Unveiling the Synergistic impact of N & P levels alone and in conjunction with Bio-inoculants (*Azotobacter* and PSB) on growth and yield of Wheat

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ABSTRACT

The experiment conducted during the *Rabi* season of 2022-23 at Rajoula Agriculture Farm of Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya in Satna, Madhya Pradesh, investigated the growth characteristics and yield attributes of wheat under various treatments. The experiment, utilizing a randomized block design with 3 replications and 13 treatments, including different levels of nitrogen (N), phosphorus (P), and bio-inoculants (*Azotobacter* and PSB). The wheat variety RAJ-4238 was grown as test crop. Based on experimental findings result revealed significant effects on wheat growth and yield attributes. Notably, plant height (91.80 cm), tiller count/ plant (7.47), and spike number/plant (14.27) at different stages were significantly improved by specific treatments, with the treatment containing T_{10} (100% N & P + PSB + Azotobacter + 100% K) consistently producing the most favorable results. Moreover, yield attributes such as number of spikelets (599), grains per spike (550), test weight (53.87 gm), grain yield (38.33 q/ha) and stover yield (51.14 q/ha) were all significantly influenced by the treatments, with the same treatment combination T_{10} (100% N & P + PSB + Azotobacter + 100% K) yielding the highest grain output. However harvest index (44.37 %) was significantly influenced by the treatment T_{12} (50% N & P + PSB + *Azotobacter* + 100% K). These findings emphasize the potential for tailored nutrient and bio-inoculant management practices to enhance wheat production in the region.

Keywords: Azotobacter, Bio-inoculants, PSB, Production, Wheat, Yield

1. INTRODUCTION

Wheat (*Triticum aestivum L.)* is one of the world's most important leading cereal crop after rice (*Oryza sativa L*), as well as in India, it can be grown in broad range of altitudes and latitudes. It is one of the most important and extensively cultivated cereal crops in Indian sub-continent. India is the world's second largest wheat producer after China surpassing Russia and India share of around 13.53% of world total production [1].

According to Government of India, in year 2021-22 all the growing wheat provinces, Uttar Pradesh stand at first position in production with 33.95 million tonnes followed by Madhya Pradesh with 22.42 million tonnes of production, Punjab at 3rd position with 14.82 million tonnes of production, Haryana at 4th place with 10.45 million tonnes of production, Rajasthan at 5th position with 9.48 million tonnes, Bihar stand at 6th position with 6.22 million tonnes of production, Gujarat and Maharashtra at 7th and 8th position with production of 3.33 and 247 million tonnes respectively and rest of state contribute about 3.69 million tonnes of production [2].

Nitrogen is an essential plant nutrient and has significant role in plant growth. It is a constituent of protein and amino acids [10]. Nitrogen application also increases the rate of grain filling [18]. Nitrogen plays a very vital role in the process of grain filling [9], increase leaf area of the crop and may result in increased dry matter production by intercepting more sun light [29]. Low soil nitrogen contents result in low protein content in wheat grain [8].

Phosphorus is an integral nutrient element in the plant system [31]. It is known as "key of life" because in the deficiency of this single element, plants cannot complete their life cycles [11]. Phosphorus makes 0.15–0.50% of the dry weight in crop plants, and is the most important limiting macronutrient after N for plant growth and development. It is a key constituent of important macromolecules (e.g., nucleic acids, phospholipids, ATP) and is intimately involved in a wide range of physiological and biochemical processes as it controls key enzymatic reactions (through reversible phosphorylation), thus regulating a number of metabolic pathways [27]; [23]. Phosphorus uptake and utilization also plays a vital role in determination of final crop yield [25].

Azotobacter inoculation in wheat results in increased plant height, tillers, and ear length and grain yield of wheat over non inoculated control [22]. *Azotobacter* is a free living N_2 fixing bacterium. It can successfully grow in the rhizospheric zone of wheat, maize, rice, sorghum, sugarcane, cotton, potato, tomato, brinjal, cabbage and many others and fix 10-20 kg N ha^1 cropping season [12]. *Azotobacter* has the ability to produce antifungal antibiotics and fungistatic compounds against pathogens like *Fusarium, Alternaria, Trichoderma* [28]. Inoculation with salinity tolerant *Azotobacter* strains significantly improved the plant biomass and grain yield of wheat. The inoculation with Azotobacter strain ST24 resulted in attaining 89.9 cms plant height, 6.1 g seed yield, 12.0 g shoot dry weight and 0.7 % total nitrogen at fertilization dose of 120 kg N ha^{-1} [6]. Phosphate solubilizing bacterias (PSB) are beneficial micro-organisms which have the capacity to solubilize organic compounds by the production of phosphatases such as phytase into inorganic phosphorus compounds. The major phosphate solubilizing genera include *Bacillus* and *Pseudomonas*, they constitute about 1 to 50% of the total microbial population in soil [14]. Phosphate solubilizing bacteria (PSB) as biofertilizers have been found effective in solubilizing the fixed soil P and applied phosphates resulting in higher crop yields [21].

2. MATERIAL AND METHODS

2.1 Experimental location

The experiment was carried out at Rajoula Agriculture farm, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya Chitrakoot Satna (M.P.). This area is under semi- arid and sub-tropical region of Madhya Pradesh between 25°14' North latitude and 80°85' East longitude. The altitude of Chitrakoot is about 190-210 meter above mean sea level. During cropping period of wheat, the corresponding mean weekly temperature fluctuations were observed during *Rabi* season in year 2023, maximum and minimum temperature ranged between 36 °C and 4°C, respectively. Mean weekly maximum and minimum relative humidity ranged between 100 and 15 per cent, respectively. The total rainfall during crop season were recorded 0.50 mm.

2.2 Treatments and Design

The experiment was conducted with 3 levels of Nitrogen and 3 levels of Phosphorus with combinations of bio-inoculants and total 13 treatment combination laid out in 3 replication under, “Randomized Block Design”. There were 39 plots and the gross size of each plot was 3.25m x 5.25m and the net plot size was 5.0m x 3m.

Table 1-Treatment combinations

Treatments	Details of Treatments
T_0	Control
T_1	100% RDF ,N:P:K: @120:60:40
T_2	75% N & P + 100% K
T_3	50% N & P + 100% K
T_4	100% N & P + PSB + 100% K
T_5	75% N & P + PSB + 100% K
T_6	50% N & P + PSB + 100% K
T_7	100% N & P + *Azotobacter* + 100% K
T_8	75% N & P + *Azotobacter* + 100% K
T_9	50% N & P + *Azotobacter* + 100% K
T_{10}	100% N & P + PSB + *Azotobacter* + 100% K
T_{11}	75% N & P + PSB + *Azotobacter* + 100% K
T_{12}	50% N & P + PSB + *Azotobacter* + 100% K

2.3 Fertilization

Recommended dose of nitrogen and potassium applied (120kg nitrogen: 60kg phosphorus: 40kg potassium/ha) in the form of Urea (46% N) and Muriate of potash (60% K2O) and phosphorus applied as per the treatments from DAP (Phosphorus (46 %), Nitrogen (18%)) obtained from instructional farm, Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (M.P.) was applied in each plot. Nitrogen is give in three splits at first application one third of nitrogen, full dose of phosphorus and potash were applied as basal application. Remaining nitrogen applied at 21 days and 45 days after sowing of wheat crop in the form of urea.

2.4 Application of bio-inoculants

In the experiment bio-inoculants i.e., *Azotobacter* and PSB was used for this investigation. Required quantity of healthy, bold, unbroken and fully developed seeds of wheat variety Raj-4238 was inoculated separately with *Azotobacter* and PSB as per treatment before sowing of the crop as per treatment. Wheat seeds were inoculated with liquid *Azotobacter* (@20ml/kg seed) and PSB (@20ml/kg seed) as per treatments. The seeds were spread on a polythene sheet after proper mixing uniformly with hands for proper coating. After drying in shade, the seed were used for sowing immediately.

2.5 Harvesting, Threshing and cleaning

When maturity signs were recognized, harvesting began. It was hand-harvested with the aid of the local name hansiya (sickle). Before harvesting, five plants were removed from each plot that had already been tagged in order to record post-harvest observations. The harvested wheat was bundle one day after harvesting according to the plot, taken to the threshing yard the threshing and cleaning processes were carried out independently for each plot, 5 Days after harvesting on 1 April 2023 due to sudden change in local weather condition and final winnowing and grain weight per plot was recorded 8-10 days after harvesting between 5 April 2023 to 8 April 2023.

2.6 Statistical Analysis

The standard procedure by applying the techniques of analysis of variance in order to test the significance of the experimental results. Where "F" test was found significant at 5 percent level of significance, the critical difference (C.D.) for treatment means were worked out. In order to establish inter relationship between various components was also computed for yield attributing characters with yield using the formula described by Panse and Sukhatme (1985).

3. RESULTS AND DISCUSSION

3.1 Growth parameters

The growth characters of wheat presented in table 2 were significantly influenced by the different treatments and plant height, no. of tillers and no. of spikes were significantly improved by different levels of N & P alone and along with bio-inoculants (*Azotobacter* and PSB). At 90 days stage ranged from 72.27 to 91.80 and maximum plant height was recorded in the treatment T_{10} (100 % N & P + PSB + *Azotobacter* + 100 % K) while minimum plant population was recorded in the treatment T_1 (Control). Whereas at 60 days stage ranged from 2.97 to 7.47. Treatment T_{10} (100 % N & P + PSB + *Azotobacter* + 100 % K) given highest number of tillers and lowest found in treatment combination T_0 (Control). The results of number of spike of wheat at 90 days stage ranged from 5.23 to 14.27 in different treatments. Treatment T_{10} (100 % N & P + PSB + *Azotobacter* + 100 % K) given the highest number of and lowest found in treatment combination T_0 (Control). The plant height increased to each higher level of fertility up to 100% RDF but it remained at par with 100% N & P + PSB + *Azotobacter* + 100% K. The highest plant height (60 DAS and 90 DAS) were observed in 100 % RDF with combine inoculation of *Azotobacter* + PSB over control and single inoculation. The increase in plant height with increase in fertility levels due to higher availability of nutrients to plant which might have enhanced growth substances and phytohormones. The higher nutrient availability in rhizosphere for plant growth at active vegetative stages which seem to have promoted metabolic activities [7]. Prolification of lateral root and root hairs that provided more surface area for nutrient and water absorption this might be due to increase in plant growth by combine inoculation of *Azotobacter* and PSB. Hence, plant height increased due to photosynthesis and production of assimilates which is enhanced by prolificated growth. The positive effects of application of *Azotobacter* and PSB in wheat on plant height have also been reported by [3], [30] and [19]. The highest number of tillers (30 DAS and 60 DAS) and highest number of spikes (90 DAS) were observed in 100 % RDF with combine inoculation of *Azotobacter* + PSB over control and single inoculation. It is because biofertilizers improve growth might be due to increasing the supply or availability of plant nutrients. The inoculation of seed with nitrogen fixer have increased the concentration of *Azotobacter* in the rhizosphere and they fixed atmospheric and organic nitrogen in becterioeds and later on oxidized to nitrate form. *Azotobacter* increases the root development and plant growth might be due to excretion of vitamins, auxins and amino acids [20]. PSB produced organic acids like malic, succinic, glyoxalic, fumaric and critic acid, which have increased the mineralization of insoluble organic phosphorus to soluble Phosphorus there why increase in ability of P in soil [5]. *Azotobacter* and PSB application have beneficial effects on wheat and they could be attributed to their ability to fix atmospheric nitrogen, phosphate solubilization and secretion of plant growth hormones [26]. The positive effects of application of *Azotobacter* and PSB in wheat on no. of tillers and no. of spike have also been reported by [13] and [15] respectively.

3.2 Yield Attributes and yield

The yield attributes and yield of wheat presented in table 3 were significantly influenced by the different treatments and Spikelets per plant, grain per spike, test weight, grain yield, stover yield and harvest index after harvesting were significantly improved by different levels of N & P alone and along with bio-inoculants (*Azotobacter* and PSB). The results of number of spikelet per plant of wheat after harvesting ranged from 263.93 to 599 in different treatments. The results of number of grains per spike of wheat after harvesting ranged from 187.33 to 550 in different treatments. The results of test weight (g) of wheat after harvesting ranged from 47.41 to 53.87 g. The straw yield ranged from 18.77 to 51.14 (q/ha) in different treatments. The treatment T_{10} (100 % N & P + PSB + *Azotobacter* + 100 % K) given the highest Spikelets per plant, grain per spike, test weight, grain yield, stover yield and lowest spikelets per plant, grain per spike, test weight, grain yield, stover yield found in treatment T_0 (Control). The harvest index after harvesting ranged from 41.18 to 44.37 % in different treatments. The treatment T_{12} (50% N & P + PSB + *Azotobacter* + 100% K) given the highest harvest index and lowest found in treatment T_0 (Control). The grain yield after harvesting ranged from 13.53 to 38.33 (q/ha) in different treatments. The treatment combination T_{10} (100 % N & P + PSB + *Azotobacter* + 100 % K) given the highest seed yield and lowest found in treatment T_0 (Control). The yield attributing parameters viz. number of spikelets/spike, number of grain/spike, test weight (g), grain yield (q/ha) and straw yield (q/ha) and yield except harvest index yield statistically significant result. The combined inoculation of *Azotobacter* and PSB works together to enhance nutrient availability and uptake by plants. *Azotobacter* converts atmospheric nitrogen into usable forms, while PSB makes phosphorus more accessible to plants. This dual action improves nutrient absorption, plant growth hormone production, and stress resistance, leading to better root systems and a balanced soil microbial community. As a result, this combine inoculation with RDF results, spikelet initiation, grain development, and overall crop yield. The highest test weight, grain yield and straw yield were observed in treatment 100% N & P + PSB + *Azotobacter* + 100% K, over control and single inoculation. [13] reported that, Inoculating seeds with *Azotobacter* leads to a yield increase by providing crops with a higher nitrogen supply. Additionally, the chelating action of PSB diminishes phosphorus fixation, making fixed phosphorus forms soluble. This boosts phosphorus uptake, resulting in improved growth indicators like total tillers meter^{-1} row length, effective tillers m^{-1} row length and test weight. [16] reported that, the combined inoculation of nitrogen-fixing and phosphorus-solubilizing microorganism's results in a synergistic impact, potentially elevating the production of growth-promoting hormones like auxins, gibberellins, and cytokinins, thus contributing to improved yield characteristics and overall crop yield. Combine inoculation of *Azotobacter* + PSB with 100 % RDF increased stover yield might be due to increase in biomass production. Similar results have been reported by [24], [4], [13] and [17].

Table 2. Effect of different level of N and P alone and along with bio-inoculants (*Azotobacter* and PSB) on growth parameters

Treatments	Plant height (cm) at 90 DAS	No. of tillers at 60 DAS	No. of spikes at 90 DAS
T_0	72.27	3.30	5.23
T_1	85.87	4.87	6.00
T_2	85.33	4.47	8.13
T_3	87.33	4.57	9.03
T_4	91.47	5.20	9.67
T_5	89.07	5.47	8.53
T_6	86.53	5.67	8.53

T_7	87.67	6.07	8.93
T_8	88.07	6.80	12.53
T_9	86.73	5.27	6.93
T_{10}	91.80	7.47	14.27
T_{11}	89.73	6.80	12.73
T_{12}	89.60	6.00	8.27
C.D. at 5%	**5.67**	**1.84**	**2.99**
S.E(m)±	**1.94**	**0.63**	**1.02**
Result	**Sig**	**Sig**	**Sig**

Table 3. Effect of different level of N and P alone and along with bio-inoculants (*Azotobacter* and PSB) on growth parameters

Treatments	Spikelet/ plant	Number of grains	1000 Seed weight (g)	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})	Harvest index (%)
T_0	236.93	187.33	47.41	13.53	18.77	41.18
T_1	250.47	221.87	50.62	28.67	36.46	43.89
T_2	311.00	264.93	51.11	21.16	28.14	42.86
T_3	353.73	293.33	52.00	17.25	21.75	44.31
T_4	399.13	352.13	52.03	33.40	43.22	43.53
T_5	435.13	312.27	52.47	33.18	42.99	43.40
T_6	367.27	326.00	50.00	27.05	35.12	43.25
T_7	436.27	413.83	50.32	34.87	44.86	43.76
T_8	443.40	432.87	51.87	27.01	36.06	42.25
T_9	298.20	261.67	50.03	25.58	32.88	43.75
T_{10}	599.00	550.00	53.87	38.33	51.14	42.83
T_{11}	521.13	463.00	52.04	34.16	44.35	43.50
T_{12}	437.87	374.53	50.56	33.78	42.20	44.37
C.D. at 5%	136.05	84.93	2.43	10.03	11.32	1.69
S.E(m)±	46.61	29.10	0.83	3.44	3.88	0.58

Result	Sig	Sig	Sig	Sig	Sig	Sig

4. CONCLUSION

On the basis of the results summarized above, it is found that treatment T_1 $T_{4,}$ $T_{5,}$ T_7 T_{11} and T_{12} were statistically at par with treatment T_{10} and it can be concluded that treatment application of 100% N & P + PSB + Azotobacter + 100% K given the most optimum yield for the "Raj-4238" variety of wheat for Chitrakoot region of Madhya Pradesh. T_{10} (100% N & P + PSB + Azotobacter + 100% K) resulted maximum seed productivity 38.33 q ha^{-1}.

5. COMPETING INTERESTS

Authors have attested that no conflicts of interest are present

6. AUTHORS' CONTRIBUTIONS

In the course of this research project, Harsh Gupta[1*] played a pivotal role as the principal investigator, overseeing and conducting the entire body of research work. Shikha rao[6] overseeing and helping during data collection. Serving as his advisor, Dr. U.S. Mishra[2] provided invaluable guidance and expertise throughout the project's duration. Additionally, Dr. Pawan Sirothia[3], a key member of the advisory committee, offered valuable insights and perspectives that enriched the research. Ashutosh Mishra[4] and Veerendra Kumar Patel[4] contributed significantly to the project by lending their expertise in statistical analysis, ensuring the robustness and accuracy of the study's findings. This collaborative effort among the team members led to the successful completion of our research endeavor.

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