

Original Research Article

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Effect of *Arthrobacter* Isolates on Germination, Chlorophyll Content, Nodulation, Yield and Nutrient Uptake by Soybean (*Glycine max*) in a Vertisol

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ABSTRACT

The present investigation was carried out with the objectives to evaluate the efficacy of thirteen short listed *Arthrobacter* isolates under field conditions with special reference to germination, photosynthetic activity, yield, nodulation characters and nutrient uptake by soybean. Field evaluation was done during kharif 2015-16 at Research Field of Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur, M.P. Outcome of the experimental findings revealed that most of the isolates showed their role for early seed germination at 4th and 6th days after sowing (DAS). A visual difference on early germination was noticed in the inoculated plots as compared to fertilizer un-inoculated isolates (FUI). Maximum chlorophyll content was recorded with isolate AR2 (2.25 mg/g fresh leaf) which was 25% higher to FUI (1.80 mg/g fresh leaf). Maximum number of nodules (28/ plant) were formed by isolates AR2 and it was 33% higher to FUI followed by AR4, AR9 and AR1 isolates. On considering the grain and straw yield after harvest it was observed that out of 13 *Arthrobacter* isolates only three were able to contribute significantly towards grain yield over FUI and these isolates were AR2, AR4, AR7. It was maximum (1658 kg/ha) with isolate AR2. It was found that total N, P and K uptake by crop was enhanced due to *Arthrobacter* seed inoculation but looking to significance of isolates over FUI, AR2 and AR7 significantly increased the total N, P and K uptake by crop over FUI. Average performance due to different isolates was 33, 27.9 and 24.8% more over FUI respectively. Looking to over all responses of thirteen isolates it was found that isolate AR2 performed best followed by AR4, AR7 and AR8.

Keywords

Soybean,
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Introduction

Soybean (*Glycine max.*) is known as the “GOLDEN BEAN” of the 20th Century. It accounts for approximately 50% of total

production of oil seed crops in the world. The name soya traces to the Chinese “CHIANG-YIU”, meaning soy sauce and also known as ‘miracle crop’. It has been cultivated since ancient times in countries like Brazil, United

States, India and others. It was introduced to India centuries ago through the Himalayan Routes, and also brought in via Burma (now Myanmar) by traders from Indonesia.

Soybean is rapidly emerging as the most important oil seed crop in India. Soybean has great potential as an exceptionally nutritive and very rich protein food. It can supply the much needed protein to human diets, soybean contains 35-40% protein, 19% oil, 35% carbohydrate (17% of which is a dietary fibre), 5% minerals and several other components including vitamins (Liu, 1997). Owing to its amino acids composition, the protein of soybean is called a complete protein. In Madhya Pradesh the area under soybean cultivation during Kharif 2014 was 55.46 lakh ha with 1086 kg ha⁻¹ yield and 60.25 lakh MT production (SOPA, 2014).

Conn (1928) described a group of bacteria, extremely numerous in certain soils, which have a distinct characteristics, that they appeared as Gram-negative rods in young cultures and as Gram-positive cocci in older cultures. For these bacteria, Conn (1928) created the species *Bacterium globiforme*, which was later renamed as *Arthrobacter globiformis*, belonging to the genus *Arthrobacter*. These were originally described as being highly aerobic, nutritionally non-exacting and capable of liquefying gelatine slowly (Conn and Dimmick, 1947).

Soybean rhizosphere presented greater proportions of *Bacillus*, *Pseudomonas* and *Arthrobacter* genera, with *Bacillus* being prevalent in the non-rhizosphere soil and *Pseudomonas* in the rhizosphere (Cattelan *et al.*, 1998). The maximum nitrogen-fixing activity was observed when wheat was inoculated with mixed cultures of diazotroph bacteria (*Xanthomonas* sp. + *Arthrobacter* sp.) (Biabani, 2012). In the rhizosphere a synergism between various bacterial genera

such as *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Rhizobium* has been shown to promote plant growth of various plants such as peanut, corn, soybean and maize (Dey *et al.*, 2004).

Madhya Pradesh is a soybean state where soybean is grown since 1969 by practicing a conventional method of seed inoculation with *Bradyrhizobia* and application of recommended dose of fertilizers. Little literature is available that *Arthrobacter* plays a beneficial role towards crop production through PGPR effect and no much work has been done on this microorganism. Therefore, several isolation were made from different crop rhizospheres and these isolates were short listed under net house conditions.

The present investigation has been planned to evaluate the efficacy of short listed isolates under field conditions with special reference to germination, yield, chlorophyll content, nodulation characters and also their role uptake of nutrients by crop. By keeping in mind, the present investigation on “Effect of *Arthrobacter* isolates on germination, chlorophyll content, nodulation, yield and nutrient uptake by Soybean (*Glycine max*) in a *Vertisol*” was planned to evaluate the effectiveness of *Arthrobacter* isolates on soybean yield and other growth associated properties.

Materials and Methods

The present investigation was conducted during kharif 2015-16 at the Research field of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur M.P. (India). The soil of the experimental site was *Vertisol* belonging to Montmorillonite, hypothemic family of *Typic Haplusterts* popularly known as “Black Cotton Soil”. To determine the initial chemical properties of soil before layout of experiment, composite sample was prepared by randomly soil sampling (0-15 cm depth)

from different spot of the experimental field. Samples were, air-dried in the shade and then grounded by wooden roller, there after sieved through 2 mm mesh and stored in polyethylene bags. The soil samples thus obtained were subjected to various chemical analyses to assess the various chemical properties of soil.

Thirteen isolates of *Arthrobacter* were provided by Indian Institute of Soil Science, Bhopal, M.P. which were proved to be highly efficient in initial screenings under glass house conditions. These isolates were used for field studies on soybean crop. The isolates were AR1, AR2, AR3, AR4, AR5, AR6, AR7, AR8, AR9, AR10, AR11, AR12 and AR13. All these 13 isolates were selected for evaluation on growth and yield of soybean and also for the uptake of nutrients by crop. 40 gm soybean seed was weighed separately for each plot in clean polythene bags. 1 ml of liquid formulation of each isolate was transferred aseptically to previously sterilized tubes (micro centrifuge tubes). Sterilized gum acacia (2%) was used as sticker solution and 1 ml of this solution was also transferred aseptically to micro centrifuge tubes separately. Seed treatment was done in shade and after it seeds were sown manually as early as possible. Nodulation studies were done at 45 days of sowing by uprooting 3 plants/plot very carefully taking sufficient care to avoid any losses or damage of nodules.

The rhizosphere soil was washed in the running water. After proper washing nodules of plants per plot were counted. After counting, the nodules were detached from the roots and were kept in small paper bags. Then the nodule fresh weight was taken. After that nodules were oven dried in hot air oven at 60°C for (18-20 hrs) 3-4 days (till constant weight) to record their oven dried weight. Plant samples were collected and oven dried

for nutrient content estimation in both grain and straw.

Results and Discussion

Effect of *Arthrobacter* isolates on germination at 4th and 6th days after sowing (DAS) of the crop

Seed inoculation with different isolates of *Arthrobacter* along with recommended dose of fertilizer proved synergistic towards increasing the germination of seed at 4th and 6th days after sowing (Table 1).

Isolates AR2, AR4, AR6, AR8 and AR12 were able to increase seed germination at 4th DAS significantly over FUI. Maximum germination was recorded with AR2 (42%) and all the above stated isolates were statistically at par to it. Considering the average of all isolates it was 35% and it was higher to FUI by 25% while at 6th DAS the highest germination of soybean seed was recorded with AR2 isolate (59%) and it was the only significant treatment over FUI. Considering the average performance of *Arthrobacter* isolates it was 50% which was 10% more to FUI. This result may be due to production of indole acetic acid (IAA), ammonia (NH₃), hydrogen cyanide (HCN), siderophore and catalase enzyme by PGPR like *Alcaligenes*, *Arthrobacter* and *Azospirillum* is mainly responsible to increase germination, and other growth attributes of soybean (Joseph *et al.*, 2007 and Yasmin *et al.*, 2007). *Arthrobacter* had been reported to enhance germination and seedling vigour in tomato (Rathaur *et al.*, 2012), maize (Shahsavani *et al.*, 2009), rice (Mia *et al.*, 2012) and soybean (Kumar *et al.*, 2012). Rhizobacterial isolates that promote seed germination have been designated as emergence promoting rhizobacteria (Chanway, 1997).

Table.1 Efficiency evaluation of *Arthrobacter* isolates on soybean seed germination at 4th and 6th days after sowing) DAS)

Treatment (Different Isolates of <i>Arthrobacter</i>)	Germination (%) of Soybean Crop	
	4 th DAS	6 th DAS
AR1	31	53
AR2	42	59
AR3	35	52
AR4	37	47
AR5	33	50
AR6	37	47
AR7	31	50
AR8	38	49
AR9	32	51
AR10	33	46
AR11	36	47
AR12	39	48
AR13	35	49
FUI	28	45
UFUI	25	40
Sem±	3.1	3.0
CD) P=0.05)	8.8	8.4
CV) %)	15.6	10.5

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Table.2 Efficiency evaluation of *Arthrobacter* isolates on total chlorophyll content and biomass of soybean at 45 DAS

Treatment (Different Isolates of <i>Arthrobacter</i>)	chlorophyll content (mg/g fresh leaf)	Fresh biomass (g/plant)		Oven dried biomass (g/plant)	
		Shoot	Root	Shoot	Root
AR1	2.06	8.34	1.45	2.64	0.53
AR2	2.25	10.27	1.73	2.91	0.63
AR3	1.99	8.71	1.35	2.57	0.45
AR4	2.05	9.92	1.51	2.40	0.52
AR5	1.93	9.85	1.58	2.48	0.59
AR6	2.04	9.75	1.37	2.59	0.52
AR7	1.95	9.76	1.58	2.55	0.55
AR8	1.85	9.70	1.52	2.61	0.48
AR9	2.02	9.11	1.70	2.69	0.55
AR10	1.84	7.77	1.60	2.58	0.53
AR11	2.14	9.50	1.64	2.26	0.59
AR12	2.19	8.91	1.38	2.66	0.52
AR13	2.17	8.77	1.50	2.50	0.52
FUI	1.80	8.64	1.26	2.22	0.54
UFUI	1.38	6.64	1.10	1.71	0.46
Sem±	0.13	0.81	0.14	0.22	0.05
CD (P=0.05)	0.39	2.32	0.39	0.64	0.14
CV (%)	11.81	15.57	15.98	15.62	16.12

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Table.3 Efficiency evaluation of *Arthrobacter* isolates on soybean nodulation and N content (%) at 45 DAS

Treatment (Different Isolates of <i>Arthrobacter</i>)	Nodulation			N content (%)
	No.of nodules /plant	Fresh weight of nodules (g/plant)	ODW of nodules (g/plant)	
AR1	23	0.55	0.20	3.25
AR2	28	0.63	0.22	3.69
AR3	20	0.57	0.17	3.53
AR4	25	0.54	0.18	3.43
AR5	19	0.52	0.21	3.30
AR6	22	0.52	0.19	3.21
AR7	21	0.52	0.21	3.47
AR8	21	0.62	0.21	3.55
AR9	24	0.59	0.22	3.21
AR10	22	0.56	0.20	3.24
AR11	19	0.57	0.19	3.51
AR12	22	0.62	0.21	3.32
AR13	22	0.53	0.17	3.27
FUI	21	0.51	0.17	3.15
UFUI	17	0.43	0.14	2.89
Sem±	2.1	0.05	0.02	0.18
CD (P=0.05)	6.2	0.13	0.06	0.51
CV (%)	17.1	14.80	18.8	9.20

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Table.4 Efficiency evaluation of *Arthrobacter* isolates on grain yield and NPK uptake by grain of Soybean

Treatment (Different Isolates of <i>Arthrobacter</i>)	Grain Yield (kg/ha)	Nutrient Uptake by Grain (kg/ha)		
		N	P	K
AR1	1420	77.23	4.95	23.2
AR2	1658	102.66	6.66	26.5
AR3	1425	80.12	4.94	25.9
AR4	1644	100.21	6.69	27.0
AR5	1459	86.44	5.18	24.3
AR6	1301	78.62	4.90	18.6
AR7	1618	96.07	6.33	28.4
AR8	1475	84.70	5.66	25.3
AR9	1440	82.52	5.08	23.9
AR10	1410	83.55	5.09	22.2
AR11	1391	82.11	4.91	21.0
AR12	1380	81.00	5.18	22.2
AR13	1377	80.22	5.25	24.0
FUI	1301	71.35	4.55	20.0
UFUI	1012	52.17	2.90	14.1
Sem±	82.66	5.7	0.47	2.66
CD(P=0.05)	236.25	16.3	1.35	7.60
CV (%)	10.08	12.0	15.66	19.94

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Table.5 Efficiency evaluation of *Arthrobacter* isolates on straw yield and NPK uptake by straw of Soybean

Treatment (Different Isolates of <i>Arthrobacter</i>)	Straw Yield (kg/ha)	Nutrient Uptake by straw (kg/ha)		
		N	P	K
AR1	5802	138.99	8.3	78.7
AR2	6842	183.38	12.1	115.9
AR3	5978	116.28	7.0	77.6
AR4	6298	167.09	10.2	99.9
AR5	6076	113.87	7.8	97.4
AR6	6366	139.12	9.9	86.3
AR7	6215	127.36	10.8	93.5
AR8	6143	141.15	8.6	77.0
AR9	6032	126.68	7.7	57.2
AR10	5701	124.14	7.2	90.6
AR11	5602	110.55	8.1	68.8
AR12	5285	116.58	7.0	81.4
AR13	5234	134.22	7.7	73.9
FUI	5211	93.84	6.6	67.2
UFUI	3787	55.10	4.1	33.2
Sem±	592.57	17.0	1.27	10.19
CD (P=0.05)	1693.63	48.5	3.62	29.12
CV (%)	17.78	23.3	26.67	22.08

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Table.6 Efficiency evaluation of *Arthrobacter* isolates on total uptake of nutrients (NPK) by crop Soybean

Treatment (Different Isolates of <i>Arthrobacter</i>)	Total NPK Uptake by Crop Soybean (Kg/ha)		
	N	P	K
AR1	216	13	102
AR2	286	19	142
AR3	196	12	104
AR4	267	17	127
AR5	200	13	122
AR6	218	15	105
AR7	223	17	122
AR8	226	14	102
AR9	209	13	81
AR10	208	12	113
AR11	193	13	90
AR12	198	12	104
AR13	214	13	98
FUI	165	11	87
UFUI	107	7	47
Sem±	16.0	1.34	10.25
CD (P=0.05)	45.6	3.84	29.28
CV (%)	13.3	17.34	17.23

FUI: Fertilized un-inoculated isolates; UFUI: Un-fertilized un-inoculated isolates

Effect of *Arthrobacter* isolates on total chlorophyll content and biomass of soybean

At 45 DAS chlorophyll content in soybean leaves was determined and it was found that only two isolates (AR2 and AR12) were able to increase chlorophyll content significantly over FUI (Table 2). Maximum chlorophyll content ('a' + 'b') was recorded with isolate AR2 (2.25 mg/g fresh leaf) which was 25% higher to FUI (1.80 mg/g fresh leaf). On considering the overall performance of *Arthrobacter* isolates the chlorophyll content was 2.03 mg/g fresh leaf which was 2.7% higher to FUI. It seems involvement *Arthrobacter* isolates accelerates the iron uptake in plant cell which helps in nucleic acids metabolism in chloroplast (Sharma *et al.*, 2016) that resulted in better chlorophyll content.

Looking up to the fresh weight of plants at 45 DAS (Table 2) it was seen that all isolates could increase the shoot fresh weight numerically non significantly over FUI. Isolates AR2 maintained its superiority by yielding maximum fresh weight of shoot. With regard to shoot oven dry weight only AR2 isolate was significantly better over FUI. However, other isolates could increase it numerically over FUI. Isolate AR2 maintained its significant superiority for root fresh biomass over FUI but for oven dry weight it was not significant but was maximum among all the isolates. It is apparent from the data that all isolates reflected better performance over FUI although it was statistically non significant. Dell'Amico *et al.*, (2005) revealed that the phytohormone IAA production offers great promise for sustaining the increased crop productivity by increasing the plant biomass. Similar results were found by Arruda *et al.*, 2013 and Upadhyaya *et al.*, 2012. Use of *Arthrobacter* isolates enhance root

development either directly by producing phytohormones or indirectly by inhabiting pathogens through the synthesis of different compounds (Benizri *et al.*, 2001).

Effect of *Arthrobacter* isolates on Nodulation and N content of nodules

Nodulation studies were carried out at 45 DAS i.e. maximum vegetative growth stage where no. of nodules, their fresh and oven dry weight were recorded and nitrogen content was estimated (Table 3). When no. of nodules was considered, it was found that most of the isolates were not able to increase it over FUI except AR2. Maximum number of nodules (28/ plant) were yielded by isolates AR2 and it was 33% higher to FUI followed by AR4, AR9 and AR1 isolates.

On considering fresh weight and dry weight of nodules, none of the isolates were found statistical superiority over FUI but isolate AR2 remained on top by scoring highest fresh weight of nodules among all of the isolates. On taking into consideration the average performance of isolates toward nodulation it was found that no of nodules was 5.4% more and their oven dried weight was 10% more to FUI which clearly indicates the impact of seed inoculation with *Arthrobacter* isolates.

Lowest nitrogen content in nodules was found with FUI (3.15%) while it was highest and statistically significant with isolate AR2 (3.69%) followed by AR8 (3.55%). Out of 13 inoculants treatments (which comprised of different isolates) all are at par with AR2. Most PGPRs belong to the genera *Arthrobacter*, *Bacillus*, *Micromonospora*, *Pseudomonas* and *Streptomyces* stimulates nodulation in legumes which may be due to the production of phytohormones such as auxin, gibberellins and cytokinins possibly a mechanism used by PGPR to enhance nodule formation. Available reports indicate

improved yield of legumes and nodulation when co-inoculated with PGPB compared to inoculation with *Rhizobium* alone (Egamberdieva *et al.*, 2010 and Yadegari *et al.*, 2010).

Effect of *Arthrobacter* isolates on Grain yield and nutrient uptake (NPK) by Soybean

On considering the grain yield (Table 4) after harvest it was observed that out of 13 *Arthrobacter* isolates only three were able to contribute significantly towards grain yield over FUI and these isolates were AR2, AR4, AR7. It was maximum (1658 kg/ha) with isolate no.AR2. When statistical resemblance of AR2 was worked out, it was found that most of the isolates were in resemblance to it except AR1, AR6, AR10, AR11, AR12 and AR13. On working the average performance of all the 13 isolates the grain yield was 1461kg/ha which was 12% more to FUI(1301kg/ha).

With regard to N, P and K uptake by grain it was found that isolates AR2, AR4 and AR7 gave statistically higher N and P uptake over FUI while potassium uptake was significant due to AR7 only. Considering the average performance of *Arthrobacter* isolates it was 20.2%, 19.7% and 20% higher over FUI.

Effect of *Arthrobacter* isolates on straw yield and nutrient uptake (NPK) by Soybean

Straw yield was found non significant (Table 5) due to isolates over FUI but the average performance of isolates (5967 kg/ha) was 14.5% higher over FUI (5211 kg/ha). All the *Arthrobacter* isolates increased NPK uptake numerically over FUI. AR2 isolate maintained its superiority by the maximum NPK uptake by straw along with isolate AR4 and these two isolates commonly increased

the N, P and K uptake by straw over FUI. On considering the average performance of all isolates towards total N,P and K uptake by straw it was 42.6%, 31.0% and 25.7% respectively more over FUI.

Phytohormone IAA production offers great promise for sustaining the increased crop productivity by increasing plant biomass (Dell'Amico *et al.*, 2005). Similar result on grain yield of barley was also observed by Belimov *et al.*, (1995). These rhizosphere bacteria enhance growth of plant and yield by nitrogen fixation, solubilization of phosphorus, production of phytohormones such as auxins (indole acetic acid (IAA)), cytokinins and gibberellins, sequestering of iron by production of siderophores, lowering of ethylene concentration (Kumar *et al.*, 2014). Combined inoculation consistently enhanced growth and yield of crop to a level equal to or greater than that achieved by single inoculation, and far greater than that of the uninoculated control plants. This might be due to the colonizing the hair, cortical cells and enhanced root surface area and consequently more acquisition of nutrients as well as plant hormones

Total nutrients (NPK) uptake by crop Soybean

On perusal of data (Table 6), it was found that total N, P and K uptake by crop was enhanced due to *Arthrobacter* seed inoculation but looking to significance of isolates over FUI no definite trend could be observed except that isolates AR2 and AR7 significantly increased the total N, P and K uptake by crop over FUI. Isolate AR2 was found the best towards total NPK uptake. Average performance due to different isolates was 33, 27.9 and 24.8% more over FUI respectively. PGPR promote the growth of the plant and increase the root surface area or the general root architecture. Plants growing better in turn

release higher amounts of C in root exudates. The release of more C prompts increase in microbial activity, and this process continues in a cycle and thus yield and nutrient uptake by crop is enhanced.

In conclusion from the present field evaluation conducted during kharif 2015-16 at Research Field of Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur, M.P was concluded that most of the isolates showed their role for early seed germination at 4th and 6th days after sowing (DAS). A visual difference on early germination was noticed in the inoculated plots as compared to fertilizer un-inoculated isolates (FUI). Maximum chlorophyll content was recorded with isolate AR2 (2.25 mg/g fresh leaf) which was 25% higher to FUI (1.80 mg/g fresh leaf). Maximum number of nodules (28/ plant) were formed by isolates AR2 and it was 33% higher to FUI followed by AR4, AR9 and AR1 isolates. Yield was maximum (1658 kg/ha) with isolate AR2. It was found that total N, P and K uptake by crop was enhanced due to *Arthrobacter* seed inoculation but looking to significance of isolates over FUI, AR2 and AR7 significantly increased the total N, P and K uptake by crop over FUI. Looking to over all responses of thirteen isolates it was found that isolate AR2 performed best followed by AR4, AR7 and AR8.

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