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ENHANCEMENT OF GROWTH ATTRIBUTES BY MICRONUTRIENTS AND BIO-INOCULANTS OF LENTIL (*LENS CULINARIS* L.) IN CENTRAL PLAIN OF U.P., INDIA

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ABSTRACT

Field experiment at the Student Instructional Farm was conducted during the *Rabi* season of 2021-22 and 2022-23; C. S. A. U. A. & T., Kanpur, India the crop lentil (Lentil KLS9-3) was grown. Experimental field soil had a sandy loam texture; there are 15 treatments with 3 replications of randomized block designs were used to analyze the combinations. The growth attributing observations *i.e.*, plant height and branch count at 30, 60, 90 DAS and harvest, as well as nodule numbers and nodule dry weight at harvest at 60 and 90 DAS were recorded. Pooled results of two year the T_{14} (RDF + $ZnSO_4$ + $FeSO_4$ (0.5%) foliar spray (Individual spray) at pre flowering and pod formation Stage + *Rhizobium*) showed the highest values of growth attributes *i.e.*, plant height 8.47, 24.24, 42.59 and 44.46 cm at 30, 60, 90 DAS and at harvest, no. of branches plant^{-1} 1.59, 4.60, 6.89 and 6.90 no. of nodules per plant at 30,60, 90 days after sowing and harvest 27.85, 31.80 at 60 and 90 DAS and at 60 and 90 DAS, nodules plant^{-1} weighed 51.38 and 64.62 mg as their dry weight.

Key words : Lentil, Growth attributes, Zinc, Iron, Bio-Inoculant.

Introduction

India accounts for 25% of worldwide pulse output, 15% of global commerce and 27% of Indian consumption, demonstrating pulses' significance to India's food and nutritional security. It is the main legume crop in eastern and central Indian rice fallow regions as well. DES, MoAF & W and the government have provided their fourth advanced estimate. Among all states of India, Uttar Pradesh produces the largest amount of lentil Madhya Pradesh followed with 0.44 million tons from 0.49 hectares, 36.43 percent of national output. Based on their contribution to national lentil production, West Bengal accounted for 34.55% of the production, Bihar accounted for 8.84% and Jharkhand accounted for 4.5%.

Lentils are hardy, cool-season legumes, capable of thriving in marginal lands with low soil fertility. Nitrogen can be fixed by them in a unique way, enhancing soil fertility and reducing the need for external nitrogen inputs (Sulieman *et al.*, 2019). This makes lentils an essential component of sustainable agricultural systems, promoting soil health and reducing environmental impacts.

The synthesis of protein in plants lacking Zn was observed to decrease. However, providing Zn to these plants improved the process (Zeng *et al.*, 2021). Zn is a crucial trace nutrient in the reproductive phase, such as propagation and pollen grain development, because pollen grain includes a lot of zinc. Plant growth, stress tolerance, cell membrane integrity, protein synthesis, chlorophyll production enhanced the level of antioxidants, etc, benefits are due to Zn. The typical symptoms of zinc deficiency include slowed growth and the appearance on both sides of the midrib, plants have greenish yellowish patches (Hashmi *et al*, 2023). Chlorophyll content increases as Zn concentration rises within plant tissues (Singh and Bhatt *et al*, 2013).

The mineral iron ranks third on the list of plant growth-limiting resources and metabolism because oxidized ferric forms are poorly soluble in aerobic conditions (Samaranayke *et al.*, 2012). During plant growth, chlorophyll is synthesized and maintained, and nucleic acids are metabolized. As an important nutrient to crop production, iron is essential for root, leaf, and other parts of the plant to grow, function and transport oxygen. The lack of iron will prevent crops from receiving enough oxygen, and the lack of oxygen will prevent them from producing sufficient chlorophyll. Specific gene expression is modulated by iron, which helps to manage gene expression related to nodulation in legumes may be influenced by iron availability (Rodríguez, 2013).

Rhizobium is a bio-inoculant, a group of nitrogen-fixing bacteria, that establishes a mutualistic relationship with leguminous plants, playing a vital role in maintaining soil fertility (Bashan *et al.*, 1997). Leguminous plants hosting *Rhizobium* exhibit vigorous growth, leading to higher crop yields. This enhanced productivity ensures food security for communities globally, particularly in regions dependent on leguminous crops (Santos *et al.*, 2019). *Rhizobium* inoculation has been shown to significantly increase the yield of legume crops such as soybeans, chickpeas and lentils (Khan *et al.*, 2019).

Materials and Methods

During *Rabi* season of 2021-22 and 2022-23, the C.S.A.U.A.T., Student's Instructional Farm, Kanpur was used for the experimentis located in western northernmost region inside the metropolis in fifth subtropical Agro-climatic zone (central plain zone). An irrigation tube well irrigates the university's main campus farm, which was levelled and irrigated.

The fifteen treatments were experimented in three repetitions- randomized block design. Table 2 provides details about the treatment.

Urea, DAP and MOP were treated equally via basal dressing, nitrogenin the case of fertilizer *i.e.* 20 kg ha^{-1}, 40 kg ha^{-1} for phosphorus and 20 kg ha^{-1} for potash. Zinc heptahydrate (0.5%) and iron heptahydrate (0.5%) were applied foliarly in accordance with the crop treatment protocol. Prior to seeding, *Rhizobium* was infected with seeds in accordance with the treatment.

Growth attributes

Growth characters

Plant height : A periodic measurement of plant heights was conducted during crop growth, such as 30, 60, 90 DAS & at harvest. To measure height of the plant, the distance between the ground and the last couple of leaves was measured in centimetres. The plants used in this case had already been selected. In order to conduct this study, we measured at each stage of the selection process, 5 selected plants average height was measured.

Number of branches plant^{-1} : It was also necessary to use a selection of plants which have already been tagged for this purpose. The branches of plants were counted when counting the plants. Five plants were selected on average to determine the average number of

Table 1 : Soil physico-chemical characteristics prior to agricultural cultivation of the crop before sowing.

S. no.	Soil characters	Value		Method employed
		2021-22	2022-23	
1.	pH (1:2.5 soil water suspension)	7.77	7..69	Glass electrode pH meter (Jackson, 1973)
2.	EC (dsm^{-1}) (1:2.5 soil water suspension)	0.34	0.35	Conductivity bridge (Jackson, 1973)
6.	Organic carbon (%)	0.36	0.37	Chromic acid digestion (Walkley and Black, 1934)
7.	Available N kg ha^{-1}	190	192	Alkaline permanganate method (Subbiah and Asija, 1956)
8.	Available P kg ha^{-1}	12.37	12.48	Olsen's calorimetrically method (Olsen *et al.*, 1954)
9	Available K kg ha^{-1}	198	199.5	Flame photometer Ammonium acetate extract (Hanwey and Heidel, 1952)
10	Available zinc(mg/kg)	0.49	0.51	DTPA (Lindsay and Norvel, 1978)
11	Available iron (mg/kg)	9.54	9.98	DTPA (Lindsay and Norvel, 1978)

Table 2 : Treatment details.

Treatment combination	Stage of Application
T_1 = Only RDF	
T_2= RDF+ $ZnSO_4$ (0.5%) Foliar spray	R_1
T_3= RDF +$FeSO_4$ (0.5%) Foliar spray	R_1
T_4= RDF + $ZnSO_4$ (0.5%) + $FeSO_4$(0.5%) Foliar spray	R_1
T_5= RDF + $ZnSO_4$ (0.5%) Foliar spray	R_2
T_6= RDF + $FeSO_4$ (0.5%) Foliar spray	R_2
T_7= RDF + $ZnSO_4$ (0.5%) + FeSO4 (0.5%) Foliar spray	R_2
T_8= RDF + $ZnSO_4$+ $FeSO_4$ (0.5%) Foliar spray (Mix tank spray)	R_2
T_9= RDF + $ZnSO_4$ (0.5%) Foliar spray + *Rhizobium*	R_1
T_{10}= RDF + $FeSO_4$(0.5%) Foliar spray + *Rhizobium*	R_1
T_{11}= RDF + $ZnSO_4$+ $FeSO_4$ (0.5%) Foliar spray + *Rhizobium*	R_1
T_{12}= RDF + $ZnSO_4$ (0.5%) Foliar spray + *Rhizobium*	R_2
T_{13}= RDF + $FeSO_4$ (0.5%) Foliar spray + *Rhizobium*	R_2
T_{14}= RDF + $ZnSO_4$(0.5%) + $FeSO_4$ (0.5%) Foliar spray (Individual spray) + *Rhizobium*	R_2
T_{15}= RDF + $ZnSO_4$(0.5%) + $FeSO_4$ (0.5%) Foliar spray (Mix tank spray) + *Rhizobium*	R_2

R_1 = Pre flowering stage, R_2 = Pre flowering + Pod development stage.

plants. Branch numbers were also analysed based on growth stage.

Number of nodules plant^{-1} : Random nodules were numbered from 5 plants per plot. Plants were also dug out using khurpi as well as adhered soil. Each plant was counted five times to determine the no. of nodules. At 60- and 90-days observation period was observed after sowing**.**

Dry weight of nodules plant^{-1} : The nodules were dried in a 60°C oven to achieve a consistent dry weight per plant.

Statistical analysis

Analyzed growth and yield parameters statistically to determine significance impacts of different treatments. The Fisher's 'F' test was utilized for this purpose. The interpretation of the results relies on the statistical significance of the derived 'F' value at a 5% significance level. A critical difference has been determined for examining significant treatments.

Results and Discussion

Growth attributes

Plant height

Data relating to height of plants at 30 DAS, 60DAS, 90DAS and harvest are depicted in Table 3 showed the height of the plant at 30 DAS, 60 DAS, 90 DAS and harvest was recorded substantial variation among the treatments over control. The maximum measured height of the plants at 30, 60, 90 DAS and stage of harvest found in treatment T_{14} (RDF + $ZnSO_4$ + $FeSO_4$ (0.5%) foliar spray (Individual spray) at pre flowering and pod formation Stage + *Rhizobium*) based on pooled analysis of *i.e.* (8.47 cm, 24.24 cm, 42.59 cm and 44.46 cm and respectively) and significant with treatment T_{15}, which found to be at par, similar trends were noticed in pooled data of both years. Increment of plant height was primarily due to higher growth of the shoots due to cell elongation, differentiation of the cells and apical dominance promoted by zinc (Marschner, 2012). Increased Iron availability to plants may have encouraged metabolic and enzymatic activity, causing the crop to develop and ultimately, reach greater heights (Masoud *et al*., 2012). The same result was examined by respective authors; Singh *et al*. (2023), Nayak and Chandrakar (2022), Sahu *et al.* (2017).

Number of branches plant^{-1}

Table 4 shows no. of branches plant^{-1} at 30DAS, 60DAS, 90DAS and harvest. There is a significant difference between the treatments over control in No. of branches plant^{-1} at 30DAS, 60DAS, 90DAS and harvest. At 30DAS, 60DAS, 90DAS, and harvest, treatment T_{14} (RDF + $ZnSO_4$ + $FeSO_4$ (0.5%) foliar spray (Individual spray) at pre-flowering and pod formation Stage + *Rhizobium*) had the maximum no. of branches plant^{-1}. Based on a pooled analysis (1.59, 4.60, 6.89 and 6.90, respectively) and significant with treatment T_{15}, which was comparable at par, both years showed similarities. In pooled data as well. Increasing the number of branches caused by iron application could be due to iron's involvement in protein synthesis, starch development, chlorophyll synthesis and chloroplast development (Kumar *et al*., 2009). Zinc is required by plants for chlorophyll synthesis and photosynthesis, a process that provides energy for the growth of plants, including the production of branches (Broadley *et al*., 2007). Identical

Table 3 : Impact of treatments on plant height at 30, 60, 90 DAS and at harvest (cm) of lentil.

S. no.	Treat-ments	Plant Height at 30 DAS			Plant Height at 60 DAS			Plant Height at 90 DAS			Plant Height at Harvest		
		2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
1.	T_1	8.25	8.27	8.26	18.75	18.78	18.77	39.10	39.12	39.11	39.52	39.54	39.53
2.	T_2	8.25	8.27	8.26	19.40	19.42	19.41	39.16	39.17	39.17	39.78	39.79	39.79
3.	T_3	8.26	8.27	8.27	19.25	19.28	19.27	39.20	39.21	39.21	40.15	40.17	40.16
4.	T_4	8.25	8.26	8.26	20.86	20.82	20.84	40.96	40.96	40.96	41.13	41.15	41.14
5.	T_5	8.25	8.27	8.26	19.41	19.43	19.42	39.24	39.26	39.25	40.23	40.26	40.25
6.	T_6	8.25	8.27	8.26	19.27	19.30	19.29	39.27	39.28	39.28	40.31	40.33	40.32
7.	T_7	8.26	8.27	8.27	22.60	22.62	22.61	41.67	41.69	41.68	42.23	42.25	42.24
8.	T_8	8.26	8.26	8.26	22.31	22.32	22.32	41.34	41.36	41.35	41.88	41.91	41.90
9.	T_9	8.45	8.45	8.45	20.00	20.03	20.02	40.12	40.14	40.13	40.50	40.52	40.51
10.	T_{10}	8.46	8.47	8.47	19.98	20.01	20.00	40.50	40.52	40.51	40.61	40.62	40.62
11.	T_{11}	8.45	8.46	8.46	22.22	22.24	22.23	41.11	41.13	41.12	41.50	41.50	41.50
12.	T_{12}	8.45	8.46	8.46	20.32	20.35	20.34	40.62	40.64	40.63	40.72	40.74	40.73
13.	T_{13}	8.45	8.47	8.46	20.37	20.38	20.38	40.85	40.87	40.86	40.96	40.98	40.97
14.	T_{14}	8.46	8.47	8.47	24.23	24.25	24.24	42.57	42.60	42.59	44.45	44.47	44.46
15.	T_{15}	8.46	8.46	8.46	23.87	23.90	23.89	42.05	42.10	42.08	43.10	43.13	43.12
SE (diff)		**1.10**	**0.156**	**0.111**	**0.50**	**0.38**	**0.34**	**0.90**	**0.95**	**0.64**	**1.00**	**0.91**	**0.60**
CD at 5.0%		**NS**	**NS**	**NS**	**1.02**	**0.78**	**0.70**	**1.85**	**1.95**	**1.32**	**2.06**	**1.89**	**1.24**

Table 4 : Impact of treatments on numbers of branches at 30, 60, 90 DAS and at harvest in lentil.

Treatments	No. of branches per plant at 30 DAS			No. of branches per plant at 60 DAS			No. of branches per plant at 90 DAS			No. of branches per plant at Harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1	1.41	1.42	1.42	2.82	2.83	2.83	4.62	4.63	4.63	4.62	4.64	4.63
T_2	1.41	1.42	1.42	2.85	2.87	2.86	4.65	4.67	4.66	4.65	4.67	4.66
T_3	1.42	1.43	1.43	2.87	2.88	2.88	4.67	4.70	4.69	4.67	4.68	4.68
T_4	1.41	1.42	1.42	3.89	3.91	3.90	4.94	5.00	4.97	4.94	4.97	4.96
T_5	1.41	1.43	1.42	2.95	2.98	2.97	4.70	4.85	4.78	4.70	4.80	4.75
T_6	1.42	1.43	1.43	2.99	3.00	3.00	4.73	4.75	4.74	4.73	4.80	4.77
T_7	1.42	1.43	1.43	4.02	4.05	4.04	5.20	5.32	5.26	5.20	5.32	5.26
T_8	1.41	1.43	1.42	3.98	4.00	3.99	5.16	5.32	5.24	5.16	5.35	5.26
T_9	1.57	1.58	1.58	3.22	3.25	3.24	4.77	4.85	4.81	4.77	4.89	4.83
T_{10}	1.57	1.58	1.58	3.64	3.67	3.66	4.80	4.92	4.86	4.80	4.98	4.89
T_{11}	1.58	1.59	1.59	3.94	3.95	3.95	5.10	5.23	5.17	5.10	5.45	5.28
T_{12}	1.57	1.58	1.58	3.81	3.84	3.83	4.84	4.90	4.87	4.84	4.95	4.90
T_{13}	1.58	1.59	1.59	3.86	3.90	3.88	4.89	4.93	4.91	4.89	4.98	4.94
T_{14}	1.58	1.59	1.59	4.58	4.62	4.60	6.89	6.88	6.89	6.89	6.90	6.90
T_{15}	1.57	1.45	1.51	4.20	4.38	4.29	5.72	5.81	5.77	5.72	5.88	5.80
SE	**0.111**	**0.109**	**0.110**	**0.06**	**0.08**	**0.05**	**0.11**	**0.10**	**0.08**	**0.11**	**0.12**	**0.07**
CD at 5.0	**NS**	**NS**	**NS**	**0.12**	**0.16**	**0.09**	**0.23**	**0.21**	**0.17**	**0.23**	**0.24**	**0.15**

outcomes were detailed with Singh *et al.* (2024), Kumar *et al.* (2023), Yadav *et al.* (2022), Kumari *et al.* (2021).

Number of nodules plant^{-1}

Nodule numbers plant^{-1} at 60 DAS, 90 DAS and harvest are shown in Table 3. The results demonstrate that there was a significant difference between the treatments at 60 DAS and 90 DAS over the control. At 60 DAS and 90 DAS periods, the greatest number of nodules was found in treatment T_{14} (RDF + $ZnSO_4$ +

Table 5 : Impact of treatments on number of nodules per plant at 60 and 90 DAS of lentil.

S. no.	Treatments	Number of nodules per (60 DAS) plant			Number of nodules per plant (90 DAS)		
		2021	2022	Pooled	2021	2022	Pooled
1.	T_1	17.20	18.40	17.80	19.40	20.52	19.96
2.	T_2	17.40	18.72	18.06	20.00	21.11	20.56
3.	T_3	17.70	18.80	18.25	20.20	21.23	20.72
4.	T_4	22.50	23.40	22.95	26.70	27.81	27.26
5.	T_5	17.50	18.58	18.04	22.00	23.22	22.61
6.	T_6	17.70	18.76	18.23	23.50	24.60	24.05
7.	T_7	24.60	25.73	25.17	28.90	30.00	29.45
8.	T_8	24.00	25.21	24.61	27.60	28.70	28.15
9.	T_9	19.40	20.57	19.99	24.40	25.51	24.96
10.	T_{10}	20.50	21.62	21.06	25.10	26.12	25.61
11.	T_{11}	23.80	25.00	24.40	26.80	27.82	27.31
12.	T_{12}	21.60	22.71	22.16	26.10	27.24	26.67
13.	T_{13}	22.20	23.21	22.71	26.10	27.21	26.66
14.	T_{14}	27.30	28.40	27.85	31.30	32.41	31.86
15.	T_{15}	25.40	26.50	25.95	29.40	30.56	29.98
SE (diff)		**0.45**	**0.41**	**0.31**	**0.62**	**0.46**	**0.44**
CD at 5.0 %		**0.93**	**0.85**	**0.64**	**1.27**	**0.95**	**0.91**

Table 6 : Impact of treatments on no. of nodules per plant at 60 and 90 DAS of lentil.

S. no.	Treatments	Dry weight of nodules $plant^{-1}$ (60 DAS)			Dry weight of nodules $plant^{-1}$ (90 DAS)		
		2021	2022	Pooled	2021	2022	Pooled
1.	T_1	41.00	41.20	41.10	46.20	47.11	46.66
2.	T_2	43.00	43.50	43.25	47.00	47.89	47.45
3.	T_3	44.00	44.82	44.41	48.00	49.00	48.50
4.	T_4	47.70	48.50	48.10	54.20	55.00	54.60
5.	T_5	44.50	45.64	45.07	48.50	49.60	49.05
6.	T_6	44.70	45.80	45.25	49.20	50.15	49.68
7.	T_7	48.90	49.80	49.35	59.00	59.90	59.45
8.	T_8	48.60	49.70	49.15	57.20	58.12	57.66
9.	T_9	45.40	46.32	45.86	49.80	50.70	50.25
10.	T_{10}	47.00	47.80	47.40	51.40	52.51	51.96
11.	T_{11}	48.40	49.50	48.95	56.00	57.11	56.56
12.	T_{12}	47.30	48.23	47.77	52.60	53.70	53.15
13.	T_{13}	47.80	48.75	48.28	53.40	54.51	53.96
14.	T_{14}	51.00	51.00	51.38	64.00	65.24	64.62
15.	T_{15}	49.40	49.40	49.76	61.00	62.32	61.66
SE (diff)		**0.45**	**0.41**	**0.31**	**0.62**	**0.46**	**0.44**
CD at 5.0%		**0.93**	**0.85**	**0.64**	**1.27**	**0.95**	**0.91**

$FeSO_4$ (0.5%) foliar spray (Individual spray) at pre flowering and pod formation stages + *Rhizobium*) based on a pooled analysis of 2021-2022 and 2022-2023 (27.85, and 31.86, respectively) and significant with treatment T_{15}, which was found to be at par, similar trends data from both years were pooled. Zinc is crucial for the synthesis of tryptophan, resulting in the synthesis of IAA, which leads to the emergence of nodules (Guilfoyle and Hagen, 2007). For nodule formation, iron is required in greater amounts and it is an essential component of leghaemoglobin that converts atmospheric nitrogen into ammonia using nitrogenase. As a result of inoculating Rhizobium with root zones, nodule formation is accelerated and atmospheric nitrogen fixation is increased. It will ultimately enhance the plant's ability to absorb nutrients (Dixon and Kahn, 2004). The Rhizobium produces signalling molecules called Nodule-initiating factors (Ferguson). The results are consistent with those reported in Prasad *et al.* (2023), Pandit *et al.* (2022), Yadav *et al.* (2022), Singh *et al.* (2013).

Dry weight of nodules plant^{-1}

The growth stages of the crop are depicted in Table 4 regarding dry weight of nodules plant^{-1}. As shown in Table-1, the dry weight of nodules plant^{-1} at 60 DAS, 90 DAS and Harvest is presented for 60 DAS and 90 DAS& harvest was recorded significantly difference within the treatments over control. Nodules plant^{-1} are weighed at their maximum dry weight, 60 DAS & 90 DAS in treatment T_{14} (RDF + $ZnSO_4$ + $FeSO_4$ (0.5%) foliar spray (Individual spray) at pre flowering and pod formation Stage + *Rhizobium*) on the basis of pooled analysis of year 2021-22 and 2022-23 *i.e.* 51.38 and 64.62, respectively) and significant with treatment T_{15}, which found to be at par, similar trends were noticed data from both years and pooled data of both years.

During nucleic acid assimilation and nitrogen fixation, iron is crucial for the function of nitrogenase enzyme (Kumawat *et al.*, 2006). As a result, a higher increase in dry weight of nodules was seen in treatments with iron to skin compared to those where it was not applied. Furthermore, zinc is also responsible for the morphogenesis of root nodules as well as its involvement in the active function of the apical meristem of a root nodule (Franssen *et al.*, 2015). Researchers reported similar results by Rawat *et al.* (2023), Katiyar *et al.* (2020).

Conclusion

The results summarized above indicate that treatment T_{14} (RDF + $ZnSO_4$ + $FeSO_4$ (0.5%) foliar spray (individual spray) at pre-flowering and pod formation stages + *Rhizobium*) produced the best growth results. During different stages growth of the plant, no. of branches, plant height, nodule weight and nodule number were assessed are recorded. We can therefore conclude that T_{14} was the best treatment, followed by T_{15}, which is statistically at par.

Conflict of interest

All authors have explicitly declared the absence of any conflicting interests.

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