



# Impact of Different Fertility Levels and Liquid Biofertilizers on Biological Properties and Nutrient Content of Soil under Wheat (*Triticum aestivum* L.) Crop

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## 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 during Rabi 2019 at the Instructional Farm of Agronomy, Rajasthan College of Agriculture, Udaipur. The treatments comprised of four levels of fertility i.e. control, 75, 100 and 125% RDF and four levels of liquid biofertilizers i.e. control, Azotobacter, PSB

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and Azotobacter + PSB. The experiment was laid out in factorial randomized block design replicated thrice taking wheat var. Raj.-4238 as test crop. The Recommended dose of fertilizer (RDF) was 100:60:40 kg ha<sup>-1</sup> of N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O. The results of the study demonstrated a significant increase in several important soil parameters in the post-harvest wheat fields. This increase was observed as we applied higher levels of fertilizers and liquid biofertilizers, specifically up to the point where we used 100% of the recommended dose of fertilizers (RDF) and a combination of Azotobacter and Phosphate Solubilizing Bacteria (PSB). The application of increased fertility levels and the use of Azotobacter + PSB as liquid biofertilizers led to significant improvements in soil microbial biomass, microbial populations and enzyme activities, enhancing the overall health and fertility of the post-harvest wheat soil. Additionally, significant increases in sulphur and micronutrients (Zn, Cu Fe and Mn) in soil with the application of 100% RDF.

**Keywords:** RDF; biofertilizers; fertility levels; Azotobacter; PSB.

## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a global staple food crop, covering approximately 17% of the total crop acreage. It contributes significantly, providing about 50% of the calories in the human diet in our country. The chemical composition of wheat grain consists of approximately 66-71.6% carbohydrates, 2.5-3.1% fats, 13-16.7% proteins and 2.5-3% crude fiber [1,2,3]. Among the various wheat species, three *Triticum* species are predominantly cultivated worldwide. Globally, wheat (*Triticum* spp.) is grown over a vast area of 220 million hectares, holding the highest acreage position compared to all other crops, with an annual production of approximately 781 million tonnes. "In India, it covers an area of 29.55 million hectares (about 13.43% of the global area) and yields around 101.20 million tonnes (approximately 12.96% of the world's production) with a productivity of 3424 kg ha<sup>-1</sup>" [4]. "In Rajasthan, wheat is cultivated on an area of 2.88 million hectares, producing 9.60 million tonnes of grain with a productivity of 3334 kg ha<sup>-1</sup>" [4].

Nitrogen is of utmost importance in facilitating various biochemical and physiological functions within plants. It plays a crucial role in enhancing processes like photosynthesis, leading to a rich, dark-green color in plants and promoting the growth and development of stems and other vegetative parts [5]. Nitrogen contributes significantly to crop growth and yield improvement [6]. Phosphorus is a vital nutrient element for plants, serving as an integral component of the plant system. "It is indispensable for cell enlargement, cell division, energy storage, and transfer. Phosphorus is also a constituent of energy-rich compounds such as ATP and ADP, NADP, phytin, nucleic acids, and

phospholipids" [7]. "Potassium acts as an activator for enzymes that play key roles in plant growth. It is essential for various physiological processes, including stomatal activity, sugar, water, and nutrient transport, as well as the synthesis of proteins and starch" [8].

"Conventional agriculture has played a significant role in meeting the food demands of a growing human population. However, heavy reliance on pesticides and chemical fertilizers, particularly urea, has resulted in soil degradation and pollution. The excessive use of phosphorus and nitrogen fertilizers has led to water bodies' eutrophication, causing air and groundwater pollution" [9]. "To address these issues, harmless alternatives like biofertilizers have been introduced. Biofertilizers play a crucial role in maintaining long-term soil fertility and sustainability. They fix atmospheric nitrogen, convert insoluble phosphorus into an available form, and mobilize essential macro and micronutrients for plants, thereby enhancing their efficiency and availability" [10]. "Moreover, biofertilizers enrich the soil environment with various macro and micronutrients through nitrogen fixation, phosphate and potassium solubilization, production of antibiotics, and degradation of organic matter" [11]. "These eco-friendly and cost-effective inputs can reduce the reliance on chemical fertilizers by 25-50%" [12]. "Liquid biofertilizers are specially formulated with viable microorganisms protected by certain cell-protecting chemicals. These chemicals enhance microbial cell survival during storage and after seed application, even under adverse soil conditions such as desiccation and high temperature. Liquid biofertilizers have a high microbial population, with up to 10<sup>9</sup> cells per ml for 12 to 24 months, and their application dosage is significantly lower than carrier-based biofertilizers" [13].

“Soil microbial communities play a crucial role in enhancing biological soil fertility and managing productivity. These communities are carefully harvested and processed to capture their beneficial effects on soil, thereby improving the soil-microbial relationship. Soil microorganisms are involved in various essential functions such as nitrogen fixation, hormonal regulation, production of siderophores and phytohormones, resistance to phytopathogens, nutrient availability, promotion of mycorrhizal functioning, and reducing pollutant toxicity” [14]. “Dehydrogenase, an enzyme present in all living microorganisms, plays a vital role in the oxidation of organic matter, contributing to soil health and nutrient cycling” [15]. “In soils with limited phosphorus, the majority of phosphorus is organically bound. Phosphatase activity becomes a significant factor in maintaining and managing the rate of phosphorus cycling through the soil” [16].

## 2. MATERIALS AND METHODS

### 2.1 Site, Soil and Climatic Conditions

The experiment was carried out at the Agronomy Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur. The site was situated at 24°35' N latitude, 73°42' E longitude and an altitude of 582.17 meters above sea level in the south-eastern part of Rajasthan. The region falls under Rajasthan's agro-climatic zone IVA (Sub-Humid Southern Plain and Aravalli Hills).

The composite soil sample was collected randomly before sowing of crop from the experimental field up to 15 cm depth. The composite sample was air dried under shade and passed through 2 mm sieve and then use for analysis. The soil of this area was clay loam (38.47%, silt 26.46% and clay 34.57 %). The soil having 8.25, electrical conductivity 0.82 dSm<sup>-1</sup>, soil organic carbon 0.56% and available nitrogen 253.80 kg ha<sup>-1</sup>, phosphorus 20.09 kg ha<sup>-1</sup>, potassium 380.03 kg ha<sup>-1</sup>. The soil microbial biomass carbon 159 mg kg<sup>-1</sup>, soil microbial biomass nitrogen 23.76 dehydrogenase activity 8.70 µg TPF g<sup>-1</sup> 24h<sup>-1</sup> soil and alkaline phosphatase activity 41.52 µg PNP g<sup>-1</sup> h<sup>-1</sup> soil. <sup>-1</sup> The microbial population was determined by serial dilution [17].

During cropping period of wheat, the corresponding mean weekly temperature fluctuations were observed during *Rabi* season in

year 2019, maximum and minimum temperature ranged between 37.3°C and 20.8°C, respectively. Mean weekly maximum and minimum relative humidity ranged between 86.7 and 16.7 per cent, respectively. Total rainfall and maximum evaporation were 42.6 and 9.9 mm was recorded during crop season.

The soil analysis confirmed that soil of experimental field was clay loam belongs to *Typic Haplustepts*, neutral alkaline in reaction, medium in available nitrogen and phosphorus and high in available potassium. Soil was low in available zinc and iron.

### 2.2 Experimental Design

The experiment consisted of 16 treatment combinations comprising of four levels of fertility (Control, 75, 100 and 125 % RDF) and four levels of liquid biofertilizers (Control, *Azotobacter*, PSB and *Azotobacter* + PSB). Experiment was laid out in factorial randomized block design replicated thrice.

### 2.3 Application of Fertilizers

In wheat crop as per treatment required dose of nitrogen by subtracting the amount of N supplied through DAP and remaining by urea, P<sub>2</sub>O<sub>5</sub> through DAP and K<sub>2</sub>O through MOP was applied to the crop. At the time of sowing, half dose of nitrogen, full dose of phosphorus, potassium and half dose of nitrogen was applied before the sowing and remaining half dose of nitrogen was applied in two equal splits during 1<sup>st</sup> and 3<sup>rd</sup> irrigation.

### 2.4 Seed Treatment with Liquid Biofertilizers

The seed treatment with liquid biofertilizers was done using a plastic bag. The bag filled with 1 kg of seeds and the required amount of biofertilizers (@ 5 to 10 ml kg<sup>-1</sup> seed of each biofertilizers) was added. Then bag was closed and squeezed until all the seeds were evenly wetted. The bag was opened and seeds dried for 20 to 30 minutes in the shade. There are plots in which seeds are treated with *Azotobacter* and PSB alone and some plots that are treated with both.

### 2.5 Soil Biological and Chemical Properties

The estimation of the microbial population was done by standard serial dilution and plate count method [18]. The chloroform-fumigated

incubation method was used for microbial biomass carbon analysis [19] and the chloroform fumigation method was used for microbial biomass nitrogen [20]. Soil acid and alkaline phosphatase activities were examined by  $\beta$  nitrophenol phosphate by spectrophotometry method [21]. An Anthrone extraction method was used for the analysis of soil dehydrogenase activity [22]. Micronutrients (Zn, Cu Fe and Mn) were determined using the standard method given by Lindsay and Norvell [23].

## 2.6 Statistical Analysis

The obtained data were statistically analysed with the techniques of analysis of variance as described by Steel and Torrie, [24]. The comparison in the treatment mean was tested by critical difference (CD) at 5% ( $P=0.05$ ) level of significance.

## 3. RESULTS AND DISCUSSION

### 3.1 Soil Biological Properties

#### 3.1.1 Effect of fertility levels

The dehydrogenase enzyme activity, alkaline phosphate activity, microbial population, soil biomass carbon and soil biomass nitrogen (Table 1) significantly affected by application of fertility levels. The dehydrogenase enzyme activity, alkaline phosphate activity, microbial population, soil biomass carbon and soil biomass nitrogen significantly increased to each higher level of fertility up to 100% RDF but it remained at par with 125% RDF. Bhatt et al. [25] reported that the native pools of soil organic carbon act as a substrate for enzymes which is utilized by microorganisms and their activity increases by adding inorganic nutrient source. Inorganic fertilizers have a catalytic effect in stimulating microbial growth, leading to higher microbial biomass C and N. This increase in microbial growth also contributes to elevated root biomass and root exudates, consequently providing additional carbon and energy to soil microbes, which can be attributed to the rise in dehydrogenase enzyme activity (Geetha Kumari and Shivashankar, 1991). These findings are also reported by Chand et al. [26], Parewa et al. [27], Bhatt et al. [28] and Sial et al. [29].

#### 3.1.2 Effect of liquid biofertilizers

The dehydrogenase enzyme activity, alkaline phosphate activity, microbial population, soil

biomass carbon and soil biomass nitrogen (Table 1) were significantly affected by inoculation of seed with different liquid biofertilizers. Soil microbial biomass carbon and nitrogen significantly increased might be due to microbial population increased by secretion of mucigel, sloughed off cells, exudates, roots remaining of previous crop, etc. The inoculation of biofertilizers increases the biological activities that might have enhanced the soil microbial biomass carbon and nitrogen [30]. Shinde and Bangar [31] reported that an increase in the enzyme activity and microbial population in soil might be due to enhancement in porosity and availability of nutrients especially P to the plant along with better installation of inoculated microorganism, which stimulates the indigenous microorganisms. Microbial inoculation might have increased the population of beneficial microorganisms in soil. A similar finding was also given by Nath et al. [32], Khandare et al. [33] and Fitriatin et al. [34].

### 3.2 Soil Chemical Properties

#### 3.2.1 Effect of fertility levels

The available sulphur, zinc, iron, manganese and copper content in the soil after the harvest of wheat (Table 2) was significantly affected by the increasing level of fertility. The highest available sulphur ( $10.18 \text{ mg ha}^{-1}$ ), zinc ( $0.74 \text{ mg kg}^{-1}$ ), iron ( $5.58 \text{ mg kg}^{-1}$ ), manganese ( $9.82 \text{ mg kg}^{-1}$ ) and copper ( $2.11 \text{ mg kg}^{-1}$ ) the content in soil was recorded with 125% RDF. However, 125% RDF remained at par with 100% RDF. The data further revealed that the percent increase in available S, Zn, Fe, Mn and Cu status of soil were in order of 11.74, 9.80, 28.48, 10.21 and 2.67 due to the application of 125% RDF in comparison to control, respectively. The increase in available S, Zn, Fe, Mn, and Cu in the soil might be attributed to the application of inorganic fertilizers, as it improves the availability of these nutrients in the soil. The increase might be due to the increased enzymatic activity, microbial population and the organic recycling of plant nutrients which leads to greater mineralization of applied nutrients and thus increases the inherent S and micronutrient content in soil Ranjitha et al. [35]. Another reason could be the improvement in physio-chemical which helps in the retention of nutrient in soil and prevent nutrient losses. The application of inorganic fertilization improves the soil pH for better nutrient availability Similar results were also reported by Zhao et al. [36] Gourav et al. [37] and Mehta et al. [38].

Table 1. Effect of fertility levels and liquid biofertilizers on biological properties of soil after crop harvest

Treatments	Microbial population			Soil microbial biomass		Enzyme activities	
	Bacterial (10 <sup>7</sup> cfu g <sup>-1</sup> of soil)	Fungal (10 <sup>5</sup> cfu g <sup>-1</sup> of soil)	Actinomycetes (10 <sup>6</sup> cfu g <sup>-1</sup> of soil)	Carbon (mg kg <sup>-1</sup> )	Nitrogen (mg kg <sup>-1</sup> )	Dehydrogenase activity (µg TPF g <sup>-1</sup> 24 h <sup>-1</sup> )	Alkaline phosphate activity (µg PNP g <sup>-1</sup> ha <sup>-1</sup> )
<b>Fertility levels (RDF)</b>							
Control (F <sub>0</sub> )	63.64	23.39	34.88	165.82	25.14	9.95	15.86
75 % RDF (F <sub>1</sub> )	69.09	24.44	38.29	176.47	26.33	11.58	18.15
100 % RDF (F <sub>2</sub> )	73.62	25.42	40.66	185.70	27.88	13.53	20.21
125 % RDF (F <sub>3</sub> )	75.29	25.81	40.86	188.98	28.65	13.77	20.61
<b>SEm±</b>	1.08	0.29	0.37	1.61	0.31	0.23	0.22
<b>C.D. (P = 0.05)</b>	3.12	0.84	1.08	4.65	0.90	0.68	0.64
<b>Liquid biofertilizers</b>							
No inoculation (B <sub>0</sub> )	63.41	22.44	34.83	169.92	24.90	9.92	15.70
<i>Azotobacter</i> (B <sub>1</sub> )	71.67	25.08	39.28	179.58	27.37	12.60	18.83
PSB (B <sub>2</sub> )	70.68	24.68	38.96	178.00	26.54	12.41	18.69
Azo + PSB (B <sub>3</sub> )	75.88	27.33	41.61	189.48	29.18	13.91	21.61
<b>SEm±</b>	1.08	0.29	0.37	1.61	0.31	0.23	0.22
<b>C.D. (P = 0.05)</b>	3.12	0.84	1.08	4.65	0.90	0.68	0.64

Table 2: Effect of fertility levels and liquid biofertilizers on Sulphur and micronutrient availability in soil after crop harvest

Treatments	Sulphur (mg ha <sup>-1</sup> )	Zinc (mg ha <sup>-1</sup> )	Iron (mg ha <sup>-1</sup> )	Manganese (mg ha <sup>-1</sup> )	Copper (mg ha <sup>-1</sup> )
<b>Fertility levels (RDF)</b>					
Control (F <sub>0</sub> )	9.11	0.67	4.35	8.91	2.05
75 % RDF (F <sub>1</sub> )	9.84	0.70	5.04	9.35	2.08
100 % RDF (F <sub>2</sub> )	10.16	0.73	5.57	9.76	2.10
125 % RDF (F <sub>3</sub> )	10.18	0.73	5.58	9.82	2.11
<b>SEm±</b>	0.10	0.01	0.02	0.13	0.01
<b>C.D. (P = 0.05)</b>	0.30	0.02	0.06	0.38	0.02
<b>Liquid biofertilizers</b>					
No inoculation (B <sub>0</sub> )	9.49	0.68	5.02	9.16	2.06
<i>Azotobacter</i> (B <sub>1</sub> )	9.72	0.69	4.67	9.32	2.07
PSB (B <sub>2</sub> )	9.79	0.69	4.65	9.21	2.08
Azo + PSB (B <sub>3</sub> )	9.92	0.69	5.05	9.36	2.08
<b>SEm±</b>	0.10	0.01	0.02	0.13	0.01
<b>C.D. (P = 0.05)</b>	0.30	NS	NS	NS	NS

### 3.1.2 Effect of liquid biofertilizers

The inoculation of seeds with different liquid biofertilizers significantly increased the availability of sulphur in the soil. However, there was no significant impact on the concentration of micronutrients in the soil after the harvest of wheat (Table 2). The maximum available sulphur (9.49 mg ha<sup>-1</sup>) was recorded with inoculation of seed with *Azotobacter* + PSB. The data further revealed that the percent increase in available S status of soil was in the order of 4.53 due to inoculation of *Azotobacter* + PSB in comparison to control. The increase in available sulphur (S) in the soil might be attributed to the application of biofertilizers, as it improves the availability of sulphur in the soil by increasing microbial activity in the soil. Similar results were also found by Mir et al. [39], Yadav et al. [40] and Subbaiah [41].

## 4. CONCLUSION

In the Sub-humid Southern Plain and Aravalli Hills of Rajasthan (Zone IVa), the study found that using 100% RDF alongside *Azotobacter* and PSB in wheat cultivation significantly improved the soil microbial biomass carbon and nitrogen, microbial populations (bacteria, fungi and actinomycetes), enzyme activity (dehydrogenase and alkaline phosphatase), sulphur content in the soil. Additionally, the application of 100% RDF led to a notable increase in micronutrient content. This approach proves effective in enhancing soil health and nutrient availability for wheat crops in these specific conditions.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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