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Biofortification potential of zinc, iron, arbuscular mycorrhiza and *trichoderma* on the growth parameters of maize

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Abstract

The investigation entitled, “Biofortification potential of zinc, iron, arbuscular mycorrhiza and *Trichoderma* on growth parameters of maize” was carried out in Navsari Agricultural University, Navsari, during the *rabi* seasons of 2021 and 2022. The experiment was laid out in randomized block design with factorial concept and replicated three times. There were seven soil application treatments of Zn and Fe and four biofertilizers seed treatments namely, S₁: Control, S₂: ZnSO₄ at 15 kg/ha, S₃: ZnSO₄ at 30 kg/ha, S₄: FeSO₄ at 15 kg/ha, S₅: FeSO₄ at 30 kg/ha, S₆: ZnSO₄ + FeSO₄ at 15 kg/ha, S₇: ZnSO₄ + FeSO₄ at 30 kg/ha and B₁: Control, B₂: Arbuscular mycorrhiza, B₃: *Trichoderma*, B₄: Arbuscular mycorrhiza + *Trichoderma*. Results revealed that among the soil applications of Zn and Fe, significantly, higher value for number of leaves per plant, dry matter accumulation per plant and plant height at different growth stages were recorded under treatment ZnSO₄ + FeSO₄ at 30 kg/ha. Significantly, lowest value for all growth parameters at different growth stages were obtained under control. Among the various bioinoculants seed treatments, significantly, higher value for growth parameters were recorded under seed treatment of Arbuscular mycorrhiza + *Trichoderma*. Significantly, lowest value for all the characters were recorded under control.

Keywords: Biofortification, zinc, iron, bioinoculants, growth parameters

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the world's agricultural economy, both as a food for human consumption and as a feed for livestock. Due to its relevance in food, feed, speciality corn, starch, and other food-related products, the maize crop holds a significant position in our nation's food grain supply and is the third most important versatile food grain crop. So, maize is a miracle crop called as “Queen of Cereals”. Micronutrient malnutrition is becoming a serious concern worldwide. Micronutrients are essential for crop production in the present situation of soil fertility and their deficiency drastically affects the growth, metabolism and reproductive phase of crop plants, animal and human beings (Daphade *et al.*, 2019) [2]. Due to monocropping and monoculture of maize, less awareness about micronutrients and indiscriminate use of major nutrients led to the imbalance soil nutrient status which led to micronutrient deficiency. Micronutrients are proven to increase the yield and quality of plants in addition to curing nutritional disorders in them. The growth and reproduction of plants depend on seventeen different nutrients, zinc is being one of them. Zinc is an important component of enzymes that drive and increase the rate of many metabolic reactions involved in crop growth and development. Zinc is the only metal that is required in all six enzyme classes (oxidoreductases, transferases, hydrolases, lysases, isomerases and ligases). Iron is essential trace element that take place in several redox states, accepting and donating electrons, which act as a cofactor for numerous plant proteins that contribute in key metabolic pathways. Biofertilizers are more environmentally friendly way to maintain soil fertility. Arbuscular mycorrhizal fungi play vital roles in plant product in agro ecosystem. Arbuscular mycorrhizal fungi helps to increase the availability of Zn in soils as a consequence of rhizospheric acidification and siderophore production besides hyphal transport of nutrients through the external mycelium. *Trichoderma* helps to increase plant hormones which helps to increase root growth and root hair formation that results in the more efficient use of nitrogen, phosphorus, potassium and micronutrient and increase seedling vigour and germination (Mahato *et al.*, 2018) [4]. Agriculture must now concentrate on a new paradigm that will produce more food while also delivering food of higher quality.

Micronutrient deficiencies in both humans and animals can be reduced through biofortification, which aims to increase the content of micronutrients in grains and increase their bioavailability.

2. Materials and Methods

2.1 Background of Study

The field experiment was conducted in *rabi* season of 2021 and 2022 at Navsari Agricultural University, Navsari. The campus of the Navsari Agricultural University is located at 20°57' N Latitude, 72°54' E Longitudes and has an altitude of 10 meters above the mean sea level. The climate of this region is warm and humid monsoon with heavy rainfall, moderately cold winter and fairly hot and humid summer. 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 0.97 mg/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).

2.2 Experimental Design

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, S₁: Control, S₂: ZnSO₄ at 15 kg/ha, S₃: ZnSO₄ at 30 kg/ha, S₄: FeSO₄ at 15 kg/ha, S₅: FeSO₄ at 30 kg/ha, S₆: ZnSO₄ + FeSO₄ at 15 kg/ha, S₇: ZnSO₄ + FeSO₄ at 30 kg/ha and B₁: Control, B₂: Arbuscular mycorrhiza, B₃: *Trichoderma*, B₄: Arbuscular mycorrhiza + *Trichoderma*.

2.3 Field Operations

2.3.1 Preparation of Land

The experimental field was prepared for seeding by cultivating it in both directions with a tractor-drawn cultivator, then harrowing and planking to level it out. Stubbles of previous crop were removed from the experimental field.

2.3.2 Fertilizers application

50% nitrogen (60 kg/ha) through urea and full dose of phosphorus (60 kg/ha) through DAP were applied at basal just before sowing as a common dose to all treatments. When irrigation was applied, remaining, 50% nitrogen (60 kg/ha) through urea was applied at 30 DAS. Application of Zn and Fe through ZnSO₄ and FeSO₄ were applied before sowing as per treatment basis.

2.3.3 Seed and sowing

Maize cultivar GM-6 was sown manually at a depth of 4-5 cm in the furrow, in which fertilizer was band placed previously of each plot at the spacing 60 cm x 20 cm with seed rate of 20 kg/ha.

2.3.4 Irrigation Management

Total six irrigation were given, first irrigation application was done immediately after sowing to ensure good and even germination of seed as well as satisfactory establishment of

the crops. Remaining irrigations were given as per need of crops to meet the requirement of moisture for proper growth and development of crops. Generally 12 to 15 days interval was kept for irrigation application as per requirement of crops.

2.3.5 Weed Management and Interculturing

For effective control of weeds in experimental plot, pre-emergence application of atrazine @ 1.5 kg/ha was made uniformly. All plots underwent one-handed weeding and interculturing at 25 days after sowing.

2.3.6 Harvesting

When the crop reached maturity, harvesting was followed by the removal of the cobs from the plant. Subsequently, the plants were harvested by sickle from each plot. Separate cobs were taken from the net plot area and boarder lines, and they were then let to dry in the sun for around five days. Following the drying of the cobs, each net plot underwent different threshing procedures, and grain yield was recorded.

2.4 Observations recorded

2.4.1 Number of leaves per plant

At 30, 60, 90 DAS and at harvest, number of leaves were counted from randomly selected five plants from each net plot. Average values were calculated and recorded for each plot at each stage.

2.4.2 Plant height

The periodical plant height of the five randomly chosen plants from each net plot was measured at 30, 60, 90, and the time of harvest from the ground to the tip of the main shoot. The average values for each plot at each stage were computed and recorded in centimeter.

2.4.3 Dry matter accumulation per plant

At 30, 60, 90 DAS and at harvest, randomly selected five plants were uprooted from each plot. After removing the roots, the above ground plant part was first sun dried and finally oven dried at 65 °C for 72 hours up to constant dry weight and recorded individually. Average values for each plant at each stage was worked out and recorded.

2.5 Statistical analysis

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) [7]. The significance of difference was tested by 'F' test. Five percent level of significance was used to test the significance of results. The critical differences were calculated when the differences among treatments were found significant in 'F' test. Pooled analysis of two years was worked out as per the procedure suggested by Panse and Sukhatme (1967) [7].

3. Results and Discussion

3.1 Number of Leaves per Plant

An assessment of data in Table 1 indicated that effect of soil application of Zn and Fe and bioinoculants seed treatments in maize did not manifested their significant influence on

number of leaves per plant at 30 DAS. At 60 DAS (12.47, 11.85 and 12.16, respectively), at 90 DAS (14.39, 14.30 and 14.35, respectively) and at harvest (15.32, 15.14 and 15.23, respectively), ZnSO₄ + FeSO₄ at 30 kg/ha produced significantly higher number of leaves per plant during both the years and in pooled results. At 60 and 90 DAS, treatment ZnSO₄ + FeSO₄ at 15 kg/ha (S₆) found statistically at par with ZnSO₄ + FeSO₄ at 30 kg/ha during both the years and remained statistically superior over rest of the treatments in pooled analysis. Control had a lowest number of leaves per plant at all the stages of crop growth. Among the bioinoculants seed treatments, significantly higher number of leaves per plant of maize at 60 DAS (12.48, 12.07 and 12.27, respectively), 90 DAS (14.39, 14.31 and 14.35, respectively) and at harvest (15.54, 15.15 and 15.35, respectively) were recorded with application of Arbuscular mycorrhiza + *Trichoderma*. Significantly lowest number of leaves per plant at all stages of crop growth obtained under control. The number of leaves per plant of maize did not exerted any significant effect due to interaction effect between soil application of Zn and Fe and bioinoculants.

3.2 Plant Height

An appraisal of data in Table 2 stated that effect of various levels of Zn and Fe and seed treatments with bioinoculants had no significant effect on plant height at 30 DAS. Among the various levels of Zn and Fe, at 60 DAS (167.58, 165.20 and 166.39 cm, respectively), at 90 DAS (195.90, 192.17 and 194.03 cm, respectively) and at harvest (203.25, 200.88 and 202.07 cm, respectively), significantly higher plant height was recorded in treatment ZnSO₄ + FeSO₄ at 30 kg/ha. Significantly lowest plant height at all stages of growth was observed under control. With regards to bioinoculants, perusal of data depicted in Table 2 indicated that, at 60 DAS (164.82, 162.09, and 163.45 cm, respectively), at 90 DAS (189.07, 187.60 and 188.33 cm, respectively) and at harvest (198.58, 196.42 and 197.50 cm, respectively) Arbuscular mycorrhiza + *Trichoderma* (B₄) recorded significantly higher plant height during both years and pooled analysis. Significantly lowest plant height was registered under control. The interaction effect between various levels of Zn and Fe and seed treatments with bioinoculants in maize was failed to reach the level of significance.

3.3 Dry Matter Accumulation

The data on dry matter accumulation/plant recorded periodically at 30, 60, 90 DAS and at harvest of the crop as influenced by various soil application of Zn and Fe and seed treatments with bioinoculants during both the years and in pooled analysis is presented in Table 3. Among the various levels of Zn and Fe, significantly maximum dry matter

accumulation/plant was recorded under treatment ZnSO₄ + FeSO₄ at 30 kg/ha at 30 DAS (79.08, 77.33 and 78.20 g, respectively), 60 DAS (91.43, 90.08 and 90.76 g, respectively), 90 DAS (121.70, 119.46 and 120.58 g, respectively) and at harvest (133.48, 129.70 and 131.59 g, respectively) during both the years and in pooled analysis. The lowest dry matter accumulation per plant was registered with control. Among the varying bioinoculants seed treatments, at 30 DAS (79.55, 77.18 and 78.37 g, respectively), at 60 DAS (91.90, 90.56 and 91.23 g, respectively), at 90 DAS (122.22, 120.47, 121.35 g, respectively) and at harvest (133.02, 130.08 and 131.55 g, respectively) treatment Arbuscular mycorrhiza + *Trichoderma* (B₄) recorded significantly higher plant dry matter accumulation/plant. Significantly lower dry matter accumulation per plant of maize was recorded under control at all stages of crop growth. Interaction effect between various levels of Zn and Fe and seed treatments with bioinoculants on dry matter production per plant at 30, 60, 90 DAS and at harvest was found non-significant

The results presented in above findings indicated that soil application of Zn and Fe and bioinoculants seed treatments showed significant effect on crop growth. Most of the photosynthetic pathways depends on enzymes and coenzymes which are synthesized by micronutrients. Increasing in growth parameters due to involvement of Zn and Fe which are involved in the synthesis of growth promoting hormones and the reduction process. The synthesis of auxin is aided by zinc, and this increased hormonal activity during key periods of crop growth ultimately led to enhanced crop growth indices. While iron, which is necessary for the synthesis of chlorophyll and is involved in the production of the pyrrole ring, a component of chlorophyll's structural makeup, has a stimulating influence on growth characteristics. The results are similar findings with Kumar and Salakinkop (2018) [3], Malav *et al.* (2019) [5], Bharti *et al.* (2020) [11] and Raja *et al.* (2020) [9].

Increased in plant height, number of leaves, dry matter production might be due to rendering insoluble phosphorous into available form by Arbuscular mycorrhiza. The enhanced availability of phosphorous favored photosynthesis and total nutrient uptake, this leads to an increased in growth parameters. *Trichoderma* is a fungus that binds with soil aggregates. It can produce phytohormones, vitamins, solubilize minerals which ultimately improves plant growth parameters. They form endophytic association and interact with other microbes in the rhizosphere, thereby influenced disease protection and plant growth attributes. Similar findings are reported by Wahid *et al.* (2016) [10], Prajapati *et al.* (2017) [8] and Masrahi *et al.* (2023) [6].

Table 1: Number of leaves at 30, 60, 90 DAS and at harvest as influenced by different treatments

Treatments	30 DAS			60 DAS			90 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Soil application of Zn and Fe												
S ₁ : Control	5.40	5.35	5.37	9.42	9.30	9.36	11.06	10.96	11.01	11.85	11.81	11.83
S ₂ : ZnSO ₄ at 15 kg/ha	5.51	5.43	5.47	10.35	10.01	10.18	12.20	12.10	12.15	13.19	12.83	13.01
S ₃ : ZnSO ₄ at 30 kg/ha	5.63	5.58	5.60	10.99	10.83	10.91	12.93	12.73	12.83	13.80	13.50	13.65
S ₄ : FeSO ₄ at 15 kg/ha	5.46	5.38	5.42	9.98	9.73	9.86	11.78	11.67	11.73	12.80	12.19	12.50
S ₅ : FeSO ₄ at 30 kg/ha	5.59	5.53	5.56	10.75	10.54	10.65	12.53	12.44	12.48	13.49	13.18	13.33
S ₆ : ZnSO ₄ + FeSO ₄ at 15 kg/ha	5.68	5.67	5.67	11.67	11.32	11.49	13.53	13.33	13.43	14.24	14.01	14.13
S ₇ : ZnSO ₄ + FeSO ₄ at 30 kg/ha	5.70	5.79	5.74	12.47	11.85	12.16	14.39	14.30	14.35	15.32	15.14	15.23
SEm±	0.15	0.18	0.11	0.32	0.30	0.22	0.36	0.37	0.26	0.31	0.33	0.23
CD at 5%	NS	NS	NS	0.92	0.85	0.62	1.02	1.04	0.72	0.87	0.94	0.63
Bioinoculants												
B ₁ : Control	5.51	5.41	5.46	9.49	9.23	9.36	11.26	11.14	11.20	12.10	11.94	12.02
B ₂ : Arbuscular mycorrhiza	5.59	5.57	5.58	11.21	10.74	10.98	13.08	12.96	13.02	13.94	13.56	13.75
B ₃ : <i>Trichoderma</i>	5.55	5.49	5.52	10.03	10.01	10.02	11.80	11.61	11.70	12.53	12.30	12.42
B ₄ : Arbuscular mycorrhiza + <i>Trichoderma</i>	5.62	5.66	5.64	12.48	12.07	12.27	14.39	14.31	14.35	15.54	15.15	15.35
SEm±	0.11	0.13	0.09	0.24	0.23	0.17	0.27	0.28	0.19	0.23	0.25	0.17
CD at 5%	NS	NS	NS	0.69	0.65	0.47	0.77	0.78	0.54	0.66	0.71	0.48
Interaction (SXB)												
SEm±	0.30	0.35	0.23	0.65	0.60	0.44	0.72	0.73	0.51	0.61	0.66	0.45
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig. interactions with Y	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)	9.23	10.98	10.14	10.36	9.93	10.15	9.86	10.13	9.99	7.87	8.65	8.26

Table 2: Plant height of maize at 30, 60, 90 DAS and at harvest as influenced by different treatments

Treatments	Plant height (cm)											
	30 DAS			60 DAS			90 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Soil application of Zn and Fe												
S ₁ : Control	52.27	50.68	51.48	141.04	139.15	140.10	161.41	160.24	160.83	167.35	165.21	166.28
S ₂ : ZnSO ₄ at 15 kg/ha	53.99	51.97	52.98	152.50	150.78	151.64	174.92	172.03	173.47	180.31	178.19	179.25
S ₃ : ZnSO ₄ at 30 kg/ha	54.60	53.15	53.88	160.24	158.48	159.36	189.61	186.67	188.14	193.69	191.45	192.57
S ₄ : FeSO ₄ at 15 kg/ha	53.48	52.37	52.92	146.79	145.67	146.23	170.13	168.75	169.44	173.91	171.52	172.71
S ₅ : FeSO ₄ at 30 kg/ha	54.46	53.33	53.90	156.66	153.88	155.27	183.36	179.59	181.48	185.13	182.83	183.98
S ₆ : ZnSO ₄ + FeSO ₄ at 15 kg/ha	54.90	53.78	54.34	164.90	162.89	163.90	192.79	189.44	191.12	198.08	195.82	196.95
S ₇ : ZnSO ₄ + FeSO ₄ at 30 kg/ha	55.31	54.03	54.67	167.58	165.20	166.39	195.90	192.17	194.03	203.25	200.88	202.07
SEm±	1.35	1.38	0.96	3.84	4.03	2.78	3.72	4.08	2.76	4.95	5.36	3.65
CD at 5%	NS	NS	NS	10.88	11.43	7.80	10.55	11.57	7.74	14.04	15.19	10.23
Bioinoculants												
B ₁ : Control	53.25	51.91	52.58	145.66	144.00	144.83	169.72	166.19	167.95	173.18	170.95	172.06
B ₂ : Arbuscular mycorrhiza	54.36	53.01	53.69	157.67	156.04	156.86	184.08	181.35	182.71	187.37	185.10	186.23
B ₃ : <i>Trichoderma</i>	53.86	52.41	53.14	154.54	152.76	153.65	181.77	178.51	180.14	184.72	182.33	183.53
B ₄ : Arbuscular mycorrhiza + <i>Trichoderma</i>	55.10	53.70	54.40	164.82	162.09	163.45	189.07	187.60	188.33	198.58	196.42	197.50
SEm±	1.02	1.04	0.73	2.90	3.05	2.10	2.81	3.08	2.09	3.74	4.05	2.76
CD at 5%	NS	NS	NS	8.22	8.64	5.90	7.98	8.74	5.85	10.61	11.48	7.73
Interaction (SXB)												
SEm±	2.69	2.75	1.92	7.67	8.06	5.57	7.44	8.16	5.52	9.90	10.71	7.30
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig. interactions with Y	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)	8.61	9.03	8.82	8.54	9.09	8.81	7.12	7.92	7.52	9.22	10.10	9.67

Table 3: Dry matter accumulation per plant 30, 60, 90 DAS and at harvest by different treatments

Treatments	Dry matter accumulation per plant (g/plant)											
	30 DAS			60 DAS			90 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Soil application of Zn and Fe												
S ₁ : Control	63.88	61.92	62.90	76.23	74.88	75.56	106.18	105.10	105.64	115.42	114.18	114.80
S ₂ : ZnSO ₄ at 15 kg/ha	68.48	66.93	67.70	80.90	79.39	80.15	111.18	110.12	110.65	121.34	119.18	120.26
S ₃ : ZnSO ₄ at 30 kg/ha	74.68	72.78	73.73	87.01	85.54	86.28	117.61	114.90	116.25	128.09	124.93	126.51
S ₄ : FeSO ₄ at 15 kg/ha	66.23	64.09	65.16	78.58	77.08	77.83	108.54	107.44	107.99	119.04	116.54	117.79
S ₅ : FeSO ₄ at 30 kg/ha	71.58	69.46	70.52	83.93	82.78	83.35	114.01	112.77	113.39	123.46	122.01	122.73
S ₆ : ZnSO ₄ + FeSO ₄ at 15 kg/ha	77.25	74.62	75.93	89.61	88.33	88.97	119.63	118.26	118.95	129.98	127.63	128.81
S ₇ : ZnSO ₄ + FeSO ₄ at 30 kg/ha	79.08	77.33	78.20	91.43	90.08	90.76	121.70	119.46	120.58	133.48	129.70	131.59
SEm±	2.15	1.89	1.43	2.67	2.54	1.84	3.67	3.51	2.54	3.79	3.59	2.61
CD at 5%	6.11	5.36	4.02	7.57	7.20	5.17	10.40	9.95	7.11	10.74	10.18	7.31
Bioinoculants												
B ₁ : Control	63.86	61.85	62.85	76.23	74.86	75.55	106.45	104.91	105.68	116.31	114.32	115.32
B ₂ : Arbuscular mycorrhiza	73.90	72.05	72.97	86.27	85.04	85.66	116.40	114.87	115.64	126.60	124.28	125.44
B ₃ : <i>Trichoderma</i>	69.07	67.28	68.17	81.42	79.87	80.65	111.40	110.07	110.74	121.67	119.40	120.54
B ₄ : Arbuscular mycorrhiza + <i>Trichoderma</i>	79.55	77.18	78.37	91.90	90.56	91.23	122.22	120.47	121.35	133.02	130.08	131.55
SEm±	1.63	1.43	1.08	2.02	1.92	1.39	2.77	2.65	1.92	2.86	2.71	1.97
CD at 5%	4.62	4.05	3.04	5.72	5.44	3.90	7.86	7.52	5.38	8.12	7.69	5.53
Interaction (SXB)												
SEm±	4.31	3.78	2.87	5.34	5.08	3.69	7.33	7.02	5.08	7.58	7.18	5.22
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sig. interactions with Y	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)	10.42	9.41	9.95	11.02	10.65	10.84	11.13	10.80	10.97	10.55	10.19	10.38

4. Conclusion

The experimental results indicated that, among the soil applications of Zn and Fe and bioinoculants seed treatments, ZnSO₄ + FeSO₄ at 30 kg/ha and seed treatment of Arbuscular mycorrhiza + *Trichoderma* gave higher value of number of leaves per plant, plant height and dry matter accumulation per plant.

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