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Impact of *Rhizobium* isolates on groundnut (*Arachis hypogaea* L.) under field condition

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Abstract
A field experiment was conducted to find out the nutritional efficiency and yield performance of different *Rhizobium* isolates in groundnut var. GG-20 during three consecutive *kharif* seasons of 2016-17, 2017-18 and 2018-19 at Junagadh Agricultural University, Junagadh. The results revealed that the seed treatment of groundnut with *Rhizobium leguminosarum* isolate-1 (10^7 cfu/ml) at the rate of 10 ml/kg of seeds along with soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 75% RD of N (9.4 kg/ha) at the time of sowing gave higher pod yield 1502 kg/ha and net return of ₹ 25439 with ICBR of 1: 8.69. But, the maximum nitrogen use efficiency (72.16) was found in seed treatment of *Rhizobium leguminosarum* isolate-1 (10^7 cfu/g) @ 10 ml/kg of seeds along with soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 50% RD of Nitrogen (6.25 kg/ha) as compared to other treatments.

Keywords: Groundnut, *Rhizobium leguminosarum*, seed treatment, NUE, ICBR

Introduction
Groundnut (*Arachis hypogaea* L.) is an important oilseed crop of the world belongs to the family Fabaceae or Leguminosae. De condole (1886) stated its origin in Brazil (South America) and was introduced in India by the Portuguese in the middle of sixteenth century. The crop is mostly confined to the geographical belt between 40°N and 40°S latitude (Pattee and Young, 1982) [6]. In India, it is one of the most important *kharif* oilseed crop and regarded as 'King of oilseeds'. The crop have a specific place in Indian agriculture because edible oil is the next to food grain in Indian diet. The crop is principally cultivated as an oilseed but considerable quantities are also used directly as human consumption such as other pulses. It is a multipurpose food crop with high nutritious value containing oil, protein and vitamins and its haulm provides a valuable fodder for the livestock.

Rhizobium bacteria are well known for their capacity to establish a symbiosis with legumes. They are inhabitant of root nodules, where they captured atmospheric nitrogen and make it available to the plant. *Rhizobium* inoculation had a pronounced effect on the growth and yield of groundnut (Sajid, *et al.* 2011) [7]. The effectiveness of *Rhizobium* spp. alone or in combination with chemical fertilizers to improve pod yield and other traits in groundnut has been reported by Muhammad *et al*. (2006) [4], Naser *et al.* (2001) [5], Sharma *et al.* (2011) [8], Michael *et al*. (2020) [2] and Mondal *et al.* (2020) [3]. Rhizobium based biofertilizer is an ecofriendly source of nitrogen input in different legume oilseed crops to reduce use of chemical fertilizers and cost of cultivation. Total nine Rhizobium isolates from different area of Saurashtra region were tested for their efficiency to supply nitrogen to groundnut plants in pot trials. Among them, promising two isolates were again evaluated for their nutritional efficiency and yield performance under field condition at the Department of Plant Pathology, JAU, Junagadh.

Materials and Methods
A field experiment was conducted at Research Farm of Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat), India to study the nutritional efficiency and yield performance of *Rhizobium* isolates in groundnut during three consecutive *kharif* seasons of 2016-17, 2017-18 and 2018-19.

The experiment was conducted with different eight treatments *viz.*, (1) seed treatment of *Rhizobium leguminosarum* isolate-1 with recommended dose (RD) of P_2O_5 and K_2O and 50% RD of Nitrogen.

(2) seed treatment of *R. leguminosarum* isolate-1 with RD of P_2O_5 and K_2O and 75% RD of Nitrogen. (3) Seed treatment of *R. leguminosarum* isolate-2 with RD of P_2O_5 and K_2O and 50% RD of Nitrogen. (4) seed treatment of *R. leguminosarum* isolate-2 with RD of P_2O_5 and K_2O and 75% RD of Nitrogen. (5) RD of P_2O_5 and K_2O and 50% RD of Nitrogen. (6) RD of P_2O_5 and K_2O and 75% RD of Nitrogen. (7) RD of N- P_2O_5- K_2O and (8) No any fertilizer (Absolute control).

The experiment was arranged in randomized block design with three replications. The groundnut variety GG-20 was used at the rate of 120 kg/ha. An isolates of *Rhizobium* was used as seed treatment before sowing of crops at the rate of 10 ml/kg seed having cfu 10^7/ml. The shed dried seeds were sown on 02 July in 2016, 28 June in 2017 and 22 July in 2018 in an open furrows spaced at 60 x 10 cm in a gross plot size of 5.00 m x 3.60 m followed by light planking. All agronomical practices were followed as and when required. The calculation of 50 and 75 per cent recommended dose of nitrogen were done by considering the recommended dose as 12.50-25.00-50.00 NPK kg/ha.

The growth characters of plants were taken from randomly selected five plants from each treatment. After harvesting and sun drying the pod and haulm yield were recorded from the net plot area of 4.00 m x 2.40 m and expressed as kg/ha. Shelling percentage was calculated by drawing the sample of 100 g of pods from each treatment using following formula:

Shelling percentage = Weight of kernels/weight of pods x 100

Assessment of nitrogen from plant and soil were determined using standard methods. The population of microbes from the soil was estimated using serial dilution technique. Whereas, nitrogen use efficiency was calculated using standard formula.

Results and Discussion

Effect on growth and yield characters

The pooled results over three consecutive *kharif* season of 2016-17, 2017-18 and 2018-19 on growth and yield attributing characters and determination of nitrogen from soil before sowing and after harvesting of crop alongwith population of microbes from the soil obtained are communicated hereunder.

The perusal of data presented in Table 1 showed that the maximum plant height (55.42 cm) was recorded in seed treatment of *Rhizobium leguminosarum* isolate-1 alongwith recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen, but it was found statistically at par with seed treatment of *R. leguminosarum* isolate-2 alongwith RD of P_2O_5 and K_2O and 75% RD of Nitrogen, seed treatment of *R. leguminosarum* isolate-1 alongwith RD of P_2O_5 and K_2O and 50% RD of Nitrogen and seed treatment of *R. leguminosarum* isolate-2, RD of P_2O_5 & K_2O and 50% RD of Nitrogen with 54.20, 53.43 and 53.11 cm, respectively.

Table 1: Impact of *Rhizobium* isolates on the growth attributing characters of groundnut

S. N.	Treatments	Characters											
		Plant height (cm)				No. of branches per plant				Shelling %			
		2016-17	2017-18	2018-19	Pooled	2016-17	2017-18	2018-19	Pooled	2016-17	2017-18	2018-19	Pooled
1.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 50% RD of N	54.53	51.13	54.63	53.43	8.40	9.40	9.40	9.07	70.33	73.29	71.07	71.56
2.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 75% RD of N	56.13	54.33	55.80	55.42	9.73	9.67	10.07	9.82	71.33	74.33	71.47	72.38
3.	Seed treatment of *Rhizobium. leguminosarum* isolate-2, RD of P & K and 50% RD of N	53.87	52.60	52.87	53.11	7.60	9.00	9.20	8.60	70.33	71.51	69.13	70.33
4.	Seed treatment of *Rhizobium leguminosarum* isolate-2, RD of P & K and 75% RD of N	54.80	52.73	55.07	54.20	8.27	9.53	9.40	9.07	71.00	72.76	71.27	71.68
5.	RD of P & K and 50% RD of N	50.20	43.20	52.40	48.60	7.00	8.53	8.93	8.16	69.67	67.52	68.47	68.55
6.	RD of P & K and 75% RD of N	51.20	47.60	50.80	49.87	7.53	8.60	9.07	8.40	70.33	68.43	67.80	68.86
7.	RD of N- P_2O_5- K_2O	53.93	48.00	54.23	52.06	8.27	9.20	9.33	8.93	71.00	69.30	70.40	70.23
8.	No any fertilizer (Absolute control)	45.67	41.87	46.33	44.62	6.53	6.47	7.47	6.82	67.67	61.54	63.60	64.27
	S.Em +	1.62	2.69	1.83	1.05	0.45	0.24	0.45	0.17	0.52	2.47	1.52	0.84
	C.D. at 5%	4.93	8.16	5.56	2.99	1.36	0.73	1.35	0.48	1.58	7.48	4.60	2.40
	C.V. %	5.35	9.52	6.02	6.11	9.81	4.74	8.49	5.91	1.29	6.12	3.80	3.62
	Y												
	S.Em +				0.64				0.10				0.51
	C.D. at 5%				1.83				0.30				NS
	Y x T												
	S.Em +				1.81				0.29				1.46
	C.D. at 5%				NS				NS				NS

Where, N = Nitrogen, P/P_2O_5 = Phosphorus and K/K_2O = Potash

Seed treatment of *Rhizobium leguminosarum* isolate-1 along with recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen found significantly superior over rest of the treatments and showed maximum number of branches per plant (9.82).

The seed treatment of *Rhizobium leguminosarum* isolate-1 along with recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen exhibited maximum shelling percentage of 72.38, but it was found statistically at par with seed treatment of *R. leguminosarum* isolate-2 along with RD of P_2O_5 and K_2O and 75% RD of Nitrogen, seed treatment of *R. leguminosarum* isolate-1 along with RD of P_2O_5 and K_2O and 50% RD of Nitrogen, seed treatment of *R. leguminosarum* isolate-2, RD of P_2O_5 and K_2O and 50% RD of Nitrogen and RD of N- P_2O_5- K_2O with 71.68, 71.56, 70.33 and 70.23, respectively.

The seed treatment of *Rhizobium leguminosarum* isolate-1 along with recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen found significantly superior over rest of the treatments with maximum pod yield of 1502 kg/ha, but it was remained at par with seed treatment of *R. leguminosarum* isolate-1 along with RD of P_2O_5 and K_2O and 50% RD of

Nitrogen and seed treatment of *R. leguminosarum* isolate-2 along with RD of P_2O_5 and K_2O and 75% RD of Nitrogen with 1411 and 1406 kg/ha of pod yield, respectively (Table 2). Similarly, the seed treatment of *Rhizobium leguminosarum* isolate-1 along with recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen exhibited 3019 kg/ha of haulm yield and remained at par with seed treatment of *R. leguminosarum* isolate-1 along with RD of P_2O_5 and K_2O and 50% RD of Nitrogen with haulm yield of 2797 kg/ha.

Table 2: Impact of *Rhizobium* isolates on yield attributing characters of groundnut

S. N.	Treatments	Characters							
		Pod yield (kg/ha)				Haulm yield (kg/ha)			
		2016-17	2017-18	2018-19	Pooled	2016-17	2017-18	2018-19	Pooled
1.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 50% RD of N	1419	1454	1360	1411	2795	2907	2690	2797
2.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 75% RD of N	1508	1556	1443	1502	3066	3111	2881	3019
3.	Seed treatment of *Rhizobium. leguminosarum* isolate-2, RD of P & K and 50% RD of N	1304	1359	1196	1287	2612	2685	2220	2506
4.	Seed treatment of *Rhizobium leguminosarum* isolate-2, RD of P & K and 75% RD of N	1403	1443	1370	1406	2748	2870	2724	2781
5.	RD of P & K and 50% RD of N	1097	1109	1047	1084	2185	2218	2077	2160
6.	RD of P & K and 75% RD of N	1192	1200	1090	1161	2339	2374	2167	2293
7.	RD of N- P_2O_5- K_2O	1276	1287	1278	1280	2494	2544	2382	2473
8.	No any fertilizer (Absolute control)	1057	926	896	960	2108	1883	1800	1930
	S.Em +	85.54	82.37	67.59	39.59	183.33	166.99	181.43	82.66
	C.D. at 5%	259	250	205	113	556	507	550	236
	C.V. %	11.56	11.05	9.67	9.42	12.48	11.24	13.27	9.94
	Y								
	S.Em +				24.24				50.62
	C.D. at 5%				69.22				145
	Y x T								
	S.Em +				68.57				143.17
	C.D. at 5%				NS				NS

Similar type of results were also reported by Muhammad *et al*. (2006) [4] while studying the efficacy of symbiotic association between three *Rhizobium* strains i.e. B (TAL-1371, C (Tal-1000) and 0 (NC-92) and two groundnut genotypes *viz.* V1 (ICG-4993) and V2 (ICG-732) in a field trial under rain-fed conditions of Pothowar in a semi-arid environment. They found that inoculation increased symbiotic and yield traits of groundnut genotypes. However, different strain showed variable positive effect with respect to genotypes.

Working with effect of different strain of *Rhizobium*, Sharma *et al.* (2011) [8] found significant variation on yield attribute traits of groundnut and revealed that native strains being found active and efficient as compared to exotic strains.

Michael *et al.* (2020) [2] recorded significantly positive interactive influence on pod yield of groundnut in combined application of phosphorus and effective strain of rhizobium (BR 3267). Whereas, Naser *et al.* (2001) [5] revealed higher pod and haul yield with combined treatment of PKS and *Rhizobium* inoculum (BAU strain).

Mondal *et al.* (2020) [3] found highest pod yield, accumulation of NPK and population of bacteria in 75% recommended dose of N and *Rhizobium* application in groundnut.

Population of microbes from soil before sowing and after harvest of crop

The data presented in Table 3 revealed that the groundnut kernels treated with *Rhizobium* isolates at the time of sowing exhibited more number of microbial population from the soil after harvesting of the crop as compared to untreated control. The initial population of microbes from soil before sowing represents more or less the similar number of microbes as it recorded in absolute control treatment where count was calculated from the soil after harvesting of the crop.

Table 3: Initial and final population of microbes from soil (before sowing and after harvesting of crop)

S. N.	Treatments	Final population of microbes (cfu•) from the soil after harvesting								
		2016-17			2017-18			2018-19		
		NA 10^8	PDA 10^5	YEMA 10^7	NA 10^8	PDA 10^5	YEMA 10^7	NA 10^8	PDA 10^5	YEMA 10^7
1.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 50% RD of N	15.8	3.4	59.7	16.9	3.7	68.0	18.3	4.9	73.5
2.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 75% RD of N	19.4	7.9	76.2	20.1	8.7	81.0	23.7	9.5	79.5
3.	Seed treatment of *Rhizobium. leguminosarum* isolate-2, RD of P & K and 50% RD of N	11.6	2.1	37.8	12.4	2.0	42.7	17.4	4.3	46.3
4.	Seed treatment of *Rhizobium leguminosarum* isolate-2, RD of P & K and 75% RD of N	14.9	2.2	52.4	15.7	2.3	57.0	18.6	3.6	45.8
5.	RD of P & K and 50% RD of N	9.4	2.4	26.3	9.9	2.7	21.7	12.4	3.7	24.8

6.	RD of P & K and 75% RD of N	8.6	2.3	18.7	8.8	2.0	17.3	10.5	3.1	13.3
7.	RD of N- P_2O_5- K_2O	8.6	1.5	17.8	9.8	0.7	18.3	11.6	2.4	12.1
8.	No any fertilizer (Absolute control)	6.0	1.1	6.4	5.8	0.3	7.7	7.8	2.0	8.8
		Initial population of microbes (cfu) from the soil before sowing								
		5.6	1.0	6.3	5.5	0.3	7.5	7.6	1.9	8.5

*cfu = colony forming unit

Determination of nitrogen from soil and plant

The perusal of data presented in Table 4 indicated that the seed treatment of *Rhizobium leguminosarum* isolate-1 along with recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen found significantly superior over rest of the treatments with maximum nitrogen content in the soil after harvest of the crop (193.58 kg/ha), but it was remained at par with seed treatment of *R. leguminosarum* isolate-1 along with RD of P_2O_5 and K_2O and 50% RD of Nitrogen, seed treatment of *R. leguminosarum* isolate-2 along with RD of P_2O_5 and K_2O and 75% RD of Nitrogen and seed treatment of *R. leguminosarum* isolate-2, RD of P_2O_5 and K_2O and 50% RD of Nitrogen with 181.38, 177.20 and 174.50 kg/ha of nitrogen from soil after harvest of the crop, respectively.

Table 4: Determination of N from soil and plant after harvesting of crop and nitrogen use efficiency

S. N.	Treatments	Determination of N from soil after harvesting (kg/ha)				Determination of N from plant after harvesting (%)				Nitrogen use efficiency (kg/kg)			
		2016-17	2017-18	2018-19	Pooled	2016-17	2017-18	2018-19	Pooled	2016-17	2017-18	2018-19	Av.
1.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 50% RD of N	177.80	179.83	186.50	181.38	1.69	1.67	1.69	1.68	57.92	84.48	74.24	72.16
2.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 75% RD of N	190.50	196.93	193.31	193.58	1.91	1.78	1.74	1.81	48.11	67.20	58.35	57.81
3.	Seed treatment of *Rhizobium. leguminosarum* isolate-2, RD of P & K and 50% RD of N	173.78	177.22	172.50	174.50	1.73	1.63	1.71	1.69	39.52	69.28	48.00	52.32
4.	Seed treatment of *Rhizobium leguminosarum* isolate-2, RD of P & K and 75% RD of N	171.59	178.45	181.57	177.20	1.83	1.75	1.64	1.74	36.91	55.15	50.56	47.57
5.	RD of P & K and 50% RD of N	150.62	145.53	134.93	143.70	1.34	1.29	1.25	1.29	6.40	29.28	24.16	19.84
6.	RD of P & K and 75% RD of N	152.99	146.05	141.93	146.99	1.36	1.30	1.26	1.31	14.40	29.23	20.69	21.44
7.	RD of N- P_2O_5- K_2O	153.23	149.73	151.70	151.56	1.37	1.32	1.28	1.32	17.52	28.88	30.56	25.60
8.	No any fertilizer (Absolute control)	140.43	139.90	131.63	137.32	1.30	1.26	1.18	1.25	--	--	--	--
	S.Em.±	18.20	10.76	6.21	7.05	0.09	0.05	0.08	0.03				
	C.D. at 5%	NS	32.65	18.85	20.12	0.26	0.16	0.23	0.10				
	C.V. %	19.24	11.35	6.65	12.95	9.45	6.05	9.11	6.63				
	Y												
	S.Em.±				4.32				0.02				
	C.D. at 5%				NS				0.06				
	Y x T												
	S.Em.±				12.21				0.06				
	C.D. at 5%				NS				NS				

Initial N from soil before sowing: 181.88 kg/ha

The seed treatment of *Rhizobium leguminosarum* isolate-1 alongwith recommended dose (RD) of P_2O_5 and K_2O and 75% RD of Nitrogen found significantly superior over rest of the treatments with maximum nitrogen content in plant after harvest of the crop (1.81%), but it was remained at par with seed treatment of *R. leguminosarum* isolate-2 alongwith RD of P_2O_5 and K_2O and 75% RD of Nitrogen with 1.74 per cent of nitrogen from plant after harvest of the crop.

Nitrogen use efficiency

The average of three year results indicated higher nitrogen use efficiency (72.16) was recorded in seed treatment of *Rhizobium leguminosarum* isolate-1 (10^7 cfu/g) @ 10 ml/kg of seeds alongwith soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 50% RD of Nitrogen (6.25 kg/ha) as compared to other treatments.

Economics

Considering the economics (Table 5) of different treatments, maximum net return of ₹ 25439 per hectare with the highest ICBR (1:8.69) was received in the seed treatment of *Rhizobium leguminosarum* isolate-1 alongwith soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 75% RD of Nitrogen (9.4 kg/ha).

Table 5: Economics of various treatments of *Rhizobium* isolates on yield of groundnut

S. N.	Treatments	Pod yield incre-ased over control (kg/ha)	Haulm yield incre-ased over control (kg/ha)	Gross inco-me (₹)	Quantity of input (Bio/fertilizer) (l or kg/ha)				Expenditure (₹)		Total expenditure [Cost of treatment with labour charge (₹/ha)]	Net realization (₹)	ICBR
					Rhizo-bium culture	UREA	SSP	MOP	Cost of Treat-Ment (Bio/ Fertilizer)	Labour Charge (₹/ha)			
1	2	3	4	5	6	7	8	9	10	11	12 (10+11)	13 (5-12)	14 (5÷12)
1.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 50% RD of N	451	867	23763	1.2	14	156	86	2721	550	3271	20492	1: 7.26
2.	Seed treatment of *Rhizobium leguminosarum* isolate-1, RD of P & K and 75% RD of N	542	1089	28746	1.2	20	156	86	2757	550	3307	25439	1: 8.69
3.	Seed treatment of *Rhizobium. leguminosarum* isolate-2, RD of P & K and 50% RD of N	327	576	17019	1.2	14	156	86	2721	550	3271	13748	1: 5.20
4.	Seed treatment of *Rhizobium leguminosarum* isolate-2, RD of P & K and 75% RD of N	446	851	23474	1.2	20	156	86	2757	550	3307	20167	1: 7.10
5.	RD of P & K and 50% RD of N	124	230	6500	1.2	14	156	86	2577	500	3077	3423	1: 2.11
6.	RD of P & K and 75% RD of N	201	363	10497	0	20	156	86	2613	500	3113	7384	1: 3.37
7.	RD of N- P_2O_5- K_2O	320	543	16572	0	27	156	86	2650	500	3150	13422	1: 5.26
8.	No any fertilizer (Absolute control)	0	0	0	0	0	0	0	0	0	0	0	-

Table 6: Price in ₹/kg or litre and labour charge ₹/ha

1.	*Rhizobium isolates*	:	120.00	5.	Selling price of groundnut pods	:	45.00
2.	Urea	:	5.34	6.	Selling price of groundnut haulm	:	4.00
3.	SSP (Single Super Phosphate)	:	8.30	7.	Labour charge for seed treatment of bioinoculants	:	50.00
4.	MOP (Muriate of Potash)	:	14.00	8.	Labour charge for soil application of chemical fertilizers in open furrow	:	500.00

Conclusion

In view of findings on net return and ICBR, seed treatment of *Rhizobium leguminosarum* isolate-1 along with soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 75% RD of Nitrogen (9.4 kg/ha) found effective and economical with maximum pod and haulm yield of groundnut. However, the maximum nitrogen use efficiency (72.16) was found in seed treatment of *Rhizobium leguminosarum* isolate-1 (10^7 cfu/g) @ 10 ml/kg of seeds along with soil application of recommended dose (RD) of P_2O_5 (25 kg/ha) and K_2O (50 kg/ha) and 50% RD of Nitrogen (6.25 kg/ha) as compared to other treatments.

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