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Implication of Zn fertilizer application on Zn biofortification in bajra (*Pennisetum glaucum* L.) and its interaction with other micro-nutrients

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Abstract
A field experiment was carried out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during *kharif* 2017 to survey the impact of Zn fertilizer application on the biofortification of bajra variety ICMV-221 and the interaction of Zn with other micronutrients in plant and soil. The experiment was laid out in a randomized block design with three replications. $ZnSO_4$. $7H_2O$ and Zn EDTA was applied @ 0, 5, 10, 15 and 20 kg ha^{-1} before sowing. The result revealed that Zn content in all plant parts of bajra increased significantly with increase in the Zn fertilizer dose recording maximum at 20 kg ha^{-1} Zn EDTA application which was a 37% increase in the grain Zn content over control and highest grain yield of 4153 kg ha^{-1} was observed. As observed from plant and soil nutrient status, Zn showed antagonistic effect on Fe, Cu and Mn at higher doses.

Keywords: Biofortification, bajra, Zinc, Iron, copper, manganese, yield

Introduction
Bajra or pearl millet (*Pennisetum glaucum* L.) widely grown in the arid and semi-arid region of the world as both feed as well as fodder due to its resilience to adverse climatic condition. In comparison to rice and wheat, pearl millet grains are rich in proteins, dietary fibers, mineral nutrients, vitamins and essential amino acids and also a rich source of energy (Hegde *et al*., 2005 and Saleh *et al*., 2013) [12, 25]. But some of the varieties of pearl millet are deficient in Zn content or even if sufficiently present they have low bioavailability because of anti-nutrients like phytates which reduce their availability for human consumption.
Zn has an extraordinary spot in the biological system as it is the only metal which is the part of enzymes of all six enzyme classes, i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Broadley *et al*., 2007) [5]. Zn plays an important role in photosynthesis, protein synthesis, gene regulation, auxin metabolism, in maintenance of structure and functions of bio-membranes and required for detoxification of reactive oxygen species (Cakmak, 2000 and Bell and Dell, 2008) [7, 4]. All this affect the biomass of crop and ultimately grain yield. This indicates the essentiality of increasing Zn concentration in the edible parts of plant. Zn also affects the growth and development of plants by interaction with other micronutrients and affecting their uptake and translocation by the plant, which is a very complex process. However, on average, 50% of the Indian soils are deficient in zinc (Zn), particularly in calcareous soils or even if it is present in high amount only a small fraction is available for plant uptake. This is due to the formation of insoluble zinc hydroxide and its carbonate (Rattan and Shukla, 1991; Singh and Abrol, 1986) [24, 27]. Zn deficiency can be corrected by the application as zinc salts e.g., zinc sulphate or as Zn chelates, such as Zinc ethylene diamine tetra acetic acid (Zn-EDTA), which supply significant amount of Zn to the plant without interacting with soil components.
Hence, the current investigation was undertaken to survey the impact of ZnSO4.7H2O and Zn-EDTA application on the biofortification of bajra grain with Zn and also its effect on the other micro nutrient status in bajra grain and soil. And ultimately how it's affecting grain yield.

Material and Methods
To uncover the implications of Zn fertilization on Zn biofortification of bajra grain and interaction with other micronutrients in plant and soil and its impact on grain yield, a field experiment was conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (latitude: 15^0 26' N, longitude: 75^0 07' E, altitude: 678 m) during *kharif* 2017.

The experiment was laid out in a randomized complete block design with three replication on a net plot size of 13.5 m^2. The experimental site contains medium deep black clay soil with 0.53 mg kg^{-1} available soil Zn content with pH -7.65 and EC-0.32 dS m^{-1}. At the time of sowing it was fertilized with 50:25:0 kg ha^{-1} N: P_2O_5: K_2O. Bajra variety ICMV-221 was used for the experiment and was supplied with two sources of Zn i.e. $ZnSO_4.7H_2O$ and Zn EDTA before sowing of the crop.

Treatment Description

T_1	RDF (Control)
T_2	RDF + basal application of $ZnSO_4.7H_2O$ @ 5 kg ha^{-1}
T_3	RDF + basal application of $ZnSO_4.7H_2O$ @ 10 kg ha^{-1}
T_4	RDF + basal application of $ZnSO_4.7H_2O$ @ 15 kg ha^{-1}
T_5	RDF + basal application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1}
T_6	RDF + basal application of Zn EDTA @ 5 kg ha^{-1}
T_7	RDF + basal application of Zn EDTA @ 10 kg ha^{-1}
T_8	RDF + basal application of Zn EDTA @ 15 kg ha^{-1}
T_9	RDF + basal application of Zn EDTA @ 20 kg ha^{-1}

***RDF-Recommended dose of fertilizer**

Micronutrient Estimation in Plant Tissue

The plant samples were collected at 50 per cent flowering and harvesting stage for estimation of micro-nutrient content. Plants were washed thoroughly with distilled water, separated into leaf, stem and grain and dried under shade. Then, samples were dried in hot air oven at 65 °C till a constant weight was obtained. Dried plant samples were made into fine powdered and stored for further chemical analysis.

Zinc content (mg kg^{-1}) in the leaf, stem and grain at 50% flowering and harvesting stage was estimated using atomic absorption spectrophotometer (GBS Avanta Ver. 2.02 Model, Germany) after di acid digestion (HNO_3: $HClO_4$) of plant sample. The similar procedure was also followed for the estimation of Fe, Cu and Mn content (mg kg^{-1}) in the bajra grain (Tandon, 1998) [28].

Micronutrient Estimation in Soil

For analysis of the micro-nutrients in the soil, soil samples were collected from the depth of 0-30 cm before sowing and after harvest and processed properly. Zn, Fe, Cu and Mn (mg kg^{-1}) were extracted from soil by using DTPA extracting solution and estimated in atomic absorption spectrophotometer (GBS Avanta Ver. 2.02 Model, Germany) as described by Lindsay and Norvell (1978) [19].

Grain Yield Estimation

Grain yield $plant^{-1}$ and grain yield ha^{-1} was taken after harvest and expressed in g $plant^{-1}$ and kg ha^{-1} respectively. Grain yield $plant^{-1}$ was calculated by weighing the total grains present in the panicle per plant.

Statistical Analysis

The data obtained from the experiment were subjected to statistical analysis using Randomized Complete Block Design (RCBD). Interpretation of data was carried out in accordance with Gomez and Gomez (1984) [11]. The level of significance used in the F and t test was P = 0.05. The critical difference values were calculated wherever the F test values were significant. The treatment means were computed by applying Duncan's Multiple Range Test (DMRT).

The mean values of treatments subjected to DMRT using the corresponding error mean sum of squares and degrees of freedom values at five per cent probability under MSTATC programme.

Result and Discussion

Effect of Zn Application on Distribution of Zn in Plant Parts of Bajra

An increasing in the Zn content in all plant parts observed at both 50% flowering and harvesting stage due to Zn application, which was found to be maximum in 20 kg ha^{-1} Zn EDTA application, followed by 20 kg ha^{-1} $ZnSO_4.7H_2O$ and lowest was recorded in the absolute control (Table 1). The soil application of Zn increased its concentration in the soil solution mostly in available form and facilitates its larger uptake through root (Apoorva, 2016) [3]. Leaf Zn content was recorded higher as compared to the stem at both 50 per cent flowering (33.40 and 29.42% respectively) and at harvest (30.02 and 24.94% respectively) and a grain Zn concentration of 36.54 per cent was observed at harvest. The reason might be ascribed to the active participation of leaf in physiological processes of plant especially photosynthesis and act as a sink for Zn during vegetative development. Decrease in the leaf and stem Zn concentration was observed at the harvesting stage than 50 per cent flowering stage. This might be due to the remobilization of Zn from leaf and stem to grain after flowering stage. This is due to the significant increase in the protein biosynthesis during the early stage of seed formation and this increase the Zn demand in grain compared to other plant parts (Martre *et al.*, 2003; Ozturk *et al.*, 2006 and Cakmak *et al.*, 2010) [21, 22]. A highest grain Zn content of 44.43 mg kg^{-1} was observed in 20 kg ha^{-1} Zn EDTA application followed by 20 kg ha^{-1} $ZnSO_4$. $7H_2O$ which showed 40.20 mg kg^{-1} Zn. The result of our study is in conformity with the findings of Prasad *et al*. (2015) [24] and Choudhary *et al*. (2016) [9].

Table 1: Effect of Zn biofortification on Zn content in plant parts of bajra at 50 per cent flowering and harvesting stage

Treatments	Zn content (mg kg^{-1})				
	50% flowering		at harvest		
	Leaf	Stem	Leaf	Stem	Grain
T_1: RDF (Control)	29.87^{d}	25.89^{e}	26.19^{d}	21.54^{e}	32.39^{d}
T_2:RDF + Basal application of $ZnSO_4.7H_2O$ @ 5 kg ha^{-1}	30.51^{d}	26.97^{e}	27.26^{cd}	22.92^{de}	33.29^{d}
T_3: RDF + Basal application of $ZnSO_4.7H_2O$ @ 10 kg ha^{-1}	31.12^{cd}	27.83^{de}	28.42^{cd}	23.97^{c-e}	34.95^{cd}
T_4: RDF + Basal application of $ZnSO_4.7H_2O$ @ 15 kg ha^{-1}	33.28^{b-d}	30.71^{b-d}	31.69^{ab}	25.47^{b-d}	36.41^{b-d}
T_5: RDF + Basal application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1}	37.04^{b}	32.30^{ab}	32.92^{a}	27.31^{ab}	40.20^{b}
T_6: RDF + Basal application of Zn EDTA @ 5 kg ha^{-1}	30.96^{d}	27.60^{e}	27.92^{cd}	23.31^{c-e}	33.81^{d}
T_7: RDF + Basal application of Zn EDTA @ 10 kg ha^{-1}	31.94^{cd}	28.65^{c-e}	29.74^{bc}	24.93^{b-d}	35.29^{cd}
T_8: RDF + Basal application of Zn EDTA @ 15 kg ha^{-1}	35.06^{bc}	31.01^{a-c}	32.03^{ab}	26.20^{a-c}	38.11^{bc}
T_9: RDF + Basal application of Zn EDTA @ 20 kg ha^{-1}	40.82^{a}	33.78^{a}	34.01^{a}	28.80^{a}	44.43^{a}
Mean	33.40	29.42	30.02	24.94	36.54
S.Em. ±	1.22	0.92	0.93	0.88	1.25
L.S.D. @ 5%	3.65	2.76	2.79	2.65	3.75

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P = 0.05).

Interaction of Zn with Other Micro Nutrients

The soil application of Zn greatly affects the concentration of micronutrients like Fe, Cu and Mn in bajra grains and interaction between Zn and these micronutrients is very complex. At higher doses of Zn, the concentration of these nutrients decreased in the plant (Table 2).

Effect of Zn Application on Micronutrient Status in Grain

A maximum grain Fe content of 75.92 mg kg^{-1} was recorded when amended with 10 kg ha^{-1} $ZnSO_4.7H_2O$ followed by 10 kg ha^{-1} Zn EDTA treated plot (74.97 mg kg^{-1}) and control recorded lowest grain Fe content of 68.47 mg kg^{-1} (Table 2). At higher doses of Zn grain Fe content seemed to be decreased. The reason might be the competition between Fe and Zn to occupy the active sites of IRT1 carrier protein (a ZIP transporter) which involves in the transport of both Fe and Zn. Therefore, the excess Zn concentration in soil solution due to Zn application suppresses the uptake of Fe by the root (Kitagishi *et al*., 1981) [17]. This was in support with the study of Abbas *et al*. (2009) [1] and Chilian *et al*. (2015) [8].

The Cu concentration was recorded maximum at 10 kg ha^{-1} Zn EDTA (7.55 mg kg^{-1}) application followed by 15 kg ha^{-1} $ZnSO_4.7H_2O$ (7.48 mg kg^{-1}) and the lowest was recorded in control (6.47 mg kg^{-1}). Whereas, highest grain Mn content (45.99 mg kg^{-1}) was observed in 15 kg ha^{-1} $ZnSO_4.7H_2O$ treatment followed by 15 kg ha^{-1} Zn EDTA application (44.96 mg kg^{-1}) and control showed lowest grain Mn content of 40.21 mg kg^{-1} (Table 2). The decrease in the Cu and Mn concentration at higher doses of Zn might be attributed to the competition of Zn with Cu and Mn for a common site of absorption (Alloway, 2008) [2]. This is in agreement with the findings of Fageria (2002) [10] and Imtiaz *et al*. (2003) [14].

Table 2: Effect of Zn biofortification on macro and micro nutrient content of bajra grain

Treatments	Fe content (mg kg^{-1})	Cu content (mg kg^{-1})	Mn content (mg kg^{-1})
T_1: RDF (Control)	68.47^{b}	6.47^{c}	40.21^{c}
T_2:RDF + Basal application of $ZnSO_4.7H_2O$ @ 5 kg ha^{-1}	72.60^{ab}	6.78^{bc}	40.80^{bc}
T_3: RDF + Basal application of $ZnSO_4.7H_2O$ @ 10 kg ha^{-1}	75.92^{a}	7.22^{ab}	42.39^{a-c}
T_4: RDF + Basal application of $ZnSO_4.7H_2O$ @ 15 kg ha^{-1}	71.13^{ab}	7.48^{ab}	45.99^{a}
T_5: RDF + Basal application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1}	69.33^{ab}	7.26^{ab}	43.79^{a-c}
T_6: RDF + Basal application of Zn EDTA @ 5 kg ha^{-1}	73.24^{ab}	6.92^{a-c}	41.80^{a-c}
T_7: RDF + Basal application of Zn EDTA @ 10 kg ha^{-1}	74.97^{ab}	7.55^{a}	42.86^{a-c}
T_8: RDF + Basal application of Zn EDTA @ 15 kg ha^{-1}	70.97^{ab}	7.39^{ab}	44.96^{ab}
T_9: RDF + Basal application of Zn EDTA @ 20 kg ha^{-1}	70.21^{ab}	7.20^{ab}	43.00^{a-c}
Mean	71.87	7.14	42.87
S.Em. ±	2.09	0.217	1.32
L.S.D. @ 5 %	6.26	0.647	3.97

Effect of Zn Application on Micronutrient Status in Soil

From the data of current investigation on soil nutrient content before sowing and after harvest we have observed that, the soil Zn content differed significantly among the treatments at harvesting stage due to Zn application and the mean Zn content was recorded to be 0.79 mg kg^{-1}. Before sowing the Zn in soil was 0.53 mg kg^{-1} (Table 3). The absorption of Zn was maximum at 20 kg ha^{-1} Zn EDTA application followed by 20 kg ha^{-1} $ZnSO_4.7H_2O$ and the lowest was observed in control plot. The residual Zn content in soil after harvest also followed the same trend. Significantly highest soil Zn content (0.95 mg kg^{-1}) was observed in 20 kg ha^{-1} Zn EDTA treatment followed by 0.84 mg kg^{-1} which was treated with 20 kg ha^{-1} $ZnSO_4.7H_2O$. Whereas, control recorded significantly lowest Zn content in soil 0.64 mg kg^{-1}. The reason might be the increased level of Zn increases the availability Zn in soil for plant uptake and also attributed to the increase in the root proliferation due to Zn application. This is conformity with the findings of Keram *et al*. (2012) [16], Hussain (2015) [13] and Apoorva (2016) [3]. Whereas, the Fe, Cu and Mn absorption by plant was observed highest in 15 kg ha^{-1} $ZnSO_4$. $7H_2O$, 10 kg ha^{-1} Zn EDTA and 15 kg ha^{-1} $ZnSO_4.7H_2O$ respectively and the lowest was recorded in control (Table 3). The decrease in the uptake of these nutrients at higher doses of Zn ascribed to the competition of Zn with Fe, Cu and Mn for the same absorption site which prevents the entry of these nutrients to the root of plant. This is supported by the study of Abbas *et al*. (2009) [1], who observed increased in the Fe and Mn content in soil at higher Zn doses. This implies the reduced uptake of Fe and Mn due to Zn application.

Table 3: Effect of Zn application on Zn, Fe, Cu and Mn status in soil after harvest

Treatments	Zn content (mg kg^{-1})		Fe content (mg kg^{-1})		Cu content (mg kg^{-1})		Mn content (mg kg^{-1})	
	BS	AH	BS	AH	BS	AH	BS	AH
T_1: RDF (Control)	0.54	0.639^{c}	4.72	4.56^{a}	2.87	2.59	3.26	3.13
T_2:RDF + Basal application of $ZnSO_4.7H_2O$ @ 5 kg ha^{-1}	0.43	0.730^{bc}	4.01	3.69^{a-c}	2.43	2.02	3.29	2.50
T_3: RDF + Basal application of $ZnSO_4.7H_2O$ @ 10 kg ha^{-1}	0.55	0.789^{a-c}	5.31	4.17^{ab}	1.99	1.20	4.41	3.19
T_4: RDF + Basal application of $ZnSO_4.7H_2O$ @ 15 kg ha^{-1}	0.50	0.814^{a-c}	5.40	2.18^{d}	2.05	1.29	3.86	2.19
T_5: RDF + Basal application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1}	0.59	0.838^{ab}	4.58	3.88^{a-c}	1.91	1.53	3.82	3.21
T_6: RDF + Basal application of Zn EDTA @ 5 kg ha^{-1}	0.62	0.732^{bc}	4.08	3.27^{b-d}	2.54	2.08	3.16	2.14
T_7: RDF + Basal application of Zn EDTA @ 10 kg ha^{-1}	0.45	0.792^{a-c}	5.55	3.15^{b-d}	2.81	1.83	3.12	1.78
T_8: RDF + Basal application of Zn EDTA @ 15 kg ha^{-1}	0.43	0.833^{a-c}	4.13	2.89^{cd}	2.05	1.44	3.55	2.04
T_9: RDF + Basal application of Zn EDTA @ 20 kg ha^{-1}	0.63	0.950^{a}	4.31	3.86^{a-c}	2.47	2.15	3.45	3.31
Mean	0.53	0.79	4.68	3.52	2.35	1.79	3.55	2.61
S.Em. ±	0.08	0.05	0.90	0.34	0.62	0.66	0.47	0.48
L.S.D. @ 5 %	NS	0.17	NS	1.01	NS	NS	NS	NS

BS: Before sowing, AH: After harvest, NS: Non significant

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P = 0.05).

Effect of Zn Application on Grain Yield of Bajra

Grain yield plant^{-1} as well as grain yield ha^{-1} increased significantly due Zn fertilizer application and recorded maximum in 20 kg ha^{-1} Zn EDTA (26.65 g plant^{-1} and 4153 kg ha^{-1} respectively) application followed by 20 kg ha^{-1} $ZnSO_4.7H_2O$ (25.55 g plant^{-1} and 4095 kg ha^{-1} respectively) and the lowest was recorded in control (21.10 g plant^{-1} and 3276 kg ha^{-1} respectively) (Table 4). This increase in yield might be due to increase in biomass, enhancement in photosynthesis and higher translocation of photosynthates towards grain which ultimately increased the yield of plant. This is supported by the findings of Kumar *et al*. (2015) [18], Prasad *et al*. (2015) [24], Choudhary *et al*. (2016) [9], Singh *et al*. (2017) [26] Potanna (2017) [22] and Jain *et al*. (2018) [15].

Table 4: Effect of Zn biofortification on grain yield, grain yield per plant

Treatments	Grain yield (kg ha^{-1})	Grain yield plant^{-1} (g)
T_1: RDF (Control)	3.276^{c}	21.10^{b}
T_2:RDF + Basal application of $ZnSO_4.7H_2O$ @ 5 kg ha^{-1}	3.379^{bc}	21.82^{b}
T_3: RDF + Basal application of $ZnSO_4.7H_2O$ @ 10 kg ha^{-1}	3.563^{a-c}	22.44^{ab}
T_4: RDF + Basal application of $ZnSO_4.7H_2O$ @ 15 kg ha^{-1}	3.983^{ab}	24.57^{ab}
T_5: RDF + Basal application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1}	4.095^{a}	25.55^{ab}
T_6: RDF + Basal application of Zn EDTA @ 5 kg ha^{-1}	3.549^{a-c}	22.37^{ab}
T_7: RDF + Basal application of Zn EDTA @ 10 kg ha^{-1}	3.763^{a-c}	23.42^{ab}
T_8: RDF + Basal application of Zn EDTA @ 15 kg ha^{-1}	4.066^{a}	25.35^{ab}
T_9: RDF + Basal application of Zn EDTA @ 20 kg ha^{-1}	4.153^{a}	26.65^{a}
Mean	3758	23.70
S.Em. ±	195.6	1.31
L.S.D. @ 5 %	586.4	3.93

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P = 0.05).

Conclusion

Zn content in different plant parts of bajra increased with increase in the Zn fertilizer dose recording maximum in 20 kg ha^{-1} Zn EDTA treated plot. Which showed 37% increase in the grain Zn content over control. Whereas, Fe, Cu and Mn content in bajra grain seemed to be declined at higher doses of Zn as well as their absorption from soil also decreased at these doses which was observed from the soil data. Increased Zn content in plant also increased the grain yield and recorded maximum yield of 4153 kg ha^{-1} in 20 kg ha^{-1} Zn EDTA treated plot.

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