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Bio fortification of zinc and iron in lentil (*Lens culinaris* Medik.) through agronomic intervention

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Abstract
A field experiment was conducted during *rabi* 2021-22 at Agriculture Instructional-cum-Research Farm, IGKV, Raipur. The experimental design was Randomized Block Design having three replications and 7 treatments. The crop lentil and variety chosen was CG Masoor-1. Recommended dose of fertilizer (RDF) *i.e.* N: P_2O_5: K_2O: S: 20: 50: 20: 20 kg ha^{-1} was applied as basal dose and seed inoculation with Phosphate Solubilizing Bacteria (PSB) and rhizobium @ 5 gm kg^{-1} seed at the time of sowing was commonly applied to all treatments. The treatment consist of T_1: RDF 100% (Control), T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation, T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation, T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation, T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours, T_6: RDF 100% + Seed priming with 0.05% $FeSO_4$ solution W/V soaked for 3-4 hours and T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. The initial phases of crop gr owth did not show a significant response to treatments; however, at 60 DAS, 90 DAS and harvest, the highest growth-related characteristics, including plant height, number of branches, number of leaves, leaf area, leaf area index and dry weight of plant were significantly increased with the application of RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. The increase in seed and stover yield of crop observed more significantly with the application of RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. Also, the highest zinc content in seed was observed with the application of RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1} and highest iron content in seed was observed with the application of RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation.

Keywords: applied to all treatments, recommended dose of fertilizer

Introduction

Pulses are one of the important source of human diet. Majority of the people in India have vegetarian based dietary habit. Pulses are cheapest source of dietary protein. As a rich source of vitamins, minerals and proteins, pulses are also known as "poor man's meat" and "rich man's vegetable," and they provide a significant contribution to the nation's nutritional security.

Lentil is one of the major *rabi* pulses. Approximately 24 to 26% of lentil is protein, 1.3% is fat, 57% is carbohydrates, 2.2% is ash, and 3.2% is fibre (Prasad, 2015) [8]. Since, lentil is rich in protein, carbohydrates, dietary fiber, calcium, phosphorous, iron and zinc have potential to eliminate hunger and malnutrition thus ensuring food security. Globally, micronutrient deficiency is a serious issue. Many people cannot maintain a healthy lifestyle due to a lack of availability to wholesome foods. Nearly one-fourth of the world's population suffers from one or more micronutrient deficiency disorders, making malnutrition a global problem (Kumssa *et al*., 2015) [6].

Among all micronutrients, deficiency of zinc and iron in human nutrition are prevalent in developing countries including India (Harvest Plus, 2021 and Yadava *et al*., 2018) [3, 10]. Continuous use of high yielding varieties of cereal crops year after year with high rate of primary nutrients has exhaust micronutrients like zinc and iron from the soil results in deteriorating of soil fertility and productivity.

Deficiency of zinc in the soil leads to the dietary malnutrition and health problems in human and animals. It is constituent of carbonic anhydrase, RNA polymerase enzymes, important in synthesis of IAA (Indole Acetic Acid) which is important for nodule formation and essential for water uptake. It help in production of superoxide dismutases which control oxidative stress in plants. Its deficiency causes poor synthesis of phyto hormones *viz*. auxins, gibberellins and cytokinins which results in poor growth and development of crop (Hassan *et al*., 2020) [4]. In humans zinc plays important role in immune system functioning, reproductive health, neurotransmission and body growth (Herschfinkel *et al*. 2007; Wessels and Rink, 2020) [5, 9]. It is essentially required for the mobilization of vitamin A from the liver. It also plays an important role in the formation of insulin. It's deficiency can lead to severe health problems like impaired learning, abnormal immune system, impaired growth rate and increased infection rate (Gibson, 2006; Prasad, 2007; Wessels and Rink, 2020) [1, 7, 9].

Iron is also important for nodule formation in legumes and helps in nitrogen fixation as it act as structural component of hemes, haematin and leg haemoglobin involved in the nitrogen fixation in pulses. It is essential in chlorophyll synthesis, protein synthesis, nucleic acid metabolism and many more. In humans iron is essential for a variety of metabolic functions which include oxygen transport and storage, assisting immunity and contributing to enzyme stress. It's deficiency can lead to severe health issues like anaemia, increased mortality rate, poor pregnancy and reduced cognitive ability.

One way to combat malnutrition or hidden hunger is by bio fortification, which offers a fairly affordable, long-term, sustainable method of supplying more micronutrients. The best way to lessen malnutrition is through increasing mineral concentrations in edible crops especially through staple or partial staple food crops so that it can reach to maximum population. Either agronomic or genetic methods can be used to increase the mineral elements in crops. The simplest, most practical and quickest method for bio-fortifying pulse grains with Fe, Zn, or other desirable micronutrients is through agronomic bio-fortification.

Materials and methods

A field trial was conducted during *Rabi* 2021-22 at Agriculture Instructional-cum-Research Farm, IGKV, Raipur which is located at 21°.25' N latitude and 81°.62' E longitude with an altitude elevation 298.15 m above mean sea level**.** The soil was neutral in reaction and having medium in organic carbon (0.71%), available nitrogen (184.72 kg ha^{-1}), phosphorus (9.14 kg ha^{-1}) and potassium (306.64 kg ha^{-1}) content were low, medium and high, respectively. The experiment was laid out in Randomized Block Design which consisting of 7 treatments and 3 replications, that are T_1: RDF 100% (Control), T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation, T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation, T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation, T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours, T_6: RDF 100% + Seed priming with 0.05% $FeSO_4$ solution W/V soaked for 3-4 hours and T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. The growth parameters of the plants were recorded at frequent intervals from 30 DAS up until harvest and finally, the yield parameters were recorded after harvest. Plant samples (grain and stover) collected at maturity and were dried, grinded and used for chemical analysis. Zn and Fe content in the grain and stover of the crop was determined by atomic absorption spectrophotometer from the extract obtained through digestion with di-acid mixture and it was expressed in ppm. The experimental data were analysed following the standard statistical method (Gomez and Gomez, 1984) [2] at 0.5% level of significance.

Results and Discussion

Plant height

The plant height was measured at 30, 60, 90 DAS and harvest. The age of the crop often causes a gradual increase in plant height. The height of the plant increased quickly over the first 60 days and then considerably at a slow rate between 60 DAS and harvest. At 30 DAS, there was no discernible variation in plant height, but at 60 DAS, 90 DAS, and at harvest, there is a noticeable difference. Among all the treatments, maximum plant height value recorded was 32.0 cm at 60 DAS, 43.3 cm at 90 DAS and 47.2 cm at harvest under the treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. However, it was at par to T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation (29.5 cm at 30 DAS, 40.2 cm at 60 DAS, 43.5 cm at 90 DAS) and at 30 DAS it was also at par to T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation (29.6 cm) 30 DAS. While, minimum plant height value was 22.3 cm recorded at 60 DAS, 29.0 cm at 90 DAS and 32.0 cm at harvest under T_1: control plot.

Dry matter accumulation $plant^{-1}$

The dry matter accumulation $plant^{-1}$ was measured at 30, 60, 90 DAS and harvest. The Zn and Fe bio fortification has significant influence on dry matter accumulation of lentil at all stages of observation except at 30 DAS as there was no significant difference in dry matter accumulation, but it differs significantly at 60 DAS, 90 DAS and at harvest. It was seen that dry matter accumulation $plant^{-1}$ increased up to 90 DAS, and after that rate of increase in dry weight was slow. Among all treatments, highest dry matter accumulation $plant^{-1}$ at 60 DAS (5.45 g), 90 DAS (15.84 g) and at harvest (18.51 g) was observed with treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, but it was at par with treatments T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation and T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation. Lowest dry matter accumulation plant $^{-1}$ (3.63 g) at 60 DAS, (9.85 g) at 90 DAS and (12.05 g) at harvest was observed with treatment T_1: control

Table 1: Effect of bio fortification of zinc and iron on plant height and dry matter accumulation $plant^{-1}$ of lentil at different interval of time

Treatments	Plant height (cm)				Dry matter accumulation $plant^{-1}$ (g)			
	30 DAS	60 DAS	90 DAS	At Harvest	30 DAS	60 DAS	90 DAS	At Harvest
T_1: RDF 100% (Control)	10.5	22.3	29.0	32.0	0.56	3.63	9.85	12.05
T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation	10.1	29.6	38.9	41.6	0.68	5.08	14.76	16.31
T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation	11.5	26.6	36.8	39.9	0.63	4.59	12.48	14.51
T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation	10.5	29.5	40.2	43.5	0.60	5.28	14.92	17.67
T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours	11.8	27.4	37.4	40.7	0.62	4.66	13.07	15.43
T_6: RDF 100% + Seed priming with 0.05% $FeSO_4$ solution W/V soaked for 3-4 hours	12.2	24.8	35.4	37.9	0.67	4.21	11.79	13.92

T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	12.0	32.0	43.3	47.2	0.66	5.45	15.84	18.15
SEm ±	0.5	0.91	1.35	1.44	0.03	0.14	0.38	0.61
CD at 5%	NS	2.80	4.17	4.43	NS	0.43	1.18	1.88

Number of pods $plant^{-1}$

The Zn and Fe bio fortification has positive effect on number of pods $plant^{-1}$. Among all treatments, highest number of pods $plant^{-1}$ (62.3) was observed with treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, but it was at par with treatments T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation, T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation, T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation and T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours. Lowest number of pods $plant^{-1}$ (42.6) was observed with treatment T_1: control.

Seed yield

The significant variation in seed yield was observed in lentil due to Zn and Fe bio fortification. Among all treatments, highest seed yield (1053 kg ha^{-1}) was observed with treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, but it was at par with treatment T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation. Lowest seed yield (770 kg ha^{-1}) was observed with treatment T_1: control.

Stover yield

The significant variation in stover yield was observed in lentil due to Zn and Fe bio fortification. Among all treatments, highest stover yield (1339 kg ha^{-1}) was observed with treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, but it was at par with treatment T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation. Lowest stover yield (974 kg ha^{-1}) was observed with treatment T_1: control.

Harvest Index

The harvest index value ranges from (44.005% to 44.064%) under different treatment. The result showed that there was no significant difference in harvest index. However, numerically highest harvest index was (44.064%) obtained under T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours and lowest (44.005%) under T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation.

Table 2: Effect of bio fortification of zinc and iron on number of pods $plant^{-1}$, seed yield, stover yield and harvest index of lentil

Treatments	Pods $plant^{-1}$ (No.)	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest Index (%)
T_1: RDF 100% (Control)	42.6	770	974	44.007
T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation	59.0	903	1147	44.063
T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation	56.3	820	1043	44.005
T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation	60.0	940	1194	44.032
T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours	58.6	901	1143	44.064
T_6: RDF 100% + Seed priming with 0.05% $FeSO_4$ solution W/V soaked for 3-4 hours	52.6	783	997	44.008
T_7: RDF 100% + Soil application of $ZnSO_4$ @20 kg ha^{-1}	62.3	1053	1339	44.024
SEm ±	2.24	45	57	0.03
CD at 5%	6.90	140	177	NS

Zinc content in seed and stover (ppm)

The significant variation was observed in lentil due to Zn and Fe bio fortification. Among all treatments, highest zinc content in seed (48.2 ppm) and in stover (45.3 ppm) was observed with treatment T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}. Lowest zinc content in seed (41.1 ppm) and in stover (23.3 ppm) was observed with treatment T_1: control.

Iron content in seed and stover (ppm)

The significant variation was observed in lentil due to Zn and Fe bio fortification. Among all treatments, highest iron content in seed (68.23 ppm) and in stover (109.5 ppm) was observed with treatment T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation. Lowest iron content in seed (64.33 ppm) and in stover (90.2 ppm) was observed with treatment T_1: control.

Table 3: Effect of bio fortification of zinc and iron content in lentil

	Zn content Fe content (ppm) (ppm)			
	Seed	Stover	Seed	Stover
T_1: RDF 100% (Control)	41.1	23.3	64.33	90.2
T_2: RDF 100% + 0.5% $ZnSO_4$ at pre flowering and pod initiation	46.8	42.1	66.30	95.8
T_3: RDF 100% + 0.5% $FeSO_4$ at pre flowering and pod initiation	41.4	33.7	68.23	109.5
T_4: RDF 100% + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at pre flowering and pod initiation	47.4	38.3	66.0	104.5
T_5: RDF 100% + Seed priming with 0.05% $ZnSO_4$ solution W/V soaked for 3-4 hours	42.2	38.7	68.17	102.1
T_6: RDF 100% + Seed priming with 0.05% $FeSO_4$ solution W/V soaked for 3-4 hours	42.6	36.7	64.68	109.1
T_7: RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	48.2	45.3	64.67	108.5
SEm ±	0.51	0.31	0.48	0.69
CD at 5%	1.6	0.96	1.5	2.15

Conclusion

Based on the findings, application of RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1} showed significantly higher plant height, dry matter accumulation, number of pods $plant^{-1}$, seed yield and stover yield. The highest zinc content in seed was observed with treatment RDF 100% + Soil application of $ZnSO_4$ @ 20 kg ha^{-1} and highest iron content in seed was observed with treatment RDF 100% + 0.5% $FeSO_4$

at pre flowering and pod initiation.

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