Co-inoculation Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Growth, Yield and Nutrient Uptake in Wheat (*Triticum aestivum*)

Original Research Article

ABSTRACT

A field experiment was conducted to study the effect of *Azospirillium brasilense* and *Bacillus subtilis* along with graded level of chemicals fertilizers on growth, yield and nutrient uptake of wheat (*Triticum aestivum*) cluring Rabi, 2018 at Agronomy farm, Rajarshee Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur. The co-inoculation effect of *Azospirillium brasilense* and *Bacillus subtilis* along with 75% nitrogen and Phosphorus and 100% potassium recorded highest plant

height at tillering (25.00),plant height at flowering (87.80cm) plant height at maturity (90.53cm), number of tillers sq.m^{1} (403.00).In addition ,ear length (14.77cm),number of spikelets ear-^{1} (42.87), number of grain ear-^{1} (38.33) ,1000 grain weight (43.88gm), gram yield (50.63q/ha) straw yield (75.93 q/ha) and uptake of nitrogen and phosphorus by wheat plant also found to be increased 120.00 kg hand 37.00kg/ha respectively.

Keywords: Wheat; rhizobacteria; PGPR; yield; nutrient uptake.

1. INTRODUCTION

Wheat (*Triticum aestivum*) belongs to family *Gramminae* is one of the most important cereals crops of the global significance. It is staple food at millions of people (36% of the world's population). Approximately one sixth of the total arable land in the world is cultivated with wheat. After green revolution (1960) extensive cultivation of wheat was started, it plays major role in Indian economy, worldwide it provides nearly 55% of the carbohydrates and 20% food calories. (Breiman & Graur, 1995). It is source of manganese, phosphorous like nutrients like niacin and other dietery fibers.it is used as major ingredient in different food i.e bread, biscuts, pasta, noodles, pancakes, pizza etc (Omara & Elbagory, 2018).

Biofertilizers play a major role in increasing nutrient availability for high yield. It also reduces production cost by limiting chemical fertilizers application (Shivashakarappa et al., 2022). Phosphorus biofertilizer help to increase the availability of phosphate for plant growth through production of plant growth promoting substances by increasing the efficiency of biological nitrogen fixation (Kucey *et al.,* 1989). Several reports have described the beneficial effect of *Azospirillium* inoculation on plant growth *Azospirilium brasilense* and a local strain clearly improved growth and increased three cultivar of wheat. (Akbari *et al.,* 2007). Co-inoculation with *Azospirilium brasilense* have been increase root and shoot biomass, nitrogenese activity, N_2 fixation and grain yield in wheat (Shaukat *et al.,* 2006). These bacteria are believed to produce various phytohormones that improve root growth, water and mineral adsorption and increase stress tolerance, leading to more vigrous and more productive plants (Bashan & Holgwin, 1997, Dobbelaere *et al*., 2001; Bashan *et al*., 2004).

Inoculation of *Azospirillium* to wheat significantly increased foliage & grain yield, nitrogen yield and fertile tillers per unit area. (Kapulnik *et al.,* 1983). *Bacillus substilis* plays important role in phosphate solubilization, hence called PSB (Rana et al., 2012). It has positive influence on plant growth, vitality, cope up with pathogens results in higher yield (Elkoca *et al*., 2010) (Valente et al., 2020). Hence bio fertilizers can be important component of integrated nutrient management systems for to sustaining agricultural productivity and a healthy environment. (Adesemoye *et al*., 2009).

Hence the objective of present investigation was to evaluate co inoculation effect of *Azospirillium Brasiliense* and *Bacillus substilis* on growth, yield and nutrient uptake of wheat.

2. MATERIALS AND METHODS

In this field experiment plant promoting rhizobacteria *viz. Bacillus subtilis* (obtained from Biofertilizer Production Unit, RCSM College of Agriculture, Kolhapur, India and *Azospirillium brasilense* (Indigenous isolate) were used as biofertilizer. Indigenous isolates Ab-3 was identified on morphological and biochemical study. Efficiency was tested in plot. The seeds (Phule Samadhan) were inoculated with *Azospirillium brasilense* @25g/kg seeds and *Bacillus subtilis* @ 25g/kg seed. The crop was fertilized chemical fertilizers @ 120:60:60; N:P_2O5:K_2O kg /ha.

The plots were arranged in a randomized block design with three replications. The obtained data were statistically analyzed using standard procedure suggested by Panse and Sukhatme (1985) for the design of experiment. Growth and yield measurement observations were recorded tillering and flowering stage and maturity stage for plant height and no. of tillers, ear length, no. of spikelet, no of grain per year, 1000 grain weight, and grain yield also observed.

The cultural operations like irrigation, weeding was uniformly carried out to all treatments.

3. RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads. The result regarding this are presented in Table 1.

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3.1 Plant Height at Tillering Stage

Among the different inoculation treatment tried in the investigation, inoculation with *Azospirillium brasilense* + *Bacillus subtilis* + 75% N and P and 100%K was found to be most effective as it recorded maximum plant height (25.00) over rest of the treatments. However, treatment consisting *Azospirillium brasilense* + *Bacillus subtilis* +100% NPK (24.83), *Azospirillium brasilense* +100 RDF (24.77cm), *Bacillus subtilis* +100% RDF (24.48) and RDF only (24.33cm) was par with above treatment. Significantly lowest plant height 19.18cm with *Bacillus subtilis* + 50% P and 100NK and *Azospirillium brasilense* +50%N and 100%PK. These result obtained in the present investigation are in close confirmation with Saber *et al.,* (2012) and Khan *et al.,* (2002). The improved plant height might be due to plant growth activity at both *Azospirillium brasilense* and *Bacillus subtilis.*

3.2 Flowering Stage

Tallest plant height (87.80cm) evident in treatment *Azospirillium brasilense* + *Bacillus subtilis* +75%NP and 100%K and on par with *Azospirillium brasilense* + *Bacillus subtilis* +100%NPK (87.63cm), *Azospirillium brasilense* 100% NPK (87.53cm) &*Bacillus subtilis* +100%NPK(87.33cm). Lowest plant height was observed in *Bacillus subtilis* +50%P& NK (78.00cm) and *Azospirillium brasilense* +50%N, 100%PK (78.18cm). Similar results were reported by Kennedy & Tehan (1992) & Cuppels *et.al.* (1999), Panwar *et.al.* (2000) in wheat. This might be due to the production of phytoharmones (such as auxin and cytokinin) and volatile growth stimulants by PGPR. (such as ethyelene & 2,3-butanediol (Vassey, 2003).

3.3 Maturity Stage

Significantly highest mean height to the tune of 90.53 cm was observed in treatment *Azospirillium brasilense* + *Bacillus subtilis* +75%NP & 100%K in comparison to rest or the treatment.

However plant height recorded with treatments were statistically at par with treatments *Azospirillium brasilense* + *Bacillus subtilis* +100% NPK (90.32cm) *Azospirillium brasilense* +100 RDF (90.18cm), *Bacillus subtilis* +100% NPK (89.90 cm) and RDF (89.23 cm). The lowest mean height 79.27cm observed in plot treatments with *Bacillus subtilis* +50% P and 100%N&K.

3.4 Number of Tillers

Data presented in Table 1, showed that inoculation with *Azospirillium brasilense* and *Bacillus subtilis* were statistically significant.

Highest number of tillers 403 tillers sq.m^{-1} observed in treatment *Azospirillium brasilense* + *Bacillus subtilis* +75%NP & 100% K. While next best treatment was *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK (402.83 tillers sq.m^{-1}), *Azospirillium brasilense* +100% NPK (402.37 tillers sq.m^{-1}), *Bacillus subtilis* +100% NPK (402.00 tillers sq.m^{-1}) and RDF (401.67 tillers sq.m^{-1}). These treatments were found to be statistically at par with each other and proved to be significantly superior over rest of the treatment. The lowest number of tillers 389.67 tillers sq.m^{-1}was recorded in treatment *Bacillus subtilis*+50% P and NK. Saber *et.al.* (2012) stated that Nitrogen fixing bacteria like *Azospirillium* and PSB like bacillus increased number of tillers in wheat plant due to dual inoculation.

3.5 Ear Length

Longest mean length of ear in treatment *Azospirillium brasilense* + *Bacillus subtilis*+75% NP and 100K (14.77) which was at par with treatment *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK(14.67cm), *Azospirillium brasilense*+100% NPK(14.63cm), *Bacillus subtilis*+100% NPK (14.53 cm) and Control RDF (14.48cm). Similar results were put forth by Saber *et al*. (2012) in wheat crop and Biari *et.al.* (2008) in Maize crop.

3.6 Number of Spikelet Ear^{-1}

Results in regard no. Of spikelet as influenced by inoculation with *Azospirillium brasilense* and *Bacillus subtili s*are presented in Table 1.

Among the different inoculation treatment evaluated in the present investigation, inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+75% NP & 100% K recorded significantly highest number of spikelet (42.87 ear^{-1}) over rest of the treatments. Nevertheless, this treatment was statistically at par with the treatment comprising inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+ 100% NPK (42.53 ear^{-1}), *Azospirillium brasilense*+ 100% NPK (42.20 ear^{-1}), *Bacillus subtilis*+ 100% NPK (42.03 ear^{-1}) and Control RDF(41.88 ear^{-1}).

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Table 1. Co-inoculation effect of *Azospirillium brasilense* & *Bacillus subtili* son growth and yield of wheat

Tr. No.	Treatment details	Plant height at tillering (cm)	Plant height at flowering (cm)	Plant height at maturity (cm)	No. of tillers (sq.m-1)	Earlength (cm)	No. of spikelets ear-1	No. of grains ear-1	1000-grain weight	Grain yield (qha-1)	Strawyield (qha-1)
T1	*Azospirillum brasilense*+100%NPK	24.77	87.53	90.18	402.37	14.63	42.20	38.00	43.53	50.40	75.20
T2	*Azospirillum brasilense*+75%Nand 100%PK	23.00	84.50	86.57	397.63	14.00	39.63	35.20	42.20	46.13	71.03
T3	*Azospirillum brasilense*+50%N and 100% PK	19.50	78.18	80.67	390.13	11.87	34.27	30.13	35.83	37.67	65.23
T4	*Bacillussubtilis*+100%NPK	24.48	87.33	89.90	402.00	14.53	42.03	37.87	43.37	49.88	74.00
T5	*Bacillussubtilis*+75%Pand 100%NK	22.67	83.23	86.03	396.23	13.82	38.18	34.87	42.10	43.33	70.53
T6	*Bacillussubtilis*+50%Pand 100 %NK	19.18	78.00	79.27	389.67	11.63	33.30	29.63	35.18	37.03	65.00
T7	*Azospirillum brasilense+Bacillus subtilis* +100%NPK	24.83	87.63	90.32	402.83	14.67	42.53	38.27	43.67	50.57	75.77
T8	*Azospirillumbrasilense+Bacilluss ubtilis*+75%NPand 100%K	25.00	87.80	90.53	403.00	14.77	42.87	38.33	43.88	50.63	75.93
T9	*Azospirillum brasilense+Bacillus subtilis*+50%NPand 100%K	21.18	81.00	83.27	393.27	12.67	36.33	32.97	38.13	40.53	68.00
T10	Control (RDF)	24.33	86.97	89.23	401.67	14.48	41.88	37.03	43.23	49.03	73.87
	S.E±	0.40	0.72	0.78	0.92	0.10	0.52	0.50	0.28	0.88	0.73
	C.D.at 5%	1.21	2.18	2.35	2.73	0.30	1.55	1.48	0.84	2.65	2.20

Note: RDF=Recommended dose of nitrogen, phosphorus and potassium

Table 2. Co-inoculation effect of *Azospirillum brasilense* and *Bacillus subtilis* on nitrogen & Phosphorus uptake

Tr. No	Treatment details	N uptake (kgha-1)	Puptake (kgha-1)
T1	*Azospirillum brasilense*+100%NPK	119.53	36.67
T2	*Azospirillum brasilense*+75%Nand 100%PK	115.23	32.13
T3	*Azospirillum brasilense*+50%Nand 100% PK	109.36	24.13
T4	*Bacillus subtilis* +100%NPK	119.03	35.13
T5	*Bacillus subtilis*+75%Pand 100%NK	114.63	30.23
T6	*Bacillus subtilis* +50%Pand 100 %NK	108.67	23.00
T7	*Azospirillum brasilense+Bacillus subtilis*+100%NPK	119.77	36.83
T8	*Azospirillum brasilense+Bacillus subtilis* +75%NP and100%K	120.00	37.00
T9	*Azospirillumbrasilense+Bacillussubtilis*+50%NP and 100%K	111.93	27.18
T10	Control (RDF)	118.00	34.83
	S.E±	0.80	0.84
	C.D.at5%	2.41	2.54

Note: RDF=Recommended dose of nitrogen, phosphorus and potassium

The lowest number of spikelets (33.30 ear^{-1}) was found in the treatment *Bacillus subtilis*+50% P and NK. Since results were reported by Saber *et.al.* (2012) and Biari *et.al.* (2008).

3.7 Number of Grains Ear^{-1}

The inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+75% NP and 100% K gave more no. of grain (38.33ear^{-1}) as compared to rest of the treatment. However, this treatment was statistically at par with those of the treatment consisting of *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK (38.27 grain ear^{-1}), *Azospirillium brasilense*+100% NPK (38.00 grain ear^{-1}), *Bacillus subtilis*+100% NPK (37.87grain ear^{-1}) & RDF (37.03 grain ear^{-1}).

The minimum grain per ear of 29.63 was obtained in treatment *Bacillus subtilis*+50%P+100% NK. This result was inconformity with those Darmwal & Guar (1988) and Cakmakci *et.al.* (2014).

3.8 1000 Grain Weight

Results indicated that highest 1000 grain weight 43.88 gm in treatment *Azospirillium brasilense* + *Bacillus subtilis*+75% NP & 100% K which was at par with treatment *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK (43.67gm) *Azospirillium brasilense*+100% NPK (43.53 gm) *Bacillus subtilis*+100% NPK (43.37 gm) and RDF(43.23 gm).

The lowest 1000 grains weight 35.18 gm was recorded in treatment *Bacillus subtilis*+50% P and 100% NK. Results of the present investigation concur with observations of the research Saber *et.al*. (2012), Jagnow (1990) and Bhattarai and Hess (1993).

3.9 Grain Yield

Among the different inoculation treatments evaluated in this investigation, the treatment comprising of seed inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+75% NP and 100% K was found to be most effective as it recorded the highest yield (50.63 q ha^{-1}) over rest of the treatments.

However, the yield obtained with these treatments was statically at par with those of the treatments consisting of seed inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+ 100% NPK (50.57 q ha^{-1}), *Azospirillium brasilense*+100% NPK (50.40 q ha^{-1}), *Bacillus subtilis*+100% NPK (49.88q ha^{-1}) and RDF (49.03 q ha^{-1}).

The lowest grain yield of the crop was obtained in the treatment of *Bacillus subtilis*+50%P and 100% NK (37.03 q ha^{-1}). The improved grain yield due to the dual inoculation was reported by Saber *et.al*. (2012). Similar results in rice reported by Khan *et al*. (2002).

3.10 Straw Yield

Straw yield recorded at harvesting of the crop produced highest yield (75.93 q ha^{-1}) in *Azospirillium brasilense* + *Bacillus subtilis*+75% NP and 100% K treatment while *Azospirillium*

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brasilense + *Bacillus subtilis*+100% NPK (75.77 q ha^{-1}) *Bacillus subtilis*+100% NPK (74.00 q ha^{-1}) and RDF (73.87 q ha^{-1}) treatment found at par with each other.

The minimum straw yield 65.00 q ha^{1}was noticed in the treatments *Bacillus subtilis*+50% P & 100%NK. The results of the present investigation are in general agreement with researcher Cakmakci *et al*. (2014), Dai et al., (2012) in wheat.

3.11 Nutrient Uptake by Wheat

Results in respect of nutrient uptake by wheat plant as influenced by inoculation with *Azospirillium brasilense* & *Bacillus subtili* sare presented in Table, 2.

Among the different inoculation treatments tried in the investigation, inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+75% NP and 100% K was found to be most effective as it recorded the highest N and P uptake (120.00 kg ha^{-1},37.00 kg ha $^{-1}$) respectively over rest of the treatments.

However, it was statistically at par with those recorded with the treatment of inoculation with *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK (119.77 kg ha^{-1},36.83 kg ha $^{-1}$) , *Azospirillium brasilense*+100% NPK (119.53 kg ha^{-1}, 36.67 kg ha^{-1}), *Bacillus subtilis*+100% NPK (119.03 kg ha^{-1},35.13 kg ha^{-1}) and RDF (118.00 kg ha^{-1}, 34.83 kg ha^{-1}).Whereas, the lowest NP uptake by wheat plants was noticed in the treatment consisting of inoculation with *Bacillus subtilis*+50% P & 100% NK(108.67 kg ha $^{-1}$, 23.00 kg ha^{-1}) respectively.

4. CONCLUSION

Results of the present investigation explicitly substantiate that co-inoculation of wheat seed with *Azospirillium brasilense*& *Bacillus subtilis*in conjunction with application of 75% recommended dose of N & P & 100 % Potash fertilizer did not differ significantly from the treatment *Azospirillium brasilense* + *Bacillus subtilis*+100% NPK. Thus it is possible to replace 25% N and P fertilizer and save the price of chemical fertilizers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that No generative AI technologies such as large language models (Chat GPT, copilot etc.) and text-to-image generators have been used during writing or editing of this manuscript**.**

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