

Integrated Nutrient Management in Wheat by use of *Azotobacter* sp. and *Streptomyces badius*

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Abstract – Wheat is one of the most essential staple food crops of India. It occupies a notable position among the food grain crops. The demand for wheat in India by 2020 is expected to increase and to meet the growing demand of food the only relevant approach is to boost production per unit area per unit time. The integrated use of biofertilizers and chemical fertilizers could play a crucial role not only for increasing yield but also for sustaining soil health. Hence, recognizing the above facts, multilocation field experiments were carried out at four different locations, to study the effects bio fertilizers (*Azotobacter* sp. and *Streptomyces badius*) inoculation on grain yield of wheat with three treatments viz. Control (Recommended dose of fertilizer, RDF), RDF +*Azotobacter* sp. +*Streptomyces badius* and 75% of RDF +*Azotobacter* sp. +*Streptomyces badius*. Most prominent findings emerged was regarding superiority of 75% of RDF with inoculation of biofertilizers over full recommended dose of fertilizer without biofertilizers in respect of grain yield. The highest Benefit: Cost ratio (B:C ratio) of 2.63 was found with 75% of RDF + *Azotobacter* sp. + *Streptomyces badius* treatment followed by RDF + *Azotobacter* sp. + *Streptomyces badius* treatment (2.55) against B:C ratio of 2.47 available with RDF only. This might be due to the nitrogen fixing and phosphate solubilising activities of inoculated biofertilizers along with plant hormone producing ability and siderophore producing ability. Thus, seed inoculation with biofertilizers i.e *Azotobacter* sp. and *Streptomyces badius* coupled with 75% of RDF brought 25% economy in chemical fertilizers with higher grain yield, net profit and B:C ratio over RDF only.

Keywords – *Azotobacter* sp., Biofertilizer, *Streptomyces badius*, Wheat, Yield.

I. INTRODUCTION

Wheat is the world's leading cereal crop, cultivated over an area of about 216.06 m ha with a production of 655.49 mt. In India, the wheat production is about 95.85 mt from an area of around 30.61 m ha. [1]. The demand for food grains is proposed to rise not only as a function of population growth but also as more and more people cross the poverty line with economic and social advancement. The requisition for wheat in India by 2020 has been projected to be between 105 to 109 mt as against 95.85 mt production of present day [2]. To meet the growing necessity of food the only applicable approach is to increase production per unit area per unit time.

In addition, expanding realization for environmental pollution, overdependence for fossil fuel derived fertilizers and sustainable crop production demands for the establishment of integrated nutrient management (INM) strategies. Biofertilizers emerged as one of the integral

component of INM. These are cost effective, eco-friendly and renewable source of plant nutrition. Biofertilizers are best defined as a substance which contains living microorganisms which when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the availability of primary nutrients to the host plant [3]. The most influential microorganisms used as biofertilizer are *Rhizobium*, Blue Green Algae (BGA) and Azolla, these are crop specific, bio-inoculants like *Azotobacter*, *Azospirillum*, Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad spectrum biofertilizers [4].

Azotobacter sp. has been observed to augment plant growth and yield by production of IAA, fixation of atmospheric nitrogen, antibiotic production and by siderophore production. Actinomycetes enhance plant growth and yield by producing biologically active substances such as indole-3-acetic acid (IAA) for greater development of roots or by producing siderophores that improves nutrient uptake. Actinomycetes also reported to produce various antibiotics (chloramphenicol, neomycin and streptomycin), biologically active material like B vitamins, auxins and solubilize phosphate. Thus, the uses of biofertilizers containing *Azotobacter* sp. and *Streptomyces badius* (Actinomycetes) have the potential for improving grain yield of wheat. Hence, considering the above facts, the present study was undertaken to study the effects of biofertilizers (*Azotobacter* sp. and *Streptomyces badius*) inoculation coupled with chemical fertilizers on grain yield of wheat.

II. MATERIAL AND METHODS

A. Methodology

The multilocation field experiments were carried out at four different locations, Location 1 (Ludhiana), Location 2 (Kapurthala), Location 3 (Gurdaspur) and Location 4 (Bathinda) during *rabi* 2015-2016 with three treatments (500m²/treatment) i.e.

1. Control (Recommended dose of fertilizer, RDF),
2. RDF +*Azotobacter* sp. +*Streptomyces badius*
3. 75% of RDF +*Azotobacter* sp. + *Streptomyces badius*.

The field was prepared well before sowing by ploughing twice with tractor followed by planking to make a fine seed bed. Seeds of wheat (*Triticum aestivum* L.) were sown in the month of November in area of 500m²/treatment with spacing of 0.2 m between rows for each treatment respectively during *rabi* 2015-16. Plots

were separated by 1.5 m unplanted distances. Whole of phosphorus in the form of DAP was drilled at the time of sowing and 1/2N in the form of urea was broadcasted and mixed well in the field before sowing of wheat as per treatments. Nitrogen applied through DAP was adjusted with the first ½ split of nitrogen applied at sowing and remaining half N was applied after 1st irrigation. All other agronomic operations except those under study were kept normal and uniform for all treatments. The plants were harvested at maturity to calculate grain yield and biological yield. Biofertilizer of *Azotobacter* sp. and *Streptomyces badius* was applied @ 250 gram/40kg of wheat seeds respectively [Charcoal carrier based biofertilizer, 1x10⁸ colony forming unit (CFU) per gram carrier]. Inoculated seeds were air dried in shade and planted within 2 hrs.

B. Chemical Analysis of Soil Samples

The soil samples before the sowing of crop and after the harvest of the crop from surface layer (0-15 cm) were collected from selected four locations and analyzed for pH, electrical conductivity (EC), organic carbon, available nitrogen, phosphorous and potassium as per standard procedures.

III. RESULTS AND DISCUSSION

Biofertilizers are paramount element of integrated nutrient management as they play key role in productivity and sustainability of soil health. Because these are ecofriendly and provide renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system [5]. The results of multilocation trials conducted during *rabi* 2015-16 also revealed the potential of biofertilizer in improving grain yield of wheat.

The wheat variety HD 2967, recorded an increase of 3.28% in grain yield of wheat by application of RDF + *Azotobacter* sp. + *Streptomyces badius* over RDF (control) in a trial conducted at location 1 (Ludhiana) [Figure 1, Table 1]. The increase of 6.97% was recorded for 75% of RDF + *Azotobacter* + *Streptomyces badius* over RDF (control) [Figure 1, Table 1].

Similarly, the increase of 2.52 % in grain yield of wheat (variety PBW 550) by application of RDF + *Azotobacter* sp. + *Streptomyces badius* over RDF (control) was recorded (Table 1) at location 2 (Kapurthala). The increase of 1.17% in grain yield of wheat was recorded for 75% of RDF + *Azotobacter* sp. + *Streptomyces badius* over RDF (control) [Figure 2, Table 1].

At location 3 (Gurdaspur) wheat varieties HD 3086, yielded an increase of 1.81 % by application of RDF + *Azotobacter* sp. + *Streptomyces badius* over control (RDF). The marginal increase of 0.5% in grain yield of wheat was recorded for 75% of RDF + *Azotobacter* sp. + *Streptomyces badius* over RDF (control) (Figure 3, Table 1).

At location 4 (Bathinda), the increase of 4.5 % in grain yield of wheat (variety HD 3086) by application of RDF + *Azotobacter* sp. + *Streptomyces badius* over RDF (control) was recorded (Table 1). The increase of 3.0% in grain yield was recorded for 75% of RDF + *Azotobacter* sp. +

Streptomyces badius over RDF (control) (Figure 4, Table 1).

On an average of 3.03% increase in grain yield with application of biofertilizer with RDF and 2.91 % increase in grain yield with the application of biofertilizer with 75% RDF over RDF (control) was recorded across the locations (Table 1).

The maximal response of biofertilizers was observed in the trial conducted at location 1 (Ludhiana). This might be because of the fact that biological nitrogen fixation depends upon the availability of N and P. The initial soil sample analysis of location 1 (Ludhiana) showed least availability of nitrogen in comparison to other location. Lower availability of nitrogen could have increase the activity of nitrogenase enzyme as biological nitrogen fixation (BNF) involves symbiotic or associative relationships between the diazotroph and the host plant, as the diazotrophic nitrogenase enzyme (encoded by the diazotroph *nif* HDK genes) catalyses the reduction of gaseous nitrogen (N₂) to NH₃ [6]. However, when an excess supply of fixed N is available, the process of BNF is suppressed in many bacterial species. In the presence of excess fixed N, the diazotrophic nitrogenase enzyme is no longer active, either due to down-regulation of protein synthesis and/or inactivation of the protein [7]. In addition available phosphorus in the initial soil sample from location 1 (Ludhiana) showed highest available phosphorus in comparison to other locations. The more availability of P also influence the process of BNF as phosphorus becomes involved as an energy source when 16 molecules of adenosine triphosphate (ATP) are converted to adenosine diphosphate (ADP) as each molecule of N₂ is reduced to NH₃.

On the basis of multilocation trials, the highest Benefit: Cost ratio (B:C ratio) of 2.63 was found with 75% of RDF + *Azotobacter* sp.+ *Streptomyces badius* treatment followed by RDF + *Azotobacter* sp. + *Streptomyces badius* treatment (2.55) against B:C ratio of 2.47 available with RDF only (Table 1). Higher economic returns (Higher B:C ratio) play a key role, to convince the farmer for adoption of technology. It is obvious that realization of higher B:C ratio in multilocation trials was the result of higher grain yield and straw yield with best treatment (75% of RDF + *Azotobacter* sp.+ *Streptomyces badius*). It implies that higher B:C ratio can be obtained even by using 75% of RDF + *Azotobacter* sp.+ *Streptomyces badius* coupled with 25% saving of RDF.

The perusal of data pertaining to chemical properties of soil samples which were taken before the sowing of crop and after the harvesting of the crop from surface layer (0-15 cm)] did not revealed any particular pattern or effect of biofertilizer on pH, EC, organic carbon, available nitrogen, phosphorous and potassium from all the four locations (Table 2). For improving soil health, massive inoculation with a premium strain many times over a period of several years is prerequisite. As native strains give rigid competition to inoculated strains (applied in the form of biofertilizer). Thus bulk inoculation of soil rather than seed inoculation is required to improve soil health. Continuous use of bio-fertilizers enables the microbial

population to remain and build up in the soil and helps in maintaining soil fertility contributing to sustainable agriculture [8]. Growing crops using bio-fertilizers is advantageous in protecting the soil from degradation. By 2018, the worldwide market for bio-fertilizers is predicted to exceed a market worth of US\$ 10.2 billion; Europe and Latin America are the end user consumers due to availability of stringent regulations imposed on chemical fertilizers, followed by Asia-Pacific which controlled 34% of the market as by 2011 [9].

The overall increase in grain yield by application of biofertilizer i.e *Azotobacter* sp. + *Streptomyces badius* might be due to the nitrogen fixing and phosphate solubilising activities of inoculated biofertilizers along with plant hormone producing ability like IAA and siderophore producing ability.

Nitrogen is a paramount element for plant. It is found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, N supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Besides, it influences the cell size, leaf area and photosynthetic activity [10]. Phosphorus is also a standout macro plant nutrient ranked next only to N. Only about 20-25% of P applied to the soil is accessible for crop in the year of application and remaining part is converted into unavailable forms. Inoculation of seed or seedling with PSB can solubilize 30-50 kg P₂O₅/ha equivalent to P applied as ssp by solubilizing soil phosphorus and also applied phosphorus [11].

Furthermore, IAA hormone stimulates root growth and development. The siderophore produced by actinomycetal strain have high affinity for iron and it scavenges most of the available iron than the siderophores produced by fungal pathogen and thus prevent the proliferation of fungal pathogens. In this way, the plants are benefited in two ways i.e suppression of pathogens as well as enhance iron nutrition that results in increasing plant growth.

IV. CONCLUSION

The seed inoculation with biofertilizer of *Azotobacter* sp. and *Streptomyces badius* in combination with 75% of RDF brought 25% economy in chemical fertilizers along with higher wheat grain yield, net profit and hence B:C ratio over RDF only.

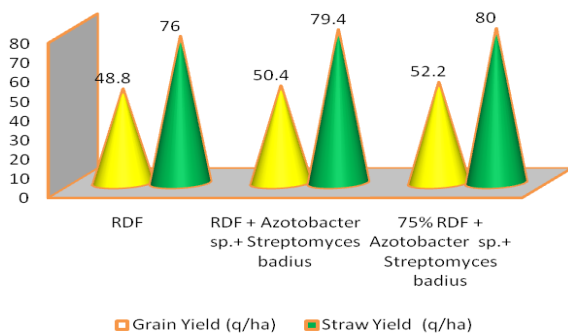


Fig. 1. Effect of *Azotobacter* sp. and *Streptomyces badius* on grain and straw yield of wheat variety HD 2967 during rabi 2015-16 (Location 1, Ludhiana)

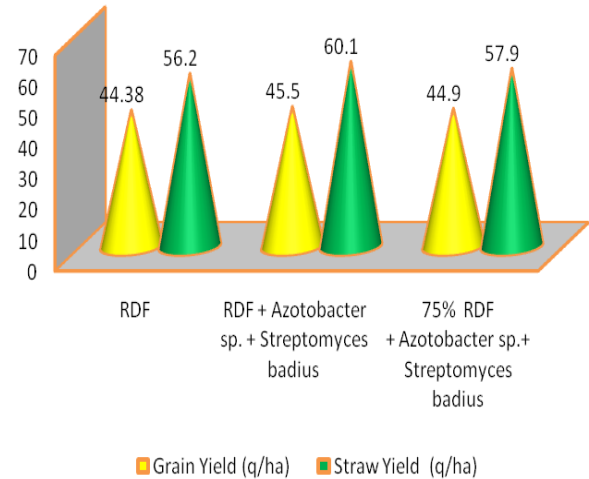


Fig. 2. Effect of *Azotobacter* sp. and *Streptomyces badius* on grain and straw yield of wheat variety PBW 550 during rabi 2015-16 (Location 2, Kapurthala)

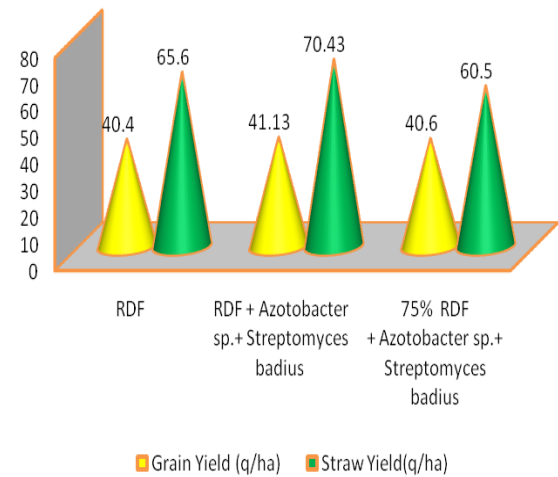


Fig. 3. Effect of *Azotobacter* sp. and *Streptomyces badius* on grain and straw yield of wheat variety HD 3086 during rabi 2015-16 (Location 3, Gurdaspur).

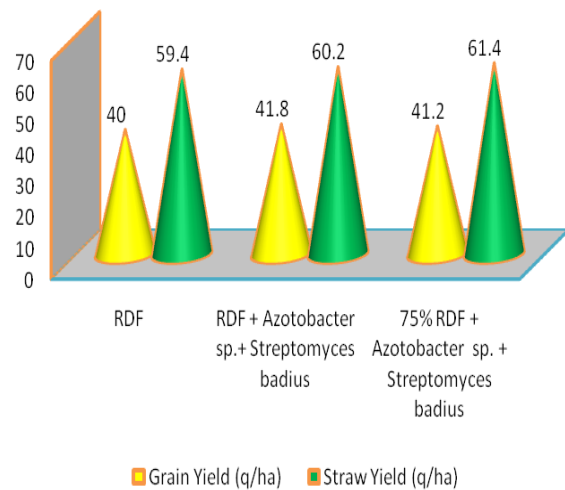


Fig. 4. Effect of *Azotobacter* sp. and *Streptomyces badius* on grain and straw yield of wheat variety HD 3086 during rabi 2015-16 (Location 4, Bathinda)

Table 1. The percentage increase in grain yield and B:C ratio of wheat in four different locations during Rabi 2015-16

S. No	Treatments	Location 1 Ludhiana	Location 1 Kapurthala	Location 3, Gurdaspur	Location 4, Bathinda	Mean
1	RDF	- (2.81)	- (2.44)	- (2.35)	- (2.28)	- (2.47)
2	RDF + <i>Azotobacter</i> sp.+ <i>Streptomyces badius</i>	3.28 (2.91)	2.52 (2.52)	1.81 (2.42)	4.5 (2.36)	3.03 (2.55)
3	75% RDF + <i>Azotobacter</i> sp.+ <i>Streptomyces badius</i>	6.97 (3.11)	1.17 (2.57)	0.50 (2.40)	3.0 (2.44)	2.91 (2.63)

Note: Percentage increase over RDF (Control) in grain yield of Wheat, values in parentheses is B/C ratio.

Table 2: Chemical properties of surface layer of soil (0-15 cm) from different four locations before sowing (initial) and after harvest of wheat (Rabi 2015-16)

Treatments	Soil Parameters (0-15 cm)					
	pH	EC (dS/m)	O.C. (%)	Av. N (kg/acre)	Av. P (kg/acre)	Av. K (kg/acre)
Location 1: Ludhiana						
Initial	7.55	0.212	0.26	38.0	20.4	115.5
RDF	7.61	0.199	0.27	38.8	20.8	114.4
RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.54	0.195	0.24	37.6	20.7	115.3
75% RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.47	0.200	0.26	38.2	20.9	115.0
Location 2: Kapurthala						
Initial	7.70	0.140	0.29	41.5	13.8	89.0
RDF	7.67	0.143	0.32	42.0	14.0	88.0
RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.71	0.142	0.32	43.9	13.5	90.0
75% RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.65	0.143	0.32	43.9	13.7	90.0
Location 3: Gurdaspur						
Initial	7.30	0.20	0.60	78.1	5.4	49.2
RDF	7.31	0.22	0.57	78.4	5.7	50.0
RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.29	0.19	0.55	76.5	5.8	50.5
75% RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	7.29	0.20	0.58	79.0	5.6	49.6
Location 4: Bathinda						
Initial	8.30	0.152	0.24	48.2	17.0	140.0
RDF	8.31	0.151	0.23	49.0	17.1	138.0
RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	8.30	0.151	0.23	48.6	18.3	135.2
75% RDF + <i>Azotobacter</i> + <i>Streptomyces badius</i>	8.29	0.147	0.24	47.9	17.2	136.4

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