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Biofortification of zinc, iron, arbuscular mycorrhiza and Trichoderma on quality parameters and available soil nutrient status in soil of maize

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Abstract
A field experiment was conducted during *rabi* seasons of 2021 and 2022 at Navsari Agricultural University, Navsari to study the "Biofortification of zinc, iron, arbuscular mycorrhiza and trichoderma on quality parameters available soil nutrient status in soil of maize". The experiment was carried out in randomized block design with factorial concept and replicated three times. The experiment comprised with treatments of soil application of Zn and Fe and seed treatments of bioinoculants namely, S_1: Control, S_2: $ZnSO_4$ at 15 kg/ha, S_3: $ZnSO_4$ at 30 kg/ha, S_4: $FeSO_4$ at 15 kg/ha, S_5: $FeSO_4$ at 30 kg/ha, S_6: $ZnSO_4$ + $FeSO_4$ at 15 kg/ha, S_7: $ZnSO_4$ + $FeSO_4$ at 30 kg/ha and B_1: Control, B_2: Arbuscular mycorrhiza, B_3: Trichoderma, B_4: Arbuscular mycorrhiza + Trichoderma. Experimental finding reported that, among the various levels of Zn and Fe, soil application of $ZnSO_4$ + $FeSO_4$ at 30 kg/ha recorded significantly higher protein yield. While, significantly lowest protein yield was observed under control. With respect to bioinoculants seed treatments, higher protein yield was found significantly higher under application of Arbuscular mycorrhiza +Trichoderma. Soil application of Zn and Fe and bioinoculants seed treatments had no significant impact on protein content, available nutrient status in soil at harvest like N, K_2O, Zn and Fe except P_2O_5.

Keywords: Biofortification, zinc, iron, bioinoculants, quality parameters, available soil nutrient status

1. Introduction
Maize (*Zea mays* L.) is the third most important cereal crop next to rice and wheat in world's agricultural economy. It is knowns as "queen of cereals" due to its maximum yield potential among cereals. Maize is a C_4 plant in nature and an efficient converter of absorbed nutrients into food. It is the main source of minerals and calories for most of the rural population. It plays important role in human diet, animal feed and provides a large amount of energy and protein.
Zinc is considered as fourth most important yield limiting nutrient after nitrogen, phosphorus and potassium (Maclean *et al*., 2002) [5]. The requirement of zinc for the function of growth regulation, gene expression and regulation, enzyme activation, kerbs cycle, energy production, phytohormone activity, photosynthesis, protein synthesis, auxin metabolism, carbohydrate metabolism seed production, defense against disease. Iron plays important role in the formation of plant chlorophyll. The decrease of chlorophyll leading to the reduction of the plant food processor and finally the yield is reduced. Iron in chloroplasts reflects the presence of cytochromes which is performing various photosynthetic reduction process. Iron in chloroplasts reflects the presence of cytochromes which is performing various photosynthetic reduction process. Trichoderma spp. in improving plant growth through different mechanism which insists degradation of toxins, resistant against pathogens, uptake of nutrients, solubilization and enhanced root development. A number of approaches exist for tackling micronutrient deficiencies. Among which, "Biofortification" offers a simple and highly effective solution to Fe and Zn deficiency problems in crop plants and too increasing their concentrations in foods. Enhancement of particular nutrient by addition of fertilizers to soil or foliage in appropriate form, time and growth stages of the crop is called as agronomic biofortification. It refers to the improvement of solubilization and mobilization of mineral elements in the soil to increase micronutrient contents in the edible part of food crops.

2. Materials and methods

Field experiments were carried out during *rabi* seasons of 2021 and 2022 at Navsari Agricultural University, Navsari (Gujarat). Navsari Agricultural University campus is situated at 20°57′ N Latitude, 72°54′ E Longitudes and has an altitude of 10 meters above the mean sea level. The climate of the experimental site was typically tropical, characterized by humid and warm monsoon with heavy rainfall, quite cold winter and relatively hot summer. The soil of South Gujarat is known to be "Deep Black Soil". The soil of experimental field is classified under the order, '*Inceptisols*' comprising of fine montmorillonitic, *isohyperthemic*, great soil group of *Vertic Ustrochrepts* and soil series Jalalpur having medium to poor drainage and good water holding capacity. The experimental site was clay in texture, low in organic carbon (0.43 and 0.39%) and available nitrogen (273.19 and 269.31 kg/ha), medium in available phosphorus (45.60 and 46.15 kg/ha), available zinc (0.95 and 0.97mg/kg), available Fe (9.20 and 8.46 mg/kg) and high in available potassium (446.35 and 440.10 kg/ha). The soil was found slightly alkaline (pH 8.1 and 7.9) with normal electrical conductivity (0.32 and 0.37 dS/m). The experiment was laid out in Randomized Block Design with Factorial concept and replicated three times. Twenty eight treatment consisting of seven soil application of Zn and Fe and four biofertilizers seed treatments namely, S_1: Control, S_2: $ZnSO_4$ at 15 kg/ha, S_3: $ZnSO_4$ at 30 kg/ha, S_4: $FeSO_4$ at 15 kg/ha, S_5: $FeSO_4$ at 30 kg/ha, S_6: $ZnSO_4$ + $FeSO_4$ at 15 kg/ha, S_7: $ZnSO_4$ + $FeSO_4$ at 30 kg/ha and B_1: Control, B_2: Arbuscular mycorrhiza, B_3: Trichoderma, B_4: Arbuscular mycorrhiza + Trichoderma.

The required quantity of Bio-compost and fertilizer was workout as per the unit area and treatments basis. The recommended dose of NPK fertilizers 120-60-00 kg/ha were followed in the form of urea and DAP. Application of Zn and Fe through $ZnSO_4$ and $FeSO_4$ were applied before sowing as per treatment basis. The required quantity of maize seeds were worked out for experimental area and treated with Thiram 75% at 1.5 to 1.87 g/kg of seeds to protect the crop against fungal diseases. Seeds were inoculated with biofertilizer (Trichoderma at 4 g/kg seed) and (Arbuscular mycorrhiza at 10 g/kg seed) before sowing as per treatments in respective plots. Maize cultivar GM 6 was sown at the spacing 60 cm x 20 cm with seed rate of 20 kg/ha. Generally 12 to 15 days interval was kept for irrigation application as per requirement of crops. When the crop reached maturity, harvesting was followed by the removal of the cobs from the plant.

Observations recorded and statistical analysis

Representative samples of seed and stover were taken from each treatment and dried in oven at 65ºC temperature for 24 hours and powdered by mechanical grinder. The protein content in seed and stover were calculated by multiplying nitrogen content of seed (%) with the conversion factor of 6.25 (Bhuiya and Chowdhary, 1974) [2].

Protein content (%) = Nitrogen content (%) x 6.25

The protein yield in seed was calculated by using the following formula.

$$\text{Protein yield (kg/ha)} = \frac{\text{Protein content in seed (\%)} \times \text{Seed yield (kg/ha)}}{100}$$

The estimation of available N, P, K, Zn and Fe in soil after harvest of crops, respective soil samples were drawn from each plot at 15 cm soil depth and analyzed for determination of content of respective nutrient using different methods. The statistical analysis of data recorded for different characters during the course of investigation was carried out through the procedure appropriate to the Randomized Block Design of the experiment as described by Panse and Sukhatme (1967) [6] . The significance of difference was tested by 'F' test. Five per cent level of significance was used to test the significance of results.

3. Results and Discussion

3.1 Protein Content

It is evident from the results that among the soil application of Zn and Fe numerically highest protein content was found in $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha (S_7) (9.77, 9.72 and 9.74%, respectively) followed by treatments $ZnSO_4$ + $FeSO_4$ each at 15 kg/ha (S_6) during both the years and pooled analysis. However, numerically lowest protein content was recorded under Control (S_1). Results summarized in Table 1 indicated that various bioinoculants seed treatments did not exerted any significant effect on protein content of maize grain. Numerically highest protein content was obtained under application of Arbuscular mycorrhiza + Trichoderma (B_4) (9.56, 9.51 and 9.53%, respectively) followed by Trichoderma (B_3) during both the years and in pooled results. Numerically lowest protein content was observed under control (B_1). The difference in protein content (%) of maize under interaction effect of various levels of Zn and Fe and seed treatments with bioinoculants to maize was found non-significant.

3.2 Protein Yield

Among the soil application of Zn and Fe, significantly higher protein yield of maize grain was recorded with treatments $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha (S_7) (415.61, 408.21 and 411.91 kg/ha, respectively) during both the years and pooled analysis. Significantly lowest protein yield was recorded under control (S_1). It might be due that effect of Zn and Fe on photosynthesis and metabolic processes augments the production of photosynthates and their translocation in different parts. Also, photosynthates can also be converted into different amino acids and which increases protein yield of the crop. Similar results are obtained by Singh & Singh (2017) [7]. An examination of data in Table 1 indicated that among different seed treatments of bioinoculants, significantly the highest protein yield was recorded under application of Arbuscular mycorrhiza +Trichoderma (B_4) (424.45, 407.00 and 415.73 kg/ha, respectively) during both the years and in pooled results. Lowest protein yield was observed under control (B_1).

3.3 Available Nutrient Status in Soil at Harvest

The data presented in Table 2 indicated that various levels of Zn and Fe was not differed significantly with respect to available nitrogen, potassium, zinc and iron in soil at harvest. Data with respect to available phosphorus in soil at harvest as influenced by soil application of Zn and Fe and seed treatments of bioinoculants. Among the soil application of Zn and Fe, numerically highest available nitrogen (268.96 and 266.66 kg/ha, respectively) and available potassium (429.65 and 427.75 kg/ha, respectively) was recorded under control (S_1) during both the years. While, numerically lowest available soil nitrogen and potassium was recorded by $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha (S_7). Among the bioinoculants treatments numerically, highest available nitrogen (271.20 and 268.93 kg/ha, respectively) and available potassium (428.58 and 426.68 kg/ha, respectively) was recorded under Arbuscular mycorrhiza + Trichoderma (B_4) during both the year of experimentation. While, numerically lowest available nitrogen and potassium was recorded by control (B_1) during both the years. Data with respect to available phosphorus showed that significantly higher available phosphorus (41.01 and 39.15 kg/ha, respectively) was recorded under control (S_1) during both the years of experiment. While, significantly, lowest available soil phosphorus was recorded by $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha (S_7). This result might be due to antagonist effect of zinc and iron with phosphorous. These findings are reported by Fulpagare and Thakare (2018) [4] and Daphade *et al*. (2019) [3]. Significantly, higher available phosphorus (39.17 and 37.74 kg/ha, respectively) was recorded under Arbuscular mycorrhiza + Trichoderma (B_4) during both the seasons. This might be due to mobility of phosphorous by Arbuscular mycorrhiza by extending mycorrhizal hyphae network beyond the rhizosphere. Similar findings are reported by Bhardwaj *et al*. (2010) [1]. Whereas, significantly lowest available phosphorus was recorded by control (B_1) during both the years.

Data pertaining to available zinc revealed that numerically highest available zinc (0.95 and 0.93 mg/kg, respectively) was recorded under $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha (S_7) during both the years. While, numerically lower available zinc was recorded under $FeSO_4$ at 15 kg/ha (S_4). Numerically highest available zinc in soil was observed under treatment Arbuscular mycorrhiza + Trichoderma (B_4) (0.96 and 0.94 mg/kg, respectively) during both the years. While, numerically lowest available zinc was recorded under control (B_1). Data on available iron in soil at harvest revealed that numerically highest available iron (9.25 and 8.63 mg/kg, respectively) was registered under $FeSO_4$ at 30 kg/ha (S_5). While, numerically lowest available iron was recorded under $ZnSO_4$ at 30 kg/ha (S_3). With respect to bioinoculants seed treatments. However, numerically highest available iron in soil was recorded under treatment Arbuscular mycorrhiza + Trichoderma (B_4) (9.17 and 8.55 mg/kg, respectively). While, numerically lowest available iron was recorded under control (B_1). The interaction between various soil application of Zn and Fe and seed treatments of bioinoculants to maize was found non-significant for available nitrogen, phosphorus, potassium, zinc and iron status in soil during both the season.

From the data mention above, it is clear that soil application of Zn and Fe with various combinations had no significant impact on available soil nutrients *viz*., available nitrogen and potassium. Availability of major nutrients was decreasing with increasing yield and uptake of major nutrients leading to more extract from soil and reduction in soil availability. While in case of Zn and Fe, it is expected to increase Zn and Fe availability after its application in the soil. However, the results showed that, the availability of Zn and Fe was found to be non-significant. Though, they were applied as basal dose. It could be attributed due to fixation of applied Zn and Fe with other minerals present in the soil such as Ca and mg as well as other. These findings are reported by Daphade *et al*. (2019) [3].

Table 1: Protein content and protein yield of maize grains as influenced by different treatments

Treatments	Protein content (%)			Protein yield (kg/ha)		
	2021	2022	Pooled	2021	2022	Pooled
Soil application of Zn and Fe						
S_1: Control	9.28	9.23	9.25	348.21	334.21	341.21
S_2: $ZnSO_4$ at 15 kg/ha	9.45	9.40	9.42	369.48	357.68	363.58
S_3: $ZnSO_4$ at 30 kg/ha	9.53	9.48	9.51	394.71	376.50	385.61
S_4: $FeSO_4$ at 15 kg/ha	9.38	9.33	9.35	357.52	346.85	352.18
S_5: $FeSO_4$ at 30 kg/ha	9.49	9.44	9.46	380.39	365.52	372.96
S_6: $ZnSO_4$ + $FeSO_4$ each at 15 kg/ha	9.59	9.54	9.56	402.84	387.07	394.96
S_7: $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha	9.77	9.72	9.74	415.61	408.21	411.91
S.Em.±	0.16	0.16	0.11	11.94	14.12	9.25
CD at 5%	NS	NS	NS	33.87	40.02	25.95
Bioinoculants						
B_1: Control	9.41	9.36	9.38	335.64	318.91	327.27
B_2: Arbuscular mycorrhiza	9.49	9.44	9.47	389.55	377.03	383.29
B_3: Trichoderma	9.53	9.48	9.50	375.37	369.08	372.23
B_4: Arbuscular mycorrhiza +Trichoderma	9.56	9.51	9.53	424.45	407.00	415.73
S.Em.±	0.12	0.12	0.09	9.03	10.67	6.69
CD at 5%	NS	NS	NS	25.60	30.25	19.59
Interaction (S×B)						
S.Em.±	0.31	0.33	0.23	23.89	28.23	18.49
CD at 5%	NS	NS	NS	NS	NS	NS
Sig. interactions with Y	-	-	-	-	-	-
CV (%)	5.69	5.98	5.84	10.85	13.29	12.09

Table 2: Available nutrients status in soil at harvest as influenced by different treatments

Treatments	N (kg/ha)		P_2O_5 (kg/ha)		K_2O (kg/ha)		Zn (mg/kg)		Fe (mg/kg)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Soil application of Zn and Fe										
S_1: Control	268.96	266.66	41.01	39.15	429.65	427.75	0.92	0.90	9.00	8.38
S_2: $ZnSO_4$ at 15 kg/ha	264.39	262.09	38.16	36.30	426.13	424.23	0.94	0.92	8.99	8.37
S_3: $ZnSO_4$ at 30 kg/ha	261.91	259.61	37.91	36.05	422.44	420.54	0.95	0.93	8.96	8.34
S_4: $FeSO_4$ at 15 kg/ha	265.18	262.88	39.11	37.25	426.11	424.21	0.91	0.89	9.09	8.47
S_5: $FeSO_4$ at 30 kg/ha	261.07	258.70	36.94	35.08	421.94	420.04	0.90	0.88	9.25	8.63
S_6: $ZnSO_4$ + $FeSO_4$ each at 15 kg/ha	258.68	256.38	34.38	32.52	420.41	418.51	0.94	0.92	9.16	8.54
S_7: $ZnSO_4$ + $FeSO_4$ each at 30 kg/ha	256.85	254.55	32.38	31.29	418.11	416.21	0.95	0.93	9.15	8.53
S.Em.±	7.81	6.99	0.99	1.16	10.25	10.65	0.02	0.02	0.25	0.21
CD at 5%	NS	NS	2.82	3.28	NS	NS	NS	NS	NS	NS
Bioinoculants										
B_1: Control	253.1	250.73	35.23	33.37	419.91	418.01	0.90	0.88	8.98	8.36
B_2: Arbuscular mycorrhiza	268.4	266.09	37.76	35.91	421.22	419.32	0.94	0.93	9.16	8.54
B_3: Trichoderma	257.1	254.75	36.35	34.49	424.45	422.55	0.92	0.90	9.04	8.42
B_4: Arbuscular mycorrhiza +Trichoderma	271.2	268.93	39.17	37.74	428.58	426.68	0.96	0.94	9.17	8.55
S.Em.±	5.91	5.28	0.75	0.88	7.75	8.05	0.02	0.02	0.19	0.16
CD at 5%	NS	NS	2.13	2.48	NS	NS	NS	NS	NS	NS
Interaction (S×B)										
S.Em.±	15.63	13.97	1.99	2.32	20.50	21.30	0.04	0.05	0.49	0.42
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	10.31	9.31	9.27	11.34	8.38	8.42	8.00	8.98	9.35	8.66

4. Conclusion

The experimental results indicated that, among the soil applications of Zn and Fe and bioinoculants seed treatments, $ZnSO_4$ + $FeSO_4$ at 30 kg/ha and seed treatment of Arbuscular mycorrhiza + Trichoderma gave higher protein yield. Soil application of Zn and Fe and bioinoculants seed treatments had no significant impact on available nutrient status in soil at harvest like nitrogen, potassium, zinc, iron except phosphorus. With respect soil application of Zn and Fe, significantly higher available phosphorus was recorded under control (S_1). Among the various seed treatments of bioinoculants, significantly, higher available phosphorus was recorded under Arbuscular mycorrhiza + Trichoderma (B_4).

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