



Studies of Nodules and Productivity with Phosphate and Zinc Solubilizing Microbes in Lentil

Monica Yaying ^a, Anil Shukla ^a, Supriya ^{b*}, Shobhana Singh ^a,
Lachha Choudhary ^c and Manish Tomar ^d

^a G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand-263 153, India.

^b ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India.

^c Swami Keshwanand Rajasthan Agriculture University, Bikaner, Rajasthan-334006, India.

^d Chaudhary Charan Singh Haryana Agriculture University, Hisar, (HR)-125004, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at GB Pant University of Agriculture and Technology, Pantnagar during Rabi 2020 on Phosphorus and Zinc efficacy with phosphate and zinc solubilizing microbes in lentil (*Lens culinaris*) in tarai region. Twelve different doses of phosphorus and zinc with phosphate and zinc solubilizing microbes viz. T₁: Absolute Control (without P and Zn), T₂: RDP @ 48 kg ha⁻¹, T₃: Soil application of ZnSO₄ @ 25 kg ha⁻¹, T₄: Biophos @ 5ml kg⁻¹ seed, T₅: Biozinc (5 ml kg⁻¹ seed), T₆: Biophos and Biozinc (5 ml kg⁻¹ seed), T₇: 50% RDP @ 24 kg + Biophos (5 ml kg⁻¹ seed),

*Corresponding author: Email: supriya.ndri5@gmail.com;

T₈: 12.5 kg ZnSO₄ + Biozinc (5 ml kg⁻¹ seed), T₉: 50% RDP @ 24 kg + Biophos + Biozinc (5 ml kg⁻¹ seed), T₁₀: 50% recommended phosphorus dose application @ 24 kg ha⁻¹ + Biophos @ 5 ml seed⁻¹ + Biozinc @ 5 ml kg⁻¹ seed, T₁₁: Recommended phosphorus dose application @ 48 kg ha⁻¹ + nutrient mobilizer (LNm 43a) @ 20 g kg⁻¹ seed and T₁₂: 50% recommended phosphorus dose application @ 24 kg ha⁻¹ + Soil application of 12.5 kg ZnSO₄ ha⁻¹ + nutrient mobilize @ 20g kg⁻¹ seed were tested in randomized block design taking three replications. Results showed that the number and dry weight of nodules and grain yield found highest in treatment 50% RDP @ 24 +12.5 kg ZnSO₄ + Biophos + Biozinc (5 ml seed⁻¹ each) whereas treatment 12.5 kg ZnSO₄ + Biozinc (5 ml seed⁻¹) showed highest harvest index compared to control.

Keywords: Biophos; biozinc; nitrogen fixation; nodules; PSB; rhizobium.

1. INTRODUCTION

Lentil (*Lens culinaris*) belongs to the family Leguminosae/ Fabaceae. About 5% of the total area under pulses is lentil of which Asia accounts for 65% in terms of production. According to FAO, Canada and India are the first and second largest producers of lentil, respectively. In India, Uttar Pradesh is the leading producer of lentil with Uttar Pradesh and Madhya Pradesh producing about 70 % of the total lentil in the country. West Bengal has the highest productivity. In India, lentil is a significant pulse crop, grown on approximately 1.42 million hectares, yielding around 1.28 million tonnes. The lentil crop accounts for about 4.9% of the total pulse cultivation area and contributes 5.03% to the country's overall pulse production (2021–22) [1]. Lentils utilize Rhizobium bacteria to fix atmospheric nitrogen, reducing the need for nitrogenous fertilizers to approximately 25-30 kg N per hectare [2]. Phosphorus and zinc are essential elements for successful plant growth, particularly in pulse production [3]. Phosphorus plays a crucial role in root development, nodules growth, plant strength, flowering, nitrogen fixation, and overall crop health. It also mitigates the effects of drought and enhances yield under water stress. Proper fertilization of crop with phosphorus is essential for improving the yield and soil quality, although excess application can lead to fixation and unavailability in acidic or alkaline soils. Introducing phosphorus-solubilizing microbes (PSB) into the rhizosphere solubilized the P in soil through the release of organic acids and enzymes, resulting enhances phosphorus availability to plants and potentially increasing crop yields by 10-30%. Zinc is a vital micronutrient that activates enzymes, affects metabolic processes, and plays a significant role in DNA transcription. Its deficiency is common in pulse-growing regions, impacting both yield and produce quality. Zinc-solubilizing microorganisms use various mechanisms, such

as acidification and chelation, to make zinc more available in the soil. Inoculating plants with beneficial bacteria like *Pseudomonas*, *Rhizobium strains*, *Bacillus* and *Azospirillum* has shown improvements in growth, phosphorus and zinc content [4]. Overall, these micronutrients and microbial interventions are crucial for optimizing crop health and yield in pulse production. The information available on the effect of phosphorus and zinc solubilizing microbes in the *tarai* region of Uttarakhand is sparse and inadequate. With respect to this objective, the present study entitled "Studies on nodules and productivity with phosphate and zinc solubilizing microbes in lentil was carried out.

2. MATERIALS AND METHODS

2.1 Experimental Site

The field experiment was carried out during the *Rabi* season of 2020 in Pulse Agronomy Block (D -6) located at N.E. Borlaug Crop Research Centre of G. B. Pant University of Agriculture and Technology. The research center lies, 30 km southern end of foot hills of Shivalik range of Himalayas at 29°N latitude and 79.3°E longitude and at an altitude of 243.83 m above the mean sea level in the *tarai* region of Uttarakhand.

2.2 Experimental Details

The experiment was conducted with twelve treatments and three replications. Lentil variety Pant L8 with RR spacing of 23cm. Experimental area was 812.16 m² with gross plot size of 13.8m² (2.76 m x 5 m). The details of the treatments have been given in Table 1. The nutrients such as N and K were applied equally in all treatments. But the P, Zn and their solubilizing organisms were varied in different treatments.

Table 1. Details of the treatments

S. No.	Treatment details
T ₁	Absolute Control (without P and Zn)
T ₂	Recommended phosphorus dose application (Control) @ 48 kg ha ⁻¹
T ₃	Soil application of ZnSO ₄ @ 25 kg ha ⁻¹
T ₄	Application of Biophos @ 5ml kg ⁻¹ seed
T ₅	Application of Biozinc @ 5ml kg ⁻¹ seed
T ₆	Application of Biophos and Biozinc @ 5 ml each kg ⁻¹ seed
T ₇	50% recommended phosphorus dose application @ 24 kg ha ⁻¹ + Biophos@ 5 ml kg ⁻¹ seed
T ₈	12.5 kg ZnSO ₄ ha ⁻¹ +Biozinc @ 5 ml kg ⁻¹ seed
T ₉	50% recommended phosphorus dose application @ 24 kg ha ⁻¹ + Biophos @ 5mlkg ⁻¹ seed + Biozinc @ 5 ml kg ⁻¹ seed
T ₁₀	50% recommended phosphorus dose application @ 24 kg ha ⁻¹ + 12.5 kg ZnSO ₄ + Biophos@ 5 ml seed ⁻¹ + Biozinc @ 5 ml kg ⁻¹ seed
T ₁₁	Recommended phosphorus dose application @ 48 kg ha ⁻¹ + nutrient mobilizer (LNm 43a) @ 20 g kg ⁻¹ seed
T ₁₂	50% recommended phosphorus dose application @ 24 kg ha ⁻¹ + Soil application of 12.5 kg ZnSO ₄ ha ⁻¹ + nutrient mobilize @ 20g kg ⁻¹ seed

The experimental field was ploughed once with disc plough drawn by a tractor which was followed by two cross harrowing. Urea and MOP were uniformly applied as basal at the rate of 20 and 40 kg respectively, while phosphorus, zinc and microbial applications were varied. The crop was harvested manually with the help of sickle when more than 80 % of the pods turned brown. The other cultural practices were followed with recommended package and practices of lentil.

2.3 Number and Dry Weight of Nodules per Plant

Nodules collected from the five roots from of plants were dried in the oven at 70°C till the constant weight. It was measured from the electronic balance and the value was averaged for each plant. Number of nodules were separated counted for the roots of five plants and then averaged.

2.4 Yield Parameters

After threshing, the grain yield from each net plot was weighed and then multiplied with suitable conversion factor to get yield (kg ha⁻¹). Straw yield was calculated by deducting the grain yield from the biological yield. Total produce of each net plot (excluding the root biomass) was dried in the sun in the field after harvest and weighed. Yield was computed by multiplying this with suitable conversion factor.

$$\text{Harvest index} = (\text{grain yield} / \text{biological yield}) \times 100$$

Grain: Straw ratio was calculated by dividing the grain yield by straw yield.

2.5 Statistical Analysis of Data

The experimental data were analyzed using OPSTAT for Randomized Block Design which is programmed by HAU, Hisar, Haryana.

3. RESULTS AND DISCUSSION

3.1 Number of Nodules per Plant

The varying treatments of phosphorus and zinc significantly affected the number of nodules at all the stages as shown in Table 2. The number of nodules increased from 30 to 60 days but the number of nodules decreased for some treatments from 60 to 90 days stage. At 30, 60 and 90 days stages, the number of nodules was significantly more in the case of the treatment receiving 50% recommended phosphorus dose @24 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀) as compared to all other treatments, while at 90 days stage, the treatments having recommended phosphorus dose @ 48 kg per hectare + nutrient mobilizer (LNm 43a) @ 20 g per kg seed (T₁₁) and 50% recommended phosphorus dose @ 24 kg per hectare + Soil of 12.5 kg ZnSO₄ per ha + nutrient mobilizer @ 20g per kg seed (T₁₂) did not differ statistically.

Table 2. Number of nodules per plant as influenced by phosphorous and zinc treatments

Treatment	Number of nodules (no. plant ⁻¹)			
	30DAS	60DAS	90DAS	
T ₁	Absolute Control (without P and Zn)	3.2	7.4	6.6
T ₂	RDP @ 48 kg per ha	5.7	12.8	14.5
T ₃	ZnSO ₄ @ 25 kg per hectare	4.8	11.2	10.0
T ₄	Biophos (5 ml per kg seed)	4.1	10.7	9.6
T ₅	Biozinc (5 ml per kg seed)	3.6	9.8	6.7
T ₆	Biophos and Biozinc (5 ml per kg seed each)	5.6	12.1	11.4
T ₇	50% RDP @ 24 kg + Biophos (5 ml per kg seed)	6.0	13.4	15.4
T ₈	12.5 kg ZnSO ₄ + Biozinc (5 ml per kg seed)	4.2	10.2	7.8
T ₉	50% RDP@ 24 kg + Biophos + Biozinc (5 ml per kg seed each)	7.0	13.7	15.5
T ₁₀	50% RDP @24 kg +12.5 kg ZnSO ₄ + Biophos + Biozinc (5 ml per kg seed each)	11.0	27.6	22.0
T ₁₁	RDP@ 48 kg + nutrient mobilizer (LNm 43a) (20 g per kg seed)	7.3	14.4	19.0
T ₁₂	50% RDP @ 24 kg + 12.5 kg ZnSO ₄ + nutrient mobilizer (20 g per kg seed)	7.3	17.9	20.1
	S.Em ±	0.4	1.2	1.3
	C.D. at 5%	1.1	3.6	3.7

Table 3. Dry weight of nodules per plant as influenced by phosphorous and zinc treatments

Treatment	Dry weight of nodules (mg plant ⁻¹)		
	30DAS	60DAS	90DAS
T ₁ Absolute Control (without P and Zn)	39.1	41.4	39.1
T ₂ RDP @ 48 kg per ha	54.6	61.3	62.7
T ₃ ZnSO ₄ @ 25 kg per hectare	48.4	57.6	57.9
T ₄ Biophos (5 ml per kg seed)	46.6	53.1	52.4
T ₅ Biozinc (5 ml per kg seed)	42.9	51.0	46.1
T ₆ Biophos and Biozinc (5 ml per kg seed each)	52.0	58.7	62.4
T ₇ 50% RDP @ 24 kg + Biophos (5 ml per kg seed)	60.4	72.0	71.3
T ₈ 12.5 kg ZnSO ₄ + Biozinc (5 ml per kg seed)	43.7	51.5	51.7
T ₉ 50% RDP @ 24 kg + Biophos + Biozinc (5 ml per kg seed each)	68.4	73.5	72.2
T ₁₀ 50% RDP @24 kg +12.5 kg ZnSO ₄ + Biophos + Biozinc(5 ml per kg seed each)	78.4	95.6	93.9
T ₁₁ RDP@ 48 kg + nutrient mobilizer (LNm 43a) (20 g per kg seed)	71.9	75.5	72.8
T ₁₂ 50% RDP @ 24 kg + 12.5 kg ZnSO ₄ + nutrient mobilizer (20 g per kg seed)	72.0	82.0	80.0
S.Em ±	3.5	4.6	1.3
C.D. at 5%	10.3	13.5	3.7

Table 4. Effect of different treatments of phosphorus and zinc on grain, straw, biological yield, grain straw ratio and harvest index

Treatment	Yield (kg ha ⁻¹)					
	Grain	Straw	Biological	Grain straw ratio	Harvest index	
T ₁	Absolute Control (without P and Zn)	1318	2591	3909	0.52	0.34
T ₂	RDP @ 48 kg per ha	1418	2894	4271	0.49	0.35
T ₃	ZnSO ₄ @ 25 kg per hectare	1381	2880	4198	0.52	0.33
T ₄	Biophos (5 ml per kg seed)	1363	2731	4179	0.49	0.34
T ₅	Biozinc (5 ml per kg seed)	1322	2668	4049	0.49	0.35
T ₆	Biophos and Biozinc (5 ml per kg seed each)	1363	2816	4216	0.48	0.33
T ₇	50% RDP @ 24 kg + Biophos (5 ml per kg seed)	1522	2890	4298	0.53	0.31
T ₈	12.5 kg ZnSO ₄ + Biozinc (5 ml per kg seed)	1341	2708	4094	0.49	0.37
T ₉	50% RDP @ 24 kg + Biophos + Biozinc (5 ml per kg seed each)	1531	2930	4475	0.53	0.33
T ₁₀	50% RDP @ 24 kg + 12.5 kg ZnSO ₄ + Biophos + Biozinc (5 ml per kg seed each)	1735	3424	5063	0.53	0.35
T ₁₁	RDP @ 48 kg + nutrient mobilizer (LNm 43a) (20 g per kg seed)	1544	2980	4502	0.52	0.32
T ₁₂	50% RDP @ 24 kg + 12.5 kg ZnSO ₄ + nutrient mobilizer (20 g per kg seed)	1563	3333	4544	0.47	0.33
	S.Em ±	65	177	130	0.03	0.01
	C.D. at 5%	191	NS	383	NS	NS

Phosphorus is essential for nitrogen fixation. About 16 molecules of ATP are required to fix each molecule of atmospheric nitrogen to ammonia. Thus, nodule formations are influenced by phosphorus. Inadequate phosphorus can, therefore, restrict the root growth, photosynthesis, sugar translocation, and other such functions. Lakhmanrao [5] found that there was a positive relationship between leghemoglobin content and N-fixation. Nodules are known to be good sinks for Phosphorus [6] and N₂ fixation is also largely influenced by phosphorus availability. Inadequate supply of Phosphorus to pulses may cause nitrogen deficiency by disturbing the symbiotic nitrogen fixation (SNF). Bolanos [7] studied the effect of micronutrient on interaction between microbes and pea and found that the Rhizobia infecting the host and number of infection thread were increased, thereby increasing the number of nodules. Different doses of phosphorus and zinc application supplied the nutrients easily and rapidly to plants which increased the nodule number (Table 2) and its dry matter. Zinc and phosphorus also help in spreading of root system, gives more sites for *rhizobia* infection and increases their proliferation in rhizosphere, thus helps in forming more effective number of nodules and their dry weight. Wani [8] also reported similar findings in chickpea crop quoting that the zinc and phosphorus mobilizer *rhizobacteria* increased the number of nodules by 15% as compared to the uninoculated plants.

3.2 Dry Weight of Root Nodules

The data followed the trend of the number of the root nodules per plant as shown in Table 3. The dry weight of nodules increased greatly between 30 to 60 days. The rate of increase declined thereafter. Nodule weight was significantly influenced due to different phosphorus and zinc treatments at all the stages. 50% recommended phosphorus dose @48 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀) showed highest weight of nodules per plant at 30, 60 and 90 DAS. However, T₉, T₁₁ and T₁₂ did not differ significantly at the earlier stage, i.e., 30 days. Different doses of phosphorus and zinc supplied to plant increased the nodule number and its dry matter. Zinc and phosphorus also helped in spreading of the root system, giving more sites for *Rhizobia* infection and increases their proliferation in rhizosphere, thus helping in forming more effective number of nodules and increasing their dry weight. The phosphorus

reduces the time required for the developing nodules to become active nitrogen fixers. It also increases the size of the nodules along with the amount of assimilates per unit weight of nodules. It also increases the density of Rhizobia surrounding the root. Jindal [9] in their experiment found that increasing doses of phosphorus increased the nodules and their dry weight per plant.

Wani [8] also reported that zinc and phosphorus mobilizer *rhizobacteria* increased the nodule dry mass by 27% when compared with the uninoculated plants in the crop of lentil.

3.3 Yield Studies

Grain yield: Grain yield per hectare differed significantly due to varying treatments of phosphorus and zinc shown in Table 4. The results figured in Table 4 reveal that the treatment getting 50% recommended phosphorus dose @24 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀) produced the maximum grain yield being significantly superior over all the other treatments.

The lowest grain yield was harvested in case of the absolute control where no phosphorus was applied which was on a par with the rest of the treatments excepting the treatment getting 50% recommended phosphorus dose @24 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀). The balanced nutrient supply and timely availability of the nutrients owing to nutrient solubilizing microbes enhanced the plant growth and consequently, increased the grain yield. Singh [10] obtained similar results supporting that increased phosphorus level enhanced the grain yield. Combined and optimum dose of P and Zn application had synergistic effect on each other and observed significant increase in grain yield. Hussain [11] reported similar results on effect of phosphorus and zinc on the grain yield.

Straw yield: Straw yield per hectare was not influenced significantly due to varying treatments of phosphorus and zinc shown in Table 4. Yet the highest yield was observed in case of 50% recommended phosphorus dose @24 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀), while the lowest yield was obtained from the absolute control (T₁) having no phosphorus and zinc. Phosphorus aids in cell division and help in

the development of new tissue. It also plays a vital role in energy transformation in plants. Thus, adding phosphorus to soil promotes root growth and winter hardiness. Enhanced plant growth due to proper availability of nutrients increased the straw yield too. The zinc helped the plant in chlorophyll production and directly contributed in plant growth. Meena [12] observed that the application of phosphorus with zinc resulted in higher grain and straw yield in soybean.

Biological yield: The total biological yield was influenced significantly due to varying treatments of phosphorus and zinc shown in Table 4. The highest biological yield was obtained from the treatment containing 50% recommended phosphorus dose @24 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀) and was statistically superior to all the other treatments. The lowest biological yield was obtained in case of the absolute control (T₁) treatment where no phosphorus and zinc were applied. Application of phosphorus and zinc to plants increased grain and straw yield and thus, the biological yield. The applied nutrients improved the various parameters of growth and development like plant height, number of branches per plant, number of compound leaves per plant, dry matter accumulation per plant, etc., the cumulative effect of which ultimately resulted in higher yields of both straw and biological parts. Singh [13] found that the biological yield increased significantly up to 50 kg P₂O₅ /ha.

Grain straw ratio: The variations in grain to straw ratio owing to different treatments was found to be non-significant as shown in Table 4. The highest ratio was found in treatment having 50% recommended phosphorus dose @48 kg per hectare +12.5 kg ZnSO₄ + Biophos@ 5 ml per kg seed + Biozinc @ 5 ml per kg seed (T₁₀), 50% recommended phosphorus dose application @ 24 kg per hectare+ Biophos @ 5ml per kg seed + Biozinc @ 5 ml per kg seed (T₉) and 50% recommended phosphorus dose application @ 24 kg per hectare + Biophos@ 5 ml per kg seed (T₇) while the lowest ratio was observed in absolute control (T₁) with no application of phosphorus and zinc.

Harvest index: Harvest index varied non significantly among the different treatment of phosphorus and zinc application as shown in Table 4. The highest harvest index was obtained from the treatment containing 12.5 kg ZnSO₄ per hectare +Biozinc @ 5 ml per kg seed (T₈) while

the lowest harvest index was found in the treatment having 50% recommended phosphorus dose @ 24 kg per hectare + Biophos@ 5 ml per kg seed (T₇). The better crop growth due to proper supply of phosphorus and zinc might have regulated starch/sucrose ratio in reproductive organs and leaves. The fruiting of plants was benefited from phosphorus and the desired metabolites were better translocated to the yield attributing parts of the plant, which might have attributed to more grain yield. Also, the increased straw yield might be because phosphorus increased growth and development such as of plant height, number of branches and plant dry matter by improving the rhizosphere environment and plant system that led to higher metabolism and photosynthetic activity in plant. Large variations in harvest index are not influenced by any of the given treatments and this might be due to character, highly associated with genetic makeup of the crop [14]. Each increment of phosphorus from (25 to 75 kg ha⁻¹) gave superior HI value of lentil [15].

4. CONCLUSION

From the above investigations, it can be inferred that application of treatment T₁₀ i.e., 50% RDP @24 +12.5 kg ZnSO₄ + Biophos + Biozinc (5 ml kg⁻¹ seed each) proved effective in significantly enhancing the number of nodules and dry matter of nodules as well as grains of lentil crop in *tarai* region of Uttarakhand. The research demonstrates the significant impact of phosphate and zinc solubilizing microbes on growth and yield of lentil. The synergistic interactions between these beneficial microbes and phosphorus and zinc fertilizers not only enhances nutrient availability but also promotes sustainable agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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