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To assess the effect of different levels of nitrogen and inoculation of *Azospirillum* on crop growth and yield of paddy

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Abstract
The present investigation entitled "Influence of different levels of nitrogen and *Azospirillum* inoculation on direct seeded rice in a Vertisol" was carried out in the experimental filed (Vertisol) of the Department of Soil Science & Agrilcultural Chmeitry, JNKVV, Jabalpur during Kharif 2015. The experiment was laid out with total 8 treatment combinations replicated thrice under randomized block design in different 24 plots each of 3 m x 4 m. The treatments were constituted with different combinations of 3 levels of fertilizer nitrogen (50, 75 and 100% of recommended dose of N) and inoculation of carrier based *Azospirillum* (as inoculated and uninoculated) including one control of unfertilized and uninoculated. The response from the treatment of RDN75%+*Azospirillum* was recorded statistically superior in enhancing growth of the crop viz., length of root and shoot, chlorophyll content, number of tiller/hill and other plant growth parameters. The same treatment combination of RDN75%+*Azospirillum* was found statistically and economically best to increase yields of grain and straw of paddy by 110.58 and 39.44%, respectively. While the treatment of RDN100%+*Azospirillum* exhibited numerically higher values for the above parameters but these were at par to that of RDN75%+*Azospirillum*.

Keywords: nitrogen inoculation *Azospirillum* yields paddy

Introduction
Rice (*Oryza sativa* L.) being one of the richest starch foods is consumed by about half of the world population. Production of rice in India ranks second among the food grain, and half of the world population sustain on rice. It provides highest (26.2%) calories (FAO 2009) [9]. India alone produces nearly one fourth of the rice produced in the world. Globally India ranks first in respect of area (45.35 m ha) and second in production 95.32 m ha^{-1} tones (Anonymous, 2010) [3]. It contains 80% carbohydrate, 7% protein and 4.74% fat. *Azospirillum* are Gram-negative free-living nitrogen-fixing rhizospheric bacteria. They display a versatile C- and N-metabolism, which makes them well adapted to establish in the competitive environment of the rhizosphere. The genus *Azospirillum* comprises free-living, nitrogen-fixing bacteria that are known as plant growth-promoting rhizobacteria (PGPR), which can colonize by adhesion, the root surface or the intercellular spaces of the host plant roots. Several species of *Azospirillum* are able to secrete phytohormones such as auxins, gibberellins, cytokinins, and nitric oxide for plant growth promotion (Fibach-Paldi, *et al.* (2012) [10] and Kochar and Srivastava, (2012) [16]. The improvement in growth parameters and yield components of paddy 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) [17]]. 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) [11].

Material and Methods
The experiment was conducted at the research field Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur. Jabalpur (MP). The field had proper drainage facility in order to remove excess irrigated water during the experimentation. The soil used for the experimental purpose belongs to the soil order Vertisols popularly known as deep black soil.

Shoot and root lengths were measured at 45, 65 DAS and maturity by carefully uprooting 3 plants per plot. The roots were washed in the running water. Shoot and root lengths were measured by using a scale. Total Leaf chlorophyll was estimated by acetone extraction method (Yoshida *et al*., 1972) [20]. Total Chlorophyll (mg g^{-1}) = CH (a) + CH (b)**.** Percent protein content in grain was estimated through assaying total nitrogen (Kjeldhal method) and protein content was computed by multiplying the nitrogen with factor 6.25 (AOAC, 1970). Number of tillers per hill was counted at 45, 65 DAS and at maturity. Number of effective tillers was counted at 65 DAS and at maturity. 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. 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.

3. Results and Discussion

Root Length

The data of root length at 45 DAS varied from 4.5 to 8.5 cm with the mean value of 6.51 cm. All the treatments (except RDN50%+uninoculated and unfertilized+inoculated with *Azospirillum*) varied significantly over the control unfertilized uninoculated (UFUI). At par was response of the treatment of RDN75%+inoculated to that of RDN100%+inoculated. Among all the treatments, RDN100%+inoculated responded maximum by 88.88%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 77.77, 62.22, 51.11, 42.25, 22.22 and 8.88%, respectively over UFUI. The data on root length at 65 DAS ranged from 7.4 to 13.5 cm while the mean value was 10.6 cm. Response of all the treatments (except RDN50%+uninoculated and unfertilized+inoculated) was found significantly distinct over the control (UFUI). The effect of treatment RDN75%+inoculated was at par to that of RDN100%+inoculated. Among all the treatments, RDN100%+inoculated responded best by 82.43%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 74.32, 64.86, 54.05, 41.89, 24.32 and 10.81%, respectively over the control of unfertilized +uninoculated. The data on root length at maturity varied from 8.5 to 15.5 cm with the mean value of 12.2 cm. Root length of paddy varied significantly with all the treatments (except unfertilized+inoculated) over the control unfertilized+ uninoculated (UFUI). Response of RDN75%+inoculated was at par to that of RDN100%+inoculated. Among all the treatments, the treatment of RDN100%+inoculated responded best by 82.35%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 74.11, 57.64, 51.76, 35.29, 28.23 and 16.47%, respectively over UFUI.

Shoot length

Table 1 presents the data on shoot length at 45 DAS, 65 DAS and at harvest. Shoot length at 45 DAS of the cop growth ranged from 23.0 to 38.1 cm with the mean value of 30.9 cm. The response with all the treatments (except RDN50%+ uninoculated and unfertilized+inoculated) varied significantly over the control of unfertilized+uninoculated (UFUI). The effect of the treatment RDN75%+inoculated was statistically at par to that of RDN100%+inoculated. Among all the treatment combinations, RDN100%+inoculated enhanced shoot length best by 65.65%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+ inoculated by 58.69, 47.82, 40.43, 30.43, 22.60 and 9.56%, respectively over unfertilized+uninoculated (UFUI). The data on shoot length at 65 DAS varied from 26.0 to 41.0 cm with the mean value of 34.1 cm. All the treatments (except RDN50%+uninoculated and unfertilized+ inoculated) performed statistically better over the control (UFUI). The effect of RDN75%+inoculated was statistically analogous or at par to that of RDN100%+inoculated. The treatment combination of RDN100%+inoculated, among all the treatments, responded best by 57.69%, followed by RDN75%+inoculated, RDN100%+ uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 51.92, 42.30, 38.46, 31.15, 19.61 and 8.07%, respectively over unfertilized+uninoculated (UFUI). The data of shoot length at maturity varied from 30.3 to 45.2 cm with the mean value of 37.7cm. Effects of all the treatments (except RDN50%+uninoculated and unfertilized+ inoculated) varied significantly over the control unfertilized+uninoculated (UFUI). Response of RDN75%+ inoculated was at par to that of RDN100%+inoculated. The treatment combination of RDN100%+inoculated responded the best by 49.17%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 40.26, 34.65, 31.35, 22.77, 9.90 and 6.27%, respectively over the control of unfertilized+ uninoculated.

Table 1: Influence of different levels of nitrogen and *Azospirillum* inoculation on root and shoot length at different growth stages of direct seeded rice

Treatment	Root length (cm)			Shoot length (cm)		
	45 DAS	65 DAS	At maturity	45 DAS	65 DAS	At maturity
UFUI	4.5	7.4	8.5	23.0	26.0	30.3
RDN50% + UI	5.5	9.2	10.9	28.2	31.1	33.3
RDN75% + UI	6.4	10.5	11.5	30.0	34.1	37.2
RDN100% + UI	7.3	12.2	13.4	34.0	37.0	40.8
UF + Azosp	4.9	8.2	9.9	25.2	28.1	32.2
RDN50% + Azosp	6.8	11.4	12.9	32.3	36.0	39.8
RDN75% + Azosp	8.0	12.9	14.8	36.5	39.5	42.5
RDN100% + Azosp	8.5	13.5	15.5	38.1	41.0	45.2

Mean	6.5	10.6	12.2	30.9	34.1	37.7
$SE_m\pm$	0.39	0.63	0.65	1.79	1.97	2.21
$CD_{5\%}$	1.19	1.92	1.98	5.41	5.97	6.71
CV(%)	10.43	10.30	10.35	10.00	10.00	10.18

Chlorophyll content

Table 2. Exhibits the data on total chlorophyll content at 45 DAS and 65 DAS. The data on chlorophyll content at 45 DAS varied from 1.60 to 2.80 mg g^{-1} leaf with the mean value of 2.21 mg g^{-1} leaf. Response of all the treatment combinations (except unfertilized+inoculated) varied significantly over the control of unfertilized+uninoculated (UFUI). Effect of RDN75%+ inoculated was at par to that of RDN100%+inoculated. Among all the treatments, the treatment combination of RDN100%+inoculated responded the best by 75.00%, followed by RDN75%+inoculated, RDN100%+ uninoculated, RDN50%+inoculated, RDN 75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 62.50, 50.00, 45.00, 39.37, 24.37 and 9.37%, respectively over the control (UFUI). The data on chlorophyll content at 65 DAS varied from 1.70 to 2.93 mg g^{-1} leaf with the mean value of 2.34 mg g^{-1} leaf. All the treatments (except unfertilized+inoculated) exhibited significantly distinct effects over the control. However, the effect of RDN 75% + inoculated was at par to that of RDN100%+inoculated. Among all the treatments RDN100%+inoculated responded best by 72.35%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 60.00, 47.05, 42.35, 36.47, 27.05 and 17.64%, respectively over UFUI. These growth parameters followed increasing trend till maturity and thereafter the trend was declining. This was concluded with the reason that vegetative growth parameters were almost progressively increased, and then marginally declined up to reproductive phase, and ultimately sharp declined towards maturity with drying and falling of leaves associated with reduced formation of photosynthates and uptake of plant nutrients from soil. Similar results were also reported by Boa *et al.* (2013) [5], Zaheen *et al.* (2006) [21],Islam *et al.* (2008) [12], Jordao *et al.* (2010) [14], and Kannan and Ponmurugan (2010) [15].

Protein content in grain

Table 2 Exhibits the data on protein content in grains of paddy at harvest. The data of protein content in grain varied from 6.04 to 9.96% with the mean value of 7.80%. All the treatment combinations varied significantly over the control unfertilized+ uninoculated (UFUI). The effect of RDN75%+inoculated with *Azospirillum* was at par to that of RDN100%+inoculated with *Azospirillum.* Among all the treatments, RDN100%+inoculated responded the best by 64.90%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 54.13, 35.59, 25.99, 21.35, 19.70 and 11.75%, respectively over the control of unfertilized+ uninoculated. Nitrogen is the essential componenet of amino acids, proteins and nucleoprotein and enzymes. Protein content was enhanced by *Azospirillum* inoculation supplemented with moderated dose of inorganic nitrogen that also increased the production of shoot biomass and yield. These results are in collaboration with the finding of Sayed El *et al.* (2015) [19], BPTPI (2010) [7] and Huergo *et al.* (2008) [11].

Table 2: Influence of different levels of nitrogen and *Azospirillum* inoculation on total chlorophyll content at different growth stages of direct seeded and Protein content in rice grain.

Treatment	Total chlorophyll content (mg g^{-1})		Protein content in grain (%)
	45 DAS	45 DAS	
UFUI	1.60	1.70	6.04
RDN50% + UI	1.99	2.16	7.23
RDN75% + UI	2.23	2.32	7.33
RDN100% + UI	2.40	2.50	8.19
UF + Azosp	1.75	2.00	6.75
RDN50% + Azosp	2.32	2.42	7.61
RDN75% + Azosp	2.60	2.72	9.31
RDN100% + Azosp	2.80	2.93	9.96
Mean	2.21	2.34	7.80
$SE_m\pm$	0.10	0.12	0.18
$CD_{5\%}$	0.33	0.36	0.66
CV (%)	10.09	10.72	10.52

No. of tiller hill^{-1} and effective tiller hill^{-1}

Table 3 presents the data on number of tillers hill^{-1} at 45 DAS and number of effective tillers hill^{-1} at 65 DAS and at maturity. The data of number of tillers at 45 DAS varied from 5.1 to 9.7 tillers hill^{-1} with the mean value of 7.3 tillers hill^{-1}. All the treatments (except RDN50%+uninoculated and unfertilized+inoculated with *Azospirillum*) effected significantly to increase number of tillers over the control of unfertilized+ uninoculated (UFUI). However, the response of treatment combination of RDN75%+inoculated showed statistically at par to that of RDN100%+ inoculated with *Azospirillum.* The treatments combination of RDN100%+inoculated responded the best by 88.49%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 75.43, 61.20, 57.30, 26.70, 18.90 and 13.64%, respectively over the control. The data on number of effective tillers at 65 DAS varied from 6.2 to 10.8 tillers hill^{-1} with the mean value of 8.4 tillers hill^{-1}. The positive response on effective tillers hill^{-1} was statistically significant with all the treatment combinations (except RDN50%+uninoculated and unfertilized+inoculated) varied significantly over the control of unfertilized+uninoculated (UFUI). However, the effect of RDN75%+inoculated was at par to that of RDN100%+inoculated. Among all the treatments, RDN100%+inoculated responded the best by 73.35%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN 75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 58.06, 51.60, 41.9, 33.87,17.74 and 11.29%, respectively over unfertilized+uninoculated. The data of no. of effective tiller hill^{-1} at maturity varied from 6.5 to 11.5 tillers hill^{-1} with the mean value of 8.9 tillers hill^{-1}. The number of effective tiller hill^{-1} was significantly increased with all the treatment combinations (except RDN50%+uninoculated and unfertilized+ inoculated) as compared to the control of unfertilized+uninoculated (UFUI). While, the effect of RDN75%+inoculated was at par to that of RDN100%+inoculated. Among all the treatment

combinations, RDN100%+inoculated responded best by 76.9%, followed by RDN75%+inoculated, RDN100%+ uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+ uninoculated and unfertilized+inoculated by 66.15, 56.92, 44.61, 30.76,13.84 and 9.23%, respectively over unfertilized+ uninoculated. The probable reason might be ascribed to greater accumulation of carbohydrates, protein and their translocation to the reproductive organs which in turn maximized the yield components. Increase in yield attributes under higher concentration of nitrogen particularly RDN75%+*Azospirillum* might be due to the favorable effect of *Azospirillum* inoculation on the availability of nutrients to the crop, which enhanced the parameters of effective tillers hill^{-1}. It means that *Azospirillum* played an important role in paddy generative growth and therefore made a significant increase in the No. of grains ear^{-1}. Similar results were also published by Boa *et al.* (2013) [5], Akhter *et al.* (2004) [2], Rothballer *et al.* (2005) [18] Bera and Pramanik (2010) [6] and Awan *et al.* (2011) [4].

Table 3: Influence of different levels of nitrogen and *Azospirillum* inoculation on number of tillers at different growth stages of direct seeded rice

Treatment	No. of tiller hill^{-1}	No. of effective tiller hill^{-1}	
	45 DAS	65 DAS	At maturity
UFUI	5.1	6.2	6.5
RDN50% + UI	6.1	7.3	7.4
RDN75% + UI	6.5	8.3	8.5
RDN100% + UI	8.3	9.4	10.2
UF + Azosp	5.8	6.9	7.1
RDN50% + Azosp	8.1	8.8	9.4
RDN75% + Azosp	9.0	9.8	10.8
RDN100% + Azosp	9.7	10.8	11.5
Mean	7.3	8.4	8.9
$SE_m\pm$	0.44	0.50	0.55
$CD_{5\%}$	1.32	1.51	1.66
CV(%)	10.29	10.24	10.61

No. of grain ear^{-1} and their test weight

Table 4 presents the data on number of grains ear^{-1} and their test weight (g) at maturity of the crop. The data of number of grains per ear varied from 60 to 90 grains ear^{-1} with the mean value 75 grains ear^{-1}. All the treatments (except RDN50%+uninoculated and unfertilized+inoculated) yielded significantly increase number of grains per ear over the control of unfertilized+ uninoculated (UFUI). While, the response of the treatment combination of RDN75%+inoculated was observed statistically at par to that of RDN 100%+inoculated with *Azospirillum.* The treatments combinations of RDN100%+inoculated responded the best by 50.00%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 43.33, 30.00, 28.33, 25.00, 16.66 and 8.33%, respectively over the control of UFUI. The data of test weight of grain (wt. of 1000 grains) varied from 15.90 to 26.23 g with the mean value 22.61 g. Statistically variability to increase in test weight of paddy grains was recorded with all the treatments (except unfertilized+inoculated with *Azospirillum*) over the control of unfertilized+ uninoculated (UFUI). However, the response of RDN75%+inoculated was at par to that of RDN100%+inoculated. The best response was obtained with the treatments combination of RDN100%+ inoculated with *Azospirillum* by 64.96%, followed by the performance of the treatment combinations of RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 60.37, 56.60, 51.63, 46.54, 39.30 and 18.23%, respectively over unfertilized+uninoculated. The probable reason might be ascribed to greater accumulation of carbohydrates, protein and their translocation to the reproductive organs which in turn maximized the yield components. Increase in yield attributes under higher concentration of nitrogen particularly RDN75%+*Azospirillum* might be due to the favorable effect of *Azospirillum* inoculation on the availability of nutrients to the crop, which enhanced the grains ear^{-1} as well as test weight of grains. It means that *Azospirillum* played an important role in paddy generative growth and therefore made a significant increase in the No. of grains ear^{-1}. Similar results were also published by Boa *et al.* (2013) [5], Akhter *et al.* (2004) [2], Rothballer *et al.* (2005) [18] Bera and Pramanik (2010) [6] and Awan *et al.* (2011) [4].

Grain and Straw yields

Table 4 exhibits the data on grain yield and straw yields and presented in kg ha^{-1}. The data of grain yield ranged from 2097 to 4486 kg ha^{-1} with the mean value 3427.78 kg ha^{-1}. All the treatments (except unfertilized+inoculated with *Azospirillum*) increased significantly the grain yield over the control unfertilized+uninoculated (UFUI). However, the treatment combination of RDN75%+inoculated did not responded statistically better as compared to that of RDN100%+inoculated. Among all the treatment combinations, RDN100%+inoculated responded the best by 113.92%, followed by the treatment combinations of RDN75%+inoculated, RDN100%+uninoculated, RDN50%+inoculated, RDN75%+ uninoculated, RDN50%+ uninoculated and unfertilized+inoculated by 110.58, 78.39, 66.23, 64.23, 58.27 and 15.87%, respectrively over unfertilized+uninoculated. The data of straw yield ranged from 3681 to 5819 kg ha^{-1} with the mean value 4870.86 kg ha^{-1}. The data on straw yield showed that all the treatments varied significantly over the control of unfertilized+uninoculated (UFUI). While, the treatment combination of RDN100%+inoculated exhibited statistically non-significant response as compared to that RDN75%+ inoculated. Among all the treatment combinations, RDN100%+inoculated responded the best by 58.08%, followed by RDN75%+inoculated, RDN100%+uninoculated, RDN50%+ inoculated, RDN75%+uninoculated, RDN50%+uninoculated and unfertilized+inoculated by 39.44, 36.94, 36.59, 30.56, 30.18 and 26.78%, respectively over the control of unfertilized+ uninoculated. Increase in the yield with these treatments plausibly due to enhancement of yield components (*viz.*, number of effective tillers hill^{-1}, grains ear^{-1}, sound grains per panicle, panicle length and test weight of grains) and growth parameters (*viz.*, plant height and number of tillers hill^{-1}). Apart from this with the higher concentration of nitrogen+*Azospirillum* might have helped in enhancing the availability of more plant nutrients from abiotic and biotic means which enhanced the yield attributes ultimately resulted in higher yield. These results are in the line of findings presented by Islam *et al.* (2005) [13], Fakir *et al.* (2007) [8] and Rodrigues *et al., (*2008) [17].

Table 4: Influence of different levels of nitrogen and *Azospirillum* inoculation on number of grains per ear and its test weight and grain and straw yields (kg ha^{-1}) at different growth stages of direct seeded rice

Treatment	No. of grains ear^{-1}	Test weight (g) (per 1000 grains)	Yield (kg ha^{-1})	
			Grain yield	Straw yield
UFUI	60	15.90	2097	3681
RDN50% + UI	70	22.15	3319	4792
RDN75% + UI	75	23.30	3444	4806
RDN100% + UI	78	24.90	3742	5041
UF + Azosp	65	18.80	2431	4667
RDN50% + Azosp	77	24.11	3486	5028
RDN75% + Azosp	86	25.50	4417	5133
RDN100% + Azosp	90	26.23	4486	5819
Mean	75	22.61	3427.78	4870.86
$SE_m\pm$	4.63	1.30	212.63	311.62
$CD_{5\%}$	14.06	3.95	644.94	945.21
CV(%)	10.67	10.62	10.74	11.08

Summary and Conclusion

The response from the treatment of RDN75%+*Azospirillum* was recorded statistically superior for enhancing root and shoot length, Chlorophyll content, number of tiller/hill and other plant growth parameters. While the treatment of RDN100%+*Azospirillum* exhibited numerically higher values but it was at par to that of the former. The performance of RDN75%+*Azospirillum* to increase grain yield of paddy was economically best by 110.58% over the control (2097 kg/ha). While the treatment of RDN100%+*Azospirillum* exhibited numerically higher yield, but it was at par. Similarly, yield of paddy straw was statistically best effected with treatment of RDN75%+*Azospirillum* by 39.44% over the control (3681 kg/ha) and the treatment of RDN100%+*Azospirillum* exhibited numerically higher but at par straw yield.

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