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Increase in growth, productivity, nutritional status of Rice (*Oryza sativa* L.) and enrichment in soil fertility applied with *Azospirillum* and nitrogen level

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ABSTRACT

Field experiments were conducted to Influence of different levels of nitrogen and *Azospirillum* inoculation on direct-seeded rice in a Vertisol, during *kharif* season of 2015 at Department of Soil Science & Agricultural Chemistry, JNKVV, Jabalpur. The experiment was laid out under randomized block design (RBD) with 3 replications and 8 treatments namely (unfertilized+uninoculated (UFUI), recommended dose of nitrogen 50%+uninoculated (RDN50%+UI), RDN75%+UI, RDN100%+UI, UF+*Azospirillum* (UF+Azosp.,), RDN50%+Azosp., RDN75%+Azosp and RDN100%+Azosp.,). It was observed that significant improvement was noticed in yield attributes and soil properties. The response from the treatment of RDN100% +Azosp., was found statistically best to increase available nitrogen (N) content in soil at 45 DAS and at harvest of the crop by 29% and 27%, respectively and N content in the plant, grain and straw by 46%, 50% and 55%, respectively over the control of UFUI. Similarly, trend was significantly enhanced total N uptake by crop with 129% over the control of UFUI. While, same treatment combination increasing azospiral population in rhizospheric soil at 45 DAS, 65 DAS and at harvest by 2.28, 2.07 and 2.05 log folds, respectively over the control of UFUI and enhanced yield attributes and yields of grain and straw of rice with 113 and 58%, respectively over the control of UFUI. While the treatment RDN100%+Azosp., exhibited numerically higher values but was statistically *at par to* RDN75%+Azosp.

KEYWORDS

Azospirillum, Inoculation, Nitrogen, Rhizospheric, Rice, Seeded, Vertisol

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The indiscriminate and excessive use of chemical fertilizers in crop production has been generally recognized as decreasing quality to the environment in ways such as polluting water basins, micro-organism, and friendly insect, decreasing the soil fertility and thus become an obstacle to soil productivity and causing irreparable damage to the overall system (Huang, 2000). However, biofertilizers an effective alternate strategy, low-cost resource have gained prime importance in recent decades and play a vital role in maintaining long term soil fertility and sustainability, cost-effective, eco-friendly and renewable sources of plant nutrients and significantly reduce inorganic chemical without compressing yield and quality of agricultural products can be produced. One such alternative that is worth investigating is the use of biofertilizers (Parr *et al*., 2000, Jakhar *et al*., 2017). The improvement in growth parameters and yield components of rice may be attributed to supplementation of nitrogen at moderate level along with inoculation of *Azospirillum* which make nitrogen and other nutrients more available either abiotic and/or biotic means (Rodrigues *et al.,* 2008). Further, *Azospirillum* inoculation may biologically increase the production of more plant biomass and development of the root system and finally the yield through a significant contribution to greater uptake ability and utilization of nutrients and water.

Development of root system may increase root length and number of lateral roots, root density and more number of root hairs and its length and consequently the volume of root zone, those favour all beneficiary characteristics of the crop (Huergo *et al.,* 2008). Nitrogen-fixing entophytes in rice were reported to be higher in stems than in roots, indicating that rice stems probably provide a suitable niche for them (Barraquio *et al*., 1997).

In this experiment, to Influence of different levels of nitrogen and *Azospirillum* inoculation on direct seeded rice in a Vertisol was investigated. The substrate of the biofertilizers used in these experiments was prepared from MRPC Laboratory, Department of soil science & agricultural chemistry, JNKVV, Jabalpur. This investigation was carried with the aim of reducing the amount of mineral N fertilizer applied to the soil while maintain healthy yield of rice.

Materials and Methods

A field experiment was conducted at research field of the Department of Soil Science and Agricultural Chemistry, JNKVV, on rice (IR-64) inoculated with *Azospirillum* and the isolate was obtained from the project AINP on Soil Biodiversity and Biofertilizers (ICAR), JNKVV, Jabalpur, M.P., India. All the technical efforts were endeavored to maintain the *Azospirillum* population to up the standard 10^{-8} to 10^{-9} cfu g^{-1} or ml^{-1} inoculants.

The recommended dose of fertilizer N: P_2O_5: K_2O was applied @ 120:60:60 kg ha^{-1} for rice crop in the form of urea, single super phosphate (SSP) and muriate of potash (MOP). Urea and MOP were supplemented as basal applications to each plot as per recommendation and SSP was applied as per scheduled dose of treatments. Rice seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide @ 2 g kg^{-1} seed. Seeds were allowed to air dry under shade. Then the seeds were inoculated individually with the bioinoculant *Azospirillum* carrier inoculants at double the recommended dose 20 g kg^{-1} of seed, respectively using sterilized gum acacia (2%) as adhesive. The soil surface (0-15 cm) samples were collected from the experimental site before sowing of rice crop and after harvest. The soil samples were air dried and crushed with wooden pestle and mortar and sieved through 2 mm sieve.

The material passed through the sieve was used for determination of various characters. Soil pH was determined in 1:2.5 soils-water suspensions, Piper (1950) using Systronics pH meter. Determination of organic carbon was done by Walkley and Black's rapid titration method (1934) as described by Piper (1950). Available N in soil was determined by using alkaline permanganate method Subbiah and Asija (1956). Total content of nitrogen was determined by the modified Kjeldahl method as described by Jackson (1967).

Number of grains per ear and their test weight were calculated at maturity. 1000 grains were counted from the sample taken from each plot. These counted grains were weighed and recorded as test weight (g) at 12% moisture level. Kjeldahl method was adopted for determination of nitrogen content in straw and grain as described by Jackson (1973).

Nutrient Uptake (kg ha^{-1})

Total N uptake of rice was calculated in kg ha^{-1} in relation to dry matter production yield ha^{-1} by using the following formula.

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content in grain \% x Grain yield (kg/ha)}}{100}$$

Crop was harvested and bundles were made plot wise, after drying in the plot for 2-3 days and then weighed. Manual threshing was done plot wise and grain and straw yields were recorded.

Microbial Study of Rhizospheric Soil Sample

Samples of soil were used as fresh as possible without grinding, sieving or any modifications. The collected samples were kept in low density polyethylene bags and stored in refrigerator at 4 ºC.

Serial Dilution (Pour Plate Method)

Plant root and soil samples collected periodically for microbial study were processed for serial dilution by suspending 10 g of soil sample in 90 ml sterilized water in flasks and were shaken thoroughly and this resulted 10^{-1} dilution. Subsequent serial dilutions were made up to10^{-9} dilution for plating purpose.

Plating

One ml aliquot of each 10^{-6} to 10^{-9} dilutions was aseptically taken in a sterilized Petri plate followed by pouring nfb molten medium (15 ml) and swirling the plate uniformly and let the plate medium solidify. The plate covered with cover plate and kept upside down in incubator at 37±2 ºC for 3-4 days till the characteristic growth of the microorganism was observed. Plating was performed in triplicate for each dilution. The medium for this purpose was prepared using the following composition.

Nitrogen free Bromothymol Blue (NFB) Medium for *Azospirillum (*Dobereiner *et al.,* 1976)

$MnSO_4.H_2O$-0.01g, $MgSO_4.7H_2O$-0.02g, NaCl-0.10g, $CaCl_2$-0.02g, Na_3MoO_4-0.002g, Distilled water-1000 ml, Bromothymol blue (0.5%)-20 ml, Agar-agar-15g, Malic acid-5.0g, KOH-4.0g, K_2HPO_4-0.5g, $FeSO_4.7H_2O$-0.05g. Adjust the pH at 6.8-7.0 by using KOH solution (4.5g KOH dissolve in 1 liter distilled water).

The serial dilutions obtained from soil samples collected initially and at different growth stages of the crop were used for azospiral counting adopting pour plate method. Aliquot of 1 ml from each soil dilution (10^{-6} to 10^{-9}) was aseptically taken in a Petri plate, to this 15 ml of melted (NFB) media was poured within an aseptic environment of laminar air flow chamber. After pouring of media, plates were rotated gently clockwise and anti-clock wise to mix the soil dilution with the media. After solidification of media the plates were incubated upside down at 37±2ºC for 3-7 days till the characteristic growth of the microorganism was observed. The colony growth in round shape with convex elevation mucilaginous was designated as *Azospirillum*. The number colony growth was expressed as cfu g^{-1} dry soil).

$$\text{Viable cells (cfu/g dry soil)} = \frac{\text{No. of colonies}}{\text{Oven dry wt. of 1 g soil}} \times \text{Dilution factor}$$

Results and Discussion

Different treatment combinations did not exert marked influence on the soil properties (*viz.,* soil pH, OC) recorded at 45 DAS and at harvest of the crop (table 1).

Since the present study was concerned to the effect of different levels of nitrogen and *Azospirillum* inoculation in experimental field for only one season, hence remarkable changes in soil properties were not observed. These all are attributed the fact that high buffering capacity of Vertisol could maintain pH of the soil. These results are similar to (Jakhar *et al*., 2018b) stated that soil reactions (soil pH) and organic carbon were statistically unaffected due to application of inorganic fertilizer with biofertilizers inoculation.

While available N at 45 DAS and at harvest stage (Table 1) in soil as influenced by treatment combination of RDN 100% + *Azospirillum* responded the best by 29% and 27% respectively, followed by RDN 75% + inoculated with *Azospirillum* was increased significantly over the control of UFUI. The slight build up of the soil available N could be attributed to increased activity of nitrogen fixing rhizobacteria thereby resulting in higher accumulation of N in the soil. Levels of these were increased over a period of time due to their residual or cumulative effect through addition of these nutrients. These results are in collaboration with the findings of (Yaduwanshi *et al*., 2019) observed increased available N content in soil after the harvest of the crop due application of microbial inoculants as compared to no inoculation.

Rice field exerted significant variation in *Azospirillum* population (table 2). The population of *Azospirillum in rhizosphere at 45, 65 DAS and at harvest of the paddy* responded maximum by 2.28, 2.07 and 2.05 log fold *increased with* RDN 100% + *Azospirillum over the control UFUI (*3.36 log cfu g^{-1} = 1.9×10^3 cfu g^{-1}, 3.80 log cfu g^{-1} = 6.76×10^3 cfu g^{-1} and 3.78 log cfu g^{-1} = 6.03×10^3 cfu $g^{-1}$of rhizospheric soil, on oven dry wt. basis), respectively.

Different *Azospirillum* species were reported to positively influence the development and productivity of a wide variety of plant species, being by far the most studied plant growth promoting biofertilizers (PGPB) group and considered a model for studies in plant-bacteria interactions (de-Bashan *et al*., 2016). It has also been stated that to promote the growth of a particular plant species, there is a need for Azospirilla build up high populations in the rhizosphere to efficiently colonize the root system.

Table 1. Effect of *Azospirillum* inoculation and nitrogen level on soil pH, organic carbon and available nitrogen of rice

Treatment	Soil pH		Organic Carbon (gkg^{-1})		Available Nitrogen ($kgha^{-1}$)	
	45 DAS	Harvest	45 DAS	Harvest	45 DAS	Harvest
UFUI	7.8	7.7	5.07	5.23	188.9	189.7
RDN50% + UI	7.8	7.7	5.27	5.75	212.5	213.3
RDN75% + UI	7.9	7.7	5.35	5.93	219.9	220.7
RDN100% + UI	7.8	7.8	5.63	6.17	236.3	236.1
UF + Azosp	7.7	7.7	5.17	5.50	199.9	201.1
RDN50% + Azosp	7.5	7.5	5.40	6.07	231.5	232.9
RDN75% + Azosp	7.6	7.6	5.80	6.35	240.0	239.1
RDN100% + Azosp	7.6	7.7	6.23	6.40	243.7	242.5
SEm±	0.46	0.46	0.40	0.39	13.74	14.01
CD at 5%	NS	NS	NS	NS	41.68	42.50

RDN = Recommended dose of nitrogen, Azosp = *Azospirillum*, NS = Non significant

Table 2. Effect of *Azospirillum* inoculation and nitrogen level on *Azospirillum* population in rhizospheric soil of rice

Treatment	*Azospirillum* Population (cfu g^{-1} soil)		
	45 DAS	65 DAS	Harvest
UFUI	3.36 (2.29×10^{3})	3.80 (6.30×10^{3})	3.78 (6.03×10^{3})
RDN50% + UI	4.58 (3.80×10^{4})	4.88 (7.59×10^{4})	4.68 (4.79×10^{4})
RDN75% + UI	5.09 (1.23×10^{5})	5.48 (3.01×10^{5})	5.46 (2.88×10^{5})
RDN100% + UI	5.67 (4.67×10^{5})	5.89 (7.76×10^{5})	5.85 (7.07×10^{5})
UF + Azosp	4.50 (3.16×10^{4})	4.60 (3.98×10^{4})	4.58 (3.80×10^{4})
RDN50% + Azosp	6.17 (1.48×10^{6})	6.46 (2.88×10^{6})	6.45 (2.82×10^{6})
RDN75% + Azosp	7.19(1.55×10^{7})	7.31(2.04×10^{7})	7.22 (1.66×10^{7})
RDN100% + Azosp	7.67 (4.67×10^{7})	7.88 (7.59×10^{7})	7.75 (5.62×10^{7})
SEm±	0.35	0.39	0.35
CD at 5%	1.06	1.18	1.06

Table 3. Effect of *Azospirillum* inoculation and nitrogen level on nitrogen content in plant and its uptake by crop

Treatment	N Content (%)			Total N Uptake by Crop (kg ha^{-1})
	Plant (at 65 DAS)	Grain	Straw	
UFUI	0.56	1.03	0.52	48
RDN50% + UI	0.65	1.16	0.61	71
RDN75% + UI	0.70	1.17	0.66	79
RDN100% + UI	0.75	1.31	0.70	89
UF + Azosp	0.60	1.08	0.55	52
RDN50% + Azosp	0.74	1.22	0.69	82
RDN75% + Azosp	0.78	1.49	0.74	99
RDN100% + Azosp	0.82	1.55	0.81	110
SEm±	0.03	0.03	0.02	2.34
CD at 5%	0.12	0.09	0.05	7.08

Table 4. Effect of *Azospirillum* inoculation and nitrogen level on number of grains ear^{-1}, test weight and yield of rice

Treatment	No. of Grains Ear^{-1}	Test Weight (g)	Yield (kg ha^{-1})	
			Grain	Straw
UFUI	60	15.90	2097	3681
RDN 50% + UI	70	22.15	3319	4792
RDN 75% + UI	75	23.30	3444	4806
RDN 100% + UI	78	24.90	3742	5041
UF + Azosp	65	18.80	2431	4667
RDN 50% + Azosp	77	24.11	3486	5028
RDN 75% + Azosp	86	25.50	4417	5133
RDN 100% + Azosp	90	26.23	4486	5819
SEm±	4.63	1.30	212.63	311.62
CD at 5%	14.06	3.95	644.94	945.21

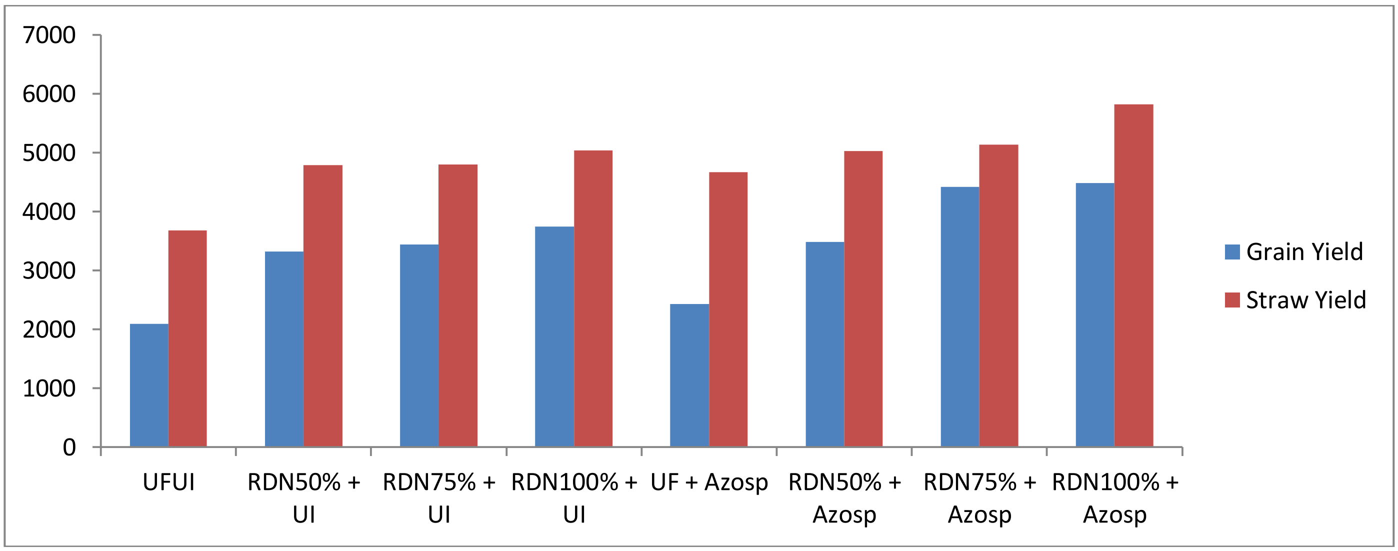

Fig 1. Influence of different levels of nitrogen and *Azospirillum* inoculation on yield of rice

In doing this, the inoculated bacteria can surpass the native soil microbial communities in the competition for the resources made available through root exudates, leading to proper establishment and cooperation with the host plant, as well as the efficient expression of the intended beneficial effect (Bashan *et al*. 2014, Pereg *et al*., 2016). Similar results were also recorded (Jakhar *et al*., 2018) they reported that biofertilizer inoculation significantly increased the population of *Rhizobium* in rhizospheric soil. *Azospirillum* significantly influenced N content in plant, grain and straw of rice. The application of RDN 100% + *Azosp* resulted increased N content in plant, grain and straw by 46%, 50% and 55% respectively, *over the control UFUI, and it was* followed by RDN 75% + *Azosp*.

The similar trend of N content in plant, grain and straw was reported by (Yaduwanshi *et al*., 2019) stated that application of microbial consortia increased N content in plant, grain ant straw of soybean. *Azospirillum* influenced total N uptake by rice plant significantly. The application of 100% + *Azosp.,* resulted significantly increased total N uptake by plant 129%, respectively *over the control UFUI, ant it was* followed by RDN 75% + inoculated. *Azospirillum* inoculation showed better results in yield contributing characters of rice like grains ear^{-1} and 1000 grain weight (tables 4). Maximum number of grains ear^{-1} was increased considerably due to application of RDN 100% + *Azosp.,* inoculated responded by 50% *over the control UFUI, and this* was followed by RDN 75% + *Azosp.,* inoculated.

Grain weight was considerably affected with the treatments combination of RDN 100% + *Azosp.,* by 64% *over the control UFUI*, followed by the performance of the treatment combinations of RDN 75% + *Azosp.* Grain and straw yield of rice were increased significantly due to chemical nitrogen and Azospirillum application. The application of RDN 100% + *Azosp.,* resulted significantly increased grain and straw by 113 and 58%, respectively over the control of UFUI followed by the performance of the RDN 75% + Azosp., Growth promoting effects of biofertilizer inoculation are mainly derived from morphological and physiological changes in inoculated sorghum roots and enhancement in water and plant nutrient uptake was described by (Sarig *et al*., 1988). Grains ear^{-1} and 1000 grain weight of rice were enhanced due to plant growth promoting bacterial inoculation.

Increment of yield components through *Azospirillum* inoculation was stated by several workers (Mathews *et al*., 2006). *Azospirillum* biofertilizer increased grain and straw yield of rice significantly due to combined effect of nitrogen fixation, growth promoting substances secretion and nutrient uptake. It was reported that significant increment of rice yield through *Azospirillum* inoculation (Ashrafuzzaman *et al*., 2009, Govindan and Varma, 2004, Subashini *et al*., 2007). They reported that 80% recommended nitrogen application along with *Azospirillum* inoculation resulted statistically similar grain and straw yield of rice through 100% N applied with inoculation. Similar results were also described by Govindan and Varma, 2004. Biofertilizer application showed significantly higher N, P and K uptake in grain and straw of rice. This higher nutrient uptake was occurred due to the activity of *Azospirillum* inoculants along with nitrogen fertilizer application which was reported by (Lucy *et al*., 2004) where they reported that increased uptake of nutrient such as N, P and K which considered as one of the mechanisms by which PGPR increased crop yield.

Conclusion

The improvement in soil, plant parameters and yield components of rice under study was plausibly attributed to supplementation of nitrogen at moderate level along with inoculation of *Azospirillum* in combination which made nitrogen and other nutrients and growth factors more available either abiotic and/or biotic means. Further, *Azospirillum* inoculation might biologically increased production of more plant biomass and development of the root system and finally the yield. Development of root system might increase root length and number of lateral roots, root density and more number of root hairs and its length and consequently the volume of root zone, these all dramatically contributed in greater uptake ability and utilization of nutrients and water.

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