: Soil application of $ZnSO_4$**
Zn_0 : No Zinc sulphate
Zn_{20} : Zinc sulphate @ 20 kg ha^{-1}
Zn_{40} : Zinc sulphate @ 40 kg ha^{-1}

Factor 2 **: Foliar spray of boric acid**
B_0 : No Boric acid
$B_{0.25}$: Boric acid @ 0.25 %
$B_{0.5}$: Boric acid @ 0.50 %
$B_{0.75}$: Boric acid @ 0.75 %

Table 4: Effect of Zn and B on ions concentration in rice roots and index leaves at panicle initiation stage (WA-TS)

Treatments	**Roots**					**Index leaves**				
	Na^+ (%)	**K^+ (%)**	**Ca^{2+} (%)**	**Na^+/K^+**	**Na^+/Ca^{2+}**	**Na^+ (%)**	**K^+ (%)**	**Ca (%)**	**Na^+/K^+**	**Na^+/Ca^{2+}**
Soil application of zinc										
Zn_0	1.02	1.19	0.75	0.87	1.36	0.51	1.37	0.30	0.38	1.75
Zn_{20}	0.91	1.30	0.82	0.70	1.11	0.43	1.45	0.33	0.29	1.34
Zn_{40}	0.76	1.41	0.93	0.55	0.83	0.36	1.80	0.36	0.20	1.00
S.Em±	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.01**	**0.03**	**0.01**	**0.01**	**0.04**
C.D @ 5 %	**0.05**	**0.07**	**0.06**	**0.06**	**0.09**	**0.04**	**0.09**	**0.02**	**0.03**	**0.11**
Foliar spray of boron										
B_0	0.95	1.24	0.80	0.78	1.21	0.47	1.49	0.31	0.33	1.56
$B_{0.25}$	0.91	1.28	0.83	0.72	1.13	0.40	1.60	0.34	0.25	1.19
$B_{0.5}$	0.88	1.32	0.84	0.67	1.06	0.42	1.55	0.33	0.27	1.29
$B_{0.75}$	0.85	1.36	0.86	0.63	1.00	0.45	1.52	0.32	0.30	1.42
S.Em±	**0.02**	**0.03**	**0.03**	**0.02**	**0.04**	**0.02**	**0.03**	**0.01**	**0.01**	**0.04**
C.D @ 5 %	**0.06**	**0.08**	**NS**	**0.07**	**0.10**	**0.05**	**0.10**	**0.02**	**0.03**	**0.13**
Interaction (Zn × B)										
Zn_0B_0	1.10	1.10	0.73	1.00	1.51	0.57	1.30	0.28	0.44	2.06
$Zn_0B_{0.25}$	1.01	1.20	0.75	0.84	1.35	0.46	1.43	0.31	0.32	1.48
$Zn_0B_{0.5}$	1.00	1.21	0.75	0.83	1.32	0.48	1.39	0.30	0.34	1.59
$Zn_0B_{0.75}$	0.98	1.24	0.77	0.79	1.26	0.53	1.35	0.29	0.40	1.86
$Zn_{20}B_0$	0.97	1.26	0.80	0.77	1.22	0.45	1.48	0.31	0.30	1.48
$Zn_{20}B_{0.25}$	0.95	1.26	0.81	0.76	1.18	0.42	1.50	0.34	0.27	1.23
$Zn_{20}B_{0.5}$	0.87	1.33	0.83	0.66	1.05	0.43	1.44	0.33	0.28	1.28
$Zn_{20}B_{0.75}$	0.83	1.33	0.83	0.61	0.98	0.44	1.40	0.32	0.30	1.37
$Zn_{40}B_0$	0.78	1.35	0.86	0.58	0.90	0.39	1.69	0.34	0.23	1.13
$Zn_{40}B_{0.25}$	0.78	1.37	0.92	0.57	0.84	0.32	1.86	0.37	0.17	0.86
$Zn_{40}B_{0.5}$	0.76	1.40	0.95	0.54	0.80	0.35	1.83	0.36	0.20	1.00
$Zn_{40}B_{0.75}$	0.74	1.50	0.98	0.50	0.76	0.36	1.82	0.35	0.19	1.02
S.Em±	**0.03**	**0.05**	**0.04**	**0.04**	**0.06**	**0.03**	**0.06**	**0.01**	**0.02**	**0.07**
C.D @ 5 %	**0.10**	**0.14**	**0.13**	**0.12**	**0.18**	**0.09**	**0.17**	**0.04**	**0.05**	**0.22**

Factor 1 **: Soil application of $ZnSO_4$**
Zn_0 : No Zinc sulphate
Zn_{20} : Zinc sulphate @ 20 kg ha^{-1}
Zn_{40} : Zinc sulphate @ 40 kg ha^{-1}

Factor 2 **: Foliar spray of boric acid**
B_0 : No Boric acid
$B_{0.25}$: Boric acid @ 0.25 %
$B_{0.5}$: Boric acid @ 0.50 %
$B_{0.75}$: Boric acid @ 0.75 %

Table 5: Effect of Zn and B on chlorophyll a, chlorophyll b and total chlorophyll in index leaves of rice at different growth stages

Treatments	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
	WA-FS	WA-SS	WA-TS	WA-FS	WA-SS	WA-TS	WA-FS	WA-SS	WA-TS
Soil application of zinc									
Zn_0	0.66	0.90	1.41	0.55	0.81	1.18	2.66	3.25	3.53
Zn_{20}	0.93	1.33	1.76	0.84	1.13	1.64	3.08	3.34	3.89
Zn_{40}	1.26	1.60	2.14	1.14	1.56	1.93	3.45	3.73	4.37
S.Em±	**0.02**	**0.03**	**0.03**	**0.02**	**0.03**	**0.03**	**0.07**	**0.09**	**0.08**
C.D @ 5 %	**0.06**	**0.10**	**0.10**	**0.07**	**0.09**	**0.08**	**0.20**	**0.27**	**0.23**
Foliar spray of boron									
B_0	0.85	1.16	1.64	0.72	1.03	1.43	2.93	3.27	3.76
$B_{0.25}$	0.92	1.31	1.88	0.82	1.24	1.70	3.03	3.56	4.07
$B_{0.5}$	0.99	1.36	1.82	0.89	1.30	1.65	3.11	3.58	3.99
$B_{0.75}$	1.05	1.27	1.75	0.94	1.10	1.56	3.18	3.34	3.91
S.Em±	**0.02**	**0.04**	**0.04**	**0.03**	**0.04**	**0.03**	**0.08**	**0.11**	**0.09**
C.D @ 5 %	**0.07**	**0.12**	**0.11**	**0.08**	**0.11**	**0.09**	**0.23**	**0.31**	**0.27**
Interaction (Zn × B)									
Zn_0B_0	0.54	0.85	1.30	0.45	0.72	1.00	2.49	3.12	3.44
$Zn_0B_{0.25}$	0.64	0.91	1.51	0.53	0.83	1.32	2.61	3.29	3.68
$Zn_0B_{0.5}$	0.68	0.95	1.43	0.57	0.92	1.28	2.71	3.32	3.55
$Zn_0B_{0.75}$	0.77	0.90	1.41	0.65	0.78	1.13	2.82	3.29	3.46
$Zn_{20}B_0$	0.85	1.21	1.63	0.75	0.95	1.45	2.99	3.33	3.77
$Zn_{20}B_{0.25}$	0.91	1.38	1.89	0.84	1.28	1.77	3.01	3.36	3.96
$Zn_{20}B_{0.5}$	0.96	1.39	1.78	0.89	1.29	1.71	3.11	3.30	3.94
$Zn_{20}B_{0.75}$	1.01	1.32	1.74	0.89	1.01	1.62	3.20	3.35	3.90
$Zn_{40}B_0$	1.15	1.43	2.00	0.97	1.41	1.83	3.32	3.37	4.06
$Zn_{40}B_{0.25}$	1.21	1.63	2.24	1.09	1.62	2.00	3.48	4.02	4.58
$Zn_{40}B_{0.5}$	1.32	1.74	2.23	1.21	1.68	1.97	3.50	4.13	4.47
$Zn_{40}B_{0.75}$	1.36	1.58	2.10	1.28	1.52	1.92	3.51	3.39	4.37
S.Em±	**0.04**	**0.07**	**0.07**	**0.05**	**0.06**	**0.05**	**0.13**	**0.18**	**0.16**
C.D @ 5 %	**0.12**	**0.21**	**0.20**	**0.14**	**0.19**	**0.16**	**0.39**	**0.54**	**0.47**

Note: WA-FS: Week after first spray, **WA-SS:** Week after second spray, **WA-TS:** Week after third spray

Factor 1 **: Soil application of $ZnSO_4$**
Zn_0 : No Zinc sulphate
Zn_{20} : Zinc sulphate @ 20 kg ha^{-1}
Zn_{40} : Zinc sulphate @ 40 kg ha^{-1}

Factor 2 **: Foliar spray of boric acid**
B_0 : No Boric acid
$B_{0.25}$: Boric acid @ 0.25 %
$B_{0.5}$: Boric acid @ 0.50 %
$B_{0.75}$: Boric acid @ 0.75 %

Plate 1 : Layout of the field experimental plot

Plate 2 : General view of experimental plot

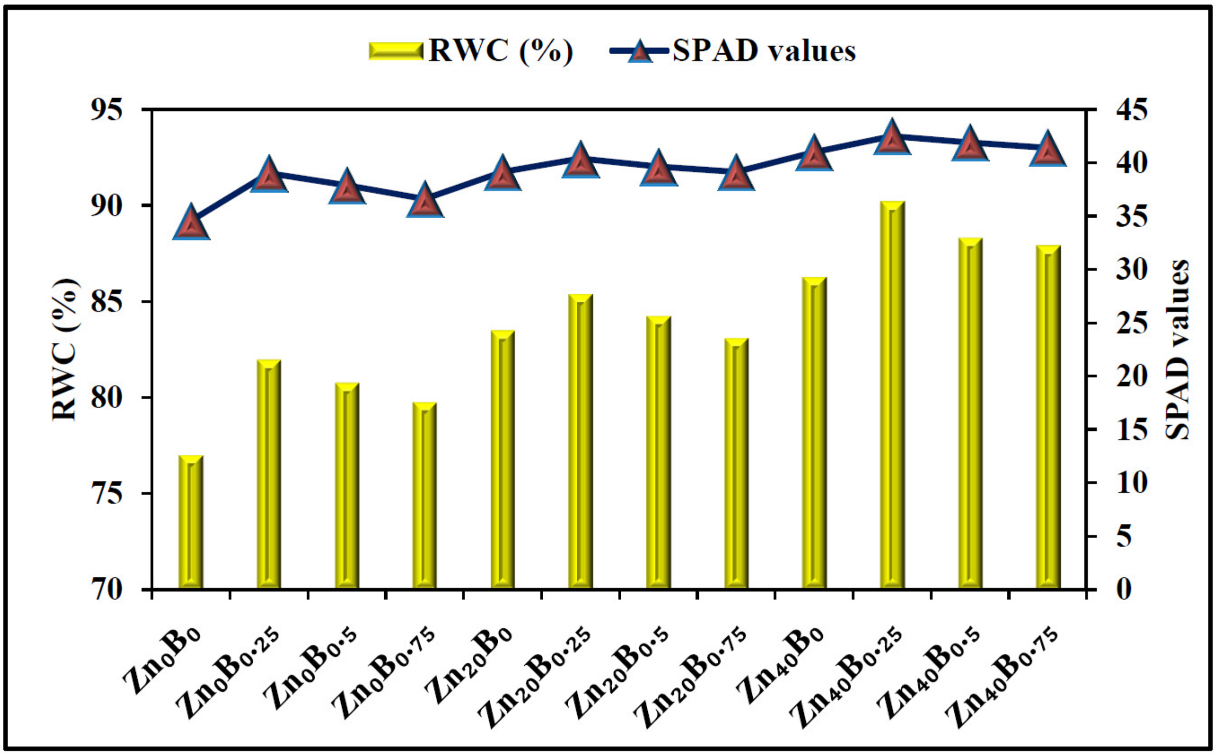

Fig. 1: Effect of Zn and B on RWC and SPAD values in rice index leaves at panicle initiation stage (WA-TS)

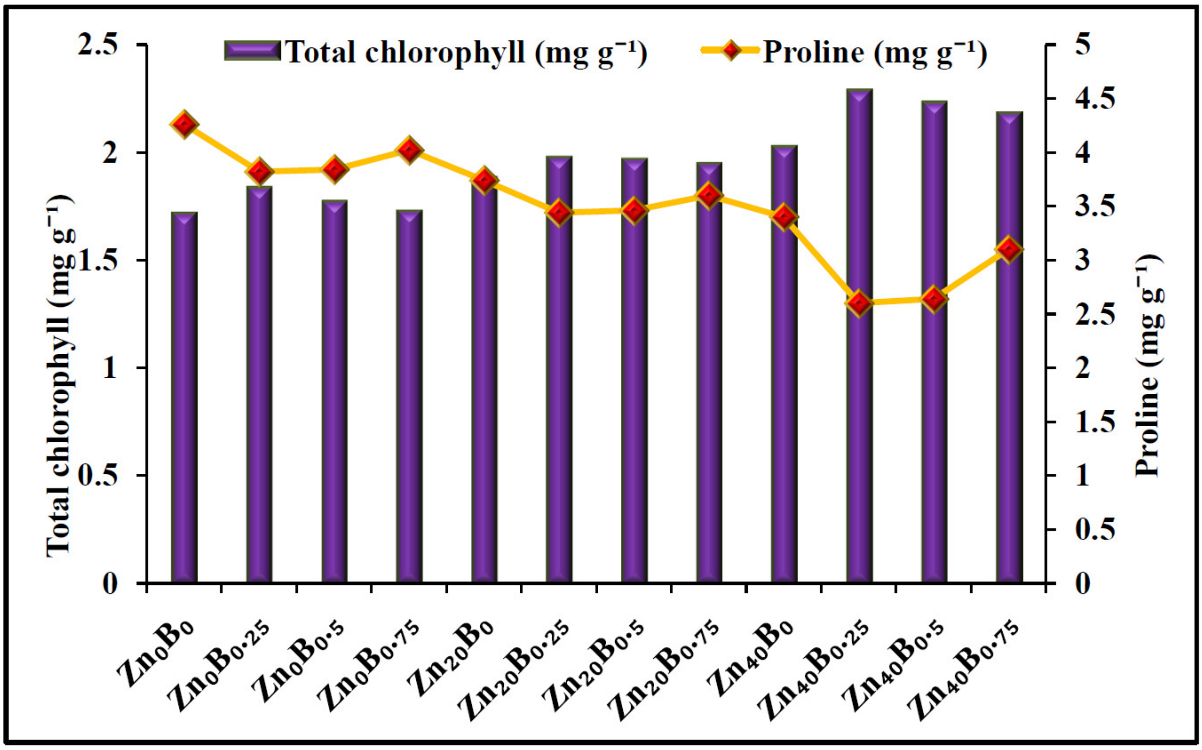

Fig. 2: Effect of Zn and B on total chlorophyll and proline content in rice index leaves at panicle initiation stage (WA-TS)

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