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Evaluation of different combination of zinc, boron and sulphur application on growth and yield of hybrid rice (*Oryza sativa L.*)

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Abstract
A pot experiment was conduct to assess the effect of various combination Zn, B and S application on growth and yield of hybrid rice. Treatments consist of T_1, absolute control; T_2, 100% RDF through inorganic source NPK; T_3, 100% RDF + Zn; T_4, 100% RDF + B; T_5, 100% RDF + S; T_6, 100% RDF + Zn + B; T_7, 100% RDF + Zn + S; T_8, 100% RDF + B + S; T_9, 100% RDF + Zn + B + S. Experimental results showed a significant increase in plant height, number of tiller, chlorophyll content, number of panicle, length of panicle, grain per panicle, straw yield, grain yield, harvest index and test weight of rice crop with combine application of Zn + B + S with RDF. Maximum chlorophyll content, plant height and number of tiller of rice were recorded in T_9 (RDF + Zn + B + S). Combination of Zn + B + S along with 100% RDF increased grain yield to the extent of 1.34 times over absolute control (no fertilizers) and 1.17 times over control (100% RDF).

Keywords: Boron, chlorophyll content, hybrid rice, test weight, zinc.

Introduction
Rice (*Oryza sativa* L) is the staple food of over half the world's population and a mainstay for the rural population and their food security, cover a highest area of 154 million ha under cultivation. It is the predominant dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. Developing countries account for 95% of the total production, with China (197 Mt) and India (131 Mt) alone responsible for nearly half of the world output of rice. Rice is vital for the nutrition of much of the population in Asia, as well as in Latin America, the Caribbean and in Africa; it is central to the food security of over half the world population. India is an important centre of rice cultivation. Indian soils have become deficient not only in major plant nutrients like nitrogen, phosphorus and in some cases, potash but also in secondary nutrients, like sulphur, calcium, and magnesium. Micronutrients such as zinc, boron and to a limited extent iron, manganese, copper and molybdenum have also been reported to be deficient. Deficiency of micronutrients during the last three decades has grown in both, magnitude and extent because of increased use of high analysis fertilizers, high yielding crop varieties and increase in cropping intensity. The problem has been compounded by soil acidity affecting large area in eastern and southern states and soil alkalinity commonly observed in north-western states as crops grown on such soils encounter nutritional disorders and toxicities. Analysis of 2.52 lakhs surface soil samples collected from different parts of India (Singh, 2002) [1] revealed the predominance of zinc deficiency in divergent soils. Of these samples 49, 12, 4, 3, 33 and 41% soils are tested to be deficient in available zinc (Zn), iron (Fe) manganese (Mn), copper (Cu), boron (B) and sulphur (S), respectively. Continuous rice cultivation over centuries in certain tracts of India depleted available Zn.

Zinc is an essential nutrient for human health. It is vital for many biological functions in the human body. The adult body contains 2–3 g of zinc. It is present in all parts of the body including organs, tissues, bones, fluids and cells. It is vital for more than 300 enzymes in the human body, activating growth (height, weight and bone development), cell division, immune system, fertility, skin, hair and nails and vision. Symptoms due to zinc deficiency in humans, especially in infants and young children include dwarfism (growth retardation), dermatitis (alopecia), impaired neurology, decreased immune function, infections and death. Zinc deficiency in humans is a critical nutritional and health problem in the world. It affects, on average, one-third of the world’s population, ranging from 4 to 73 % in different countries (Hotz and Brown, 2004) [2].

Boron (B) is an essential nutrient for normal growth of higher plants and its availability in soil and irrigation water is an important determinant of agricultural production (Saleem *et al*., 2011) [3]. Boron deficiency has been identified as one of the most important factors causing sterility in cereals because of poor development of anthers and pollen and failure of pollen germination (Cheng and Rerkasem, 1993) [4]. Boron and Zn deficiency upset the order of grains on the corns and make them deformed so that some parts of the corn ear are free from grains (Marschner, 1995) [5]. Application of zinc increases boron uptake by plants in soil with sufficient stores (Renegal *et al.*, 1998) [6].

Sulphur is an essential plant nutrient and plays a vital role in the synthesis of amino acids (methionine, cystein and cystine), proteins, chlorophyll and certain vitamins. Plants absorb S mainly in the form of inorganic sulphate (SO_4^{2-}) ions through the roots, thus sulphate S must be present in soils in sufficient amount in order to meet crop S requirements (Brady and Weil, 2002) [7]. Insufficient availability of sulphur to crop plants not only declines their growth and yield but can also deteriorate nutritional quality of the produce (Hawkesford, 2000 and Schonhof *et al.,* 2007) [8, 9]. Sulphur deficiency in crops results in a reduction of leaf area, seed number, seed weight, delayed floral initiation and anthesis. It reduces growth rate and plant protein, chlorophyll content and photosynthetic CO_2 fixation. Nitrogen assimilation is hampered due to inadequate supply of S containing amino acids and thus nitrogen uptake and translocation are impeded. It has been reported that 56 % soils of Varanasi district are deficient in sulphur (Singh *et al.,* 2015) [10].

Material and Methods

Experimental site and soil properties

A pot experiment was conducted with hybrid rice (*Oryza sativa L.*) variety Arize-6444 during *Kharif* season of 2015-2016 in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. Varanasi is situated at an altitude of 80.71 meters above mean sea level and located between 25^0 14 and 25^0 23 N latitude and 82^0 56 and 83^0 30 E longitude has a semi-arid to sub-humid climate with moisture deficit index between 20-40%. The average annual rainfall of this region is about 1100 mm. Generally, the maximum and the minimum temperature ranged between 20^0 - 42^0 C and 9^0 - 28^0 C, respectively. The mean relative humidity is about 68% which rise to 82% during wet season and goes down to 30% during dry season. The bulk soil (0-15 cm depth) sample was collected from from Lal Pahadi, Village-Kanbehri, Block-Aurangabad, District-Aurangabad (Bihar) had pH 5.5, EC 0.15 dS m^{-1}, OC 0.52%, available N 185.8 kg ha^{-1}, available P 13.5 kg ha^{-1} and available K 175.95 kg ha^{-1}. The DTPA-extractable zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) contents in soil were 0.84, 5.3 46.1 and 64.2 mg kg^{-1}, respectively (Table 1).

Crop management

Treatments consist of T_1, absolute control; T_2, 100% RDF through inorganic source NPK; T_3, 100% RDF + Zn; T_4, 100% RDF + B; T_5, 100% RDF + S; T_6, 100% RDF + Zn + B; T_7, 100% RDF + Zn + S; T_8, 100% RDF + B + S; T_9, 100% RDF + Zn + B + S. The recommended dose of fertilizer (RDF) for hybrids rice is 150:60:60 N, P_2O_5 and K_2O. Required quantities of fertilizers for 10 kg soil was calculated and applied in liquid form using urea CO $(NH_2)_2$, potassium dihydrogenphosphate (KH_2PO_4) and muriate of potash (KCl) as source of N, P_2O_5 and K_2O, respectively. Full dose of P_2O_5 and K_2O and half dose of N were applied before transplanting and remaining nitrogen was added in two equal splits at 30 and 60 days after transplanting in each pot. Zinc was applied at the rate of 5 kg ha^{-1} through zinc sulphate ($ZnSO_4.7H_2O$), boron was applied at the rate of 1.5 kg ha^{-1} through borax ($Na_2B_4O_7.10H_2O$) and sulphur was applied at the rate of 30 kg ha^{-1} through gypsum ($CaSO_4.2H_2O$). The growth and yield attribute recorded at 30, 60 and 90 days after transplanting of hybrid rice.

Results and Discussion

Plant height

At 30 DAT, plant height significantly varied from **(Table 2)** 75.13 to 90.27 cm. It was found maximum (90.27) in T_9 (RDF + Zn + B + S) followed by 88.50 and 88.07 cm in T_6 (RDF + Zn + B) and T_8 (RDF + B + S) which were 12, 10 and 9% higher over T_2 (RDF), respectively. At 60 DAT, the plant height significantly varied from 90.8 to 116.47 cm. Treatment T_9 showed the maximum plant height (116.47) followed by 116.17 cm in T_6 with respective increase of 13 and 12 % over T_2 (RDF). Similar results were also noticed at 90 DAT, where it varied from 100.53 to 127.47 cm. The maximum plant height was recorded in T_9 (127.47 cm) followed by 124.00 and 122.93 cm in T_6 and T_8 which showed a respective increase by 11, 8 and 7% over T_2 (RDF). Combined application of any two nutrients out of Zn, B and S significantly increased the plant height. The results showed that application of Zn, B and S with recommended dose of fertilizer has a significant role in increasing of plant height. It might be attributed to balanced supply of nutrients through chemical fertilizers resulting in higher plant canopy which in turn, increased photosynthetic processes during development. Balu Ram *et al.* (2014) [11] reported that application of S, Zn and B with RDF significantly increased plant height by 27.7 and 21.6% over control at 30 and 60 days after transplanting (DAT), respectively. Increase in plant height and yield of rice was also reported due to the combine application of Zn, P (Rasavel and Ravichandran, 2013) [12].

Chlorophyll content

At 30 DAT, all treatments showed significantly higher chlorophyll content over T_2 (RDF) except T_1, T_3 and T_4 which ranged from 31.97 to 36.43 SPAD **(Table 2)**. The maximum SPAD value (36.43) was recorded in T_5 (RDF + S) followed by 36.37 and 36.33 with T_9 (RDF + Zn + B + S) and T_8 (RDF + B + S) which showed an increase of 7% over RDF. At 60 DAT, similar trend was observed *i.e.,* chlorophyll content increased by combined application of Zn, B and S along with RDF. It is observed that chlorophyll content varied from 33.70 to 40.13 SPAD and the maximum was noticed with T_9 which received Zn, B and S along with RDF, followed by 40.11 and 40.09 with T_7 (RDF + Zn + S) and T_8 (RDF + B + S), respectively. At 90 DAT, the plants showed maturity and the SPAD values decreased irrespective of treatments due to the loss of protein nitrogen from the leaves. The chlorophyll content significantly varied from 18.65 to 27.95 SPAD. The maximum chlorophyll content (27.95) was noticed in T_9 which received sulphur along with Zn and B followed by 27.80 and 27.20 SPAD with T_8 and T_7, respectively. It was noticed that T_9 and T_8 increased by 13% and T_7 by 10% over T_2 (RDF). It might be attributed to increased sulphur availability that has got a significant role in synthesis of chlorophyll. The fact was reported by Bera and Ghosh (2015) [13]. Bhutto *et al.* (2013) [14] reported that application of Zn and B also showed increased chlorophyll content.

Number of tillers

The number of tillers varied from (Table 2) 4.37 to 7.27 at 30 DAT. The maximum (7.27) tiller number pot^{-1} at 30 DAT in T_9 (RDF + Zn + B + S) followed by 7.13 and 6.70 in T_8 (RDF + B + S) and T_6 (RDF + Zn + B) which were 24, 21 and 14% higher over T_2 (RDF), respectively. At 60 DAT, the number of tillers significantly varied from 4.40 to 7.73. Treatment T_9 showed the maximum number of tillers (7.73) followed by 7.33 and 7.30 in T_8 (RDF + B + S) and T_6 (RDF + Zn + B) which showed a respective increase by 21, 14 and 14 % over T_2 (RDF). Similar results were also noticed at 90 DAT and number of tillers varied from 3.27 to 6.10. It was maximum (6.10) in T_9 followed by 5.93 and 5.73 in T_8 and T_6 which showed a respective increase by 35, 31 and 26% over T_2 (RDF). The increase in number of tillers of rice might be attributed to balanced fertilization (Latare and Singh, 2013) [15]. Dash *et al.* (2015) [16] reported similar results which showed that application of Zn, B, S along with RDF showed the maximum numbers of tillers. Tejnuava *et al.* (2014) [17] observed that combined application of sulphur and boron promoted the number of effective tillers hill^{-1}. Hussain *et al*. (2012) [18] found maximum number of tillers m^{-2} in the plot where B was applied in soil at 1.5 kg ha^{-1} at flowering stage.

Number of panicle per pot

The number of panicle varied from 3.27 to 6.10 (Table 3). The maximum number of panicle (6.10) was found in T_9 (RDF + Zn + B + S) followed by 5.93 with T_8 (RDF + B + S) which showed an increased by 35 and 31% over T_2 (RDF), respectively. It was noticed that conjoint application of any of the two or more nutrients among Zn, B and S significantly increased number of panicle pot^{-1} (T_6, T_7, T_8 and T_9).

Length of panicle

It is obvious from the data (Table 3) that the panicle length in rice did not increase significantly except T_6 (RDF + Zn + B) and T_9 (RDF + Zn + B + S) as compared to T_2 (RDF). The length of panicle varied from 20.43 to 23.03 cm. The maximum length of panicle (23.03 cm) was found in T_9 followed by 23.01 cm with T_6 which showed an increased by 8% over T_2 (RDF). Similar results were also noticed by Balu Ram *et al*. (2014) [11]. They reported that panicle length of rice significantly increased with application of S, Zn and B. Dash *et al.* (2015) [16] also reported that an increase in length of panicle with combined application of Zn and B.

Grains per panicle

The number of grains per panicle varied from 84.83 to 114.33 (Table 3). The number of grains per panicle was maximum (114.33) in treatment T_9 (RDF + Zn + B + S) followed by 112.73 with T_6 (RDF + Zn + B) which showed a respective increased by 18 and 16% over T_2 (RDF). It was noticed that combined application Zn, B and S with RDF gave the highest number of grains per panicle. Zinc is involved in the synthesis of growth promoting hormones and the reproductive process of many plants which are vital for grain formation. Boron is associated with the rate of water absorbance and translocation of sugar in plant (Dhane and Shukla 1995) [19]. Hence, the increase in number of grains per panicle might be attributed to the application of Zn, B and S. This is also in conformity with the results of Balu Ram *et al.* (2014) [11]. Tajnuava *et al.* (2014) [17] reported that interaction effect of sulphur and boron showed significant variation in the number of filled grains per panicle. Uddin *et al.* (2002) [20] reported the similar findings that combined application of Zn, B and S along with RDF gave maximum grains per panicle in rice.

Grain yield

The grain yield was ranged from 22.91 to 62.53 g pot^{-1} (Table 3). The maximum grain yield (62.53 g pot^{-1}) was produced in T_9 (RDF + Zn + B + S), followed by 58.01 and 57.17 g pot^{-1} in T_8 (RDF + B + S) and T_7 (RDF + Zn + S) which resulted an increased by 45, 34 and 32% over T_2 (RDF), respectively. The grain yield increased significantly with the sole application of Zn (T_3), B (T_4) and S (T_5) with RDF or in combination (T_6, T_7, T_8 and T_9) over RDF (T_2). Similar effect was also noticed with respect to grain yield of rice by S, Zn and B application (Gaur *et al.*, 2015 and Balu Ram *et al*., 2014) [21, 11], growth and yield attributes of rice by Zn, P and S application (Rasavel and Ravichandran 2013) [12], S and Zn on growth, yield and nutrient uptake by rice and B application on yield of rice crop in Pakistan (Ahmad and Irshad, 2011) [22].

Straw yield

The straw yield varied significantly from 24.97 to 74.60 g pot^{-1} (Table 3). The maximum straw yield (74.60 g pot^{-1}) was found in T_9 which received Zn, B and S along with RDF followed by 62.29 and 58.03 g pot^{-1} with T_8 and T_7. All treatments significantly varied over T_2 (RDF) except T_1 (control). It was observed that straw yield in T_9, T_8 and T_7 significantly increased by 55, 30 and 21% over T_2 (RDF). It is evident from the results that supply of Zn, B and S in combination with RDF in T_9 might have facilitated the growth of the plant, due to its involvement in many enzyme system, regulatory functions and auxin production (Sachdev *et al*. 1988) [23], increased synthesis and transport of carbohydrates to the sink (Peda Babu *et al*. 2007) [24]. Muthukumararaja *et al.* (2012) [25], Saha *et al.* (2013)) [26]. Wang *et al*. (2014)) [27], and Imran *et al.* (2015)) [28], also reported increase in straw yield with application of Zn. Boron is associated with the rate of water absorption and translocation of sugar in plant (Dhane and Shukla, 1995)) [19]. Sulphur has significant role in synthesis of chlorophyll (Bera and Ghosh, 2015)) [13],. Ultimately, combined application of Zn, B and S increased the straw yield of rice. This is also conformity with the results found by Uddin *et al.* (2002)) [20].

Harvest Index

Harvest index (HI) denotes the proportion of economically produced part to the above ground biomass. Significant increase in HI suggests that plants maintained a higher supply of photosynthates to reproductive part as compared to vegetative biomass. The harvest index (HI) varied from 45.06 to 49.59% (Table 3) and the variation was statistically non-significant. The maximum HI (49.59%) was observed in T_6 (RDF + Zn + B) and the minimum in T_9 (45.06%) (RDF + Zn + B + S) followed by 47.35% in T_2 (RDF).

1000 grain weight

Thousand grain weight varied from 19.47 to 20.90 g (Table 3) with the maximum in treatment T_9 (RDF + Zn + B + S) which increased by 4% over T_2 (RDF). Application of Zn, B and S in various combinations appeared to increase 1000 grains weight over T_2 (RDF) as evident in T_6, T_7, T_8, and T_9. The increase in test weight might be attributed due to supply of S, Zn and B to plants as they take part in energy formation and translocation from sink to source. Balu Ram *et al.* (2014)) [11], and Uddin *et al.* (2002)) [20], also reported that application of S + Zn + B applied singly or in combination give higher 1000 grain weight over RDF. Kumar *et al.* (2002)) [29], also reported significant increase in test weight of rice, yield attributes like number of panicles, panicle length and fertile spikelet's with application of 25 kg $ZnSO_4$ ha^{-1}.

Table 1: Initial soil properties of experimental soil

Parameters	Initial soil
pH	5.5
EC (dS m^{-1})	0.15
Organic carbon (%)	0.52
Macronutrient (kg ha^{-1})	
N	185.8
P	13.5
K	150.6
S (mg kg^{-1})	32.2
Micronutrient (mg kg^{-1})	
B	0.62
Fe	46.08
Mn	34.2
Cu	5.3
Zn	0.84
Mechanical composition (%)	
Sand	40.55
Silt	25.33
Clay	34.12
Texture class	Clay loam

Table 2: Response of Zn, B and S application on plant height, chlorophyll content and number of tillers in hybrid rice

Treatment	Plant height (cm)			Chlorophyll content (SPAD)			Number of tillers pot^{-1}		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	75.13	90.80	100.53	31.86	33.74	18.65	4.37	4.40	3.27
T_2	80.80	103.43	114.80	33.91	38.41	24.66	5.87	6.40	4.53
T_3	81.83	104.87	118.00	35.00	39.09	24.74	6.00	6.53	4.80
T_4	83.33	110.87	119.53	34.03	38.55	24.49	6.30	7.27	4.93
T_5	80.87	104.60	115.53	36.43	40.03	27.48	6.27	6.67	4.60
T_6	88.50	116.17	124.00	36.07	39.76	24.74	6.70	7.30	5.73
T_7	85.07	107.60	122.40	36.15	40.11	27.86	6.33	7.00	5.60
T_8	88.07	115.73	122.93	36.33	40.09	27.21	7.13	7.33	5.93
T_9	90.27	116.47	127.47	36.37	40.13	27.95	7.27	7.73	6.10
SEm±	0.68	1.01	2.21	0.77	0.51	1.07	0.22	0.23	0.29
CD (P=0.05)	1.99	2.93	6.42	2.25	1.49	3.11	0.65	0.67	0.84

Treatments:- T_1: Absolute Control (No fertilizer), T_2: Control (RDF), T_3: RDF + Zn, T_4: RDF + B, T_5: RDF + S, T_6: RDF + Zn + B, T_7: RDF + Zn + S, T_8: RDF + B + S, T_9: RDF + Zn + B + S

Table 3: Response of Zn, B and S application on number of panicles, length of panicle, grain per panicle, straw yield, grain yield, harvest index and 1000 grains weight of hybrid rice

Treatment	Number of panicles pot^{-1}	Length of panicle (cm)	Grain per panicle	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	Harvest index (%)	Weight of 1000 grains
T_1	3.27	20.43	84.83	22.91	24.97	47.49	19.47
T_2	4.53	21.33	97.07	43.20	48.03	47.35	20.20
T_3	4.80	22.57	99.72	49.67	52.93	48.41	20.30
T_4	4.93	22.63	105.77	50.59	51.83	49.55	20.41
T_5	4.60	21.67	101.83	50.72	51.80	49.50	20.47
T_6	5.73	23.01	112.73	52.43	55.47	48.63	20.60
T_7	5.60	22.17	110.23	57.17	58.03	49.59	20.63
T_8	5.93	22.31	111.40	58.01	62.29	48.21	20.63
T_9	6.10	23.02	114.33	62.53	74.60	45.06	20.90
SEm±	0.29	0.49	0.88	1.78	1.08	0.80	0.12
CD (P=0.05)	0.84	1.43	2.56	5.17	3.15	2.32	0.35

Treatments:- T_1: Absolute Control (No fertilizer), T_2: Control (RDF), T_3: RDF + Zn, T_4: RDF + B, T_5: RDF + S, T_6: RDF + Zn + B, T_7: RDF + Zn + S, T_8: RDF + B + S, T_9: RDF + Zn + B + S

Conclusions

The study revealed that application of micronutrients (Zn and B) along with major nutrients significantly increased the yield and yield attributes of rice. The growth parameters like plant height, chlorophyll content, number of tillers pot^{-1} were significantly higher in T_9 which received Zn, B and S along with RDF. The number of panicle, length of panicle, grain per panicle, grain and straw yield, and weight of 1000 grains were also found maximum with combined application of Zn, B and S along with RDF. Based on the results of the present investigation, it was concluded that the combined application of 5, 1.5 and 30 kg ha^{-1} Zn, B and S along with recommended dose of NPK possess higher yield of rice in acid soils of Bihar.

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