E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; 7(8): 181-184
Received: 18-05-2024
Accepted: 27-06-2024

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Selection of effective *Rhizobium* isolates of chickpea (*Cicer arietinum* L.) from Bilaspur and Mungeli districts of Chhattisgarh Plain region

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DOI: https://doi.org/10.33545/2618060X.2024.v7.i8c.1212

Abstract
The study was conducted at Instructional farm, Barrister Thakur Chhedilal College of Agriculture and Research Station, Bilaspur (C.G.), aiming to study the "Selection of effective *Rhizobium* isolates of chickpea (*Cicer arietinum* L.) from Bilaspur and Mungeli Districts of Chhattisgarh Plain Region". The experiment was laid out in Complete Randomized Design (CRD) with 12 treatments, replicated three times. The study assessed the performance of *Rhizobium* seed priming on chickpea growth under pot soil conditions (Unsterilized soil). Treatment with isolate 4 (T_2) resulted in superior plant height (18.20 cm, 26.67 cm and 34.10 cm) 30, 45 and 55 DAS respectively, fresh shoot weight (g/plant) (23.55 g/plant), dry shoot weight (g/plant) (6.01 g/plant), *Rhizobium* population dynamics (5.29 X 10^4 and 6.19 X 10^4), no. of nodules/plant (17.66), fresh weight of nodules (285.17 mg/plant), dry weight of nodules (mg/plant) (113.12 mg/plant), N content (1.35%) and N uptake (8.11 mg/plant) compared to un-inoculated control (T_{12}) at different growth stages. Isolate 4 consistently showed the best performance in terms of plant height, shoot weight, nodule parameters, *Rhizobial* population and N uptake at 55 DAS stages. Therefore, this isolate is recommended as the most effective inoculant for chickpea cultivation in the region.

Keywords: Rhizobium isolate, superior chickpea growth, chickpea, isolate selection, nodulation, nitrogen fixation and yield improvement

1. Introduction
Chickpea (*Cicer arietinum* L.) is commonly called Bengal gram, Chana and also known as the king of pulses, belongs to Leguminosae or Fabaceae family. It originated from South West Asia. It contains 21.1% protein, 61.5% carbohydrate, 4.5% fat, vitamins and minerals such as phosphorus and potassium are also rich in calcium, magnesium, iron, niacin, riboflavin, thiamin, folate and rich in unsaturated fatty acids like oleic and linoleic acid. (Merga and Haji, 2019) [6].
The total chickpea area in India was 11.7 million hectares with a production of 12.5 million metric tons. Madhya Pradesh, Maharashtra, Rajasthan and Karnataka are the major chickpea producing states. The average chickpea productivity in India was 1,276 kg per hectare (Anonymous, 2021) [2].
Chhattisgarh contribution in total area chickpea of India 0.25 million tones (2.72%). Chickpea area, production and yield of Chhattisgarh 302.59 thousand ha, production 267.51 thousand tones and yield 887 kg ha^{-1} (Source: Agricultural statistics Division, Directorate of Economics & Statistics, Department of Agriculture & Cooperation, 2022) [1].
Rhizobia are soil inhabiting, gram-negative bacteria, which are able to establish a nitrogen-fixing symbiotic relationship with legume plants (Young, 1992) [12]. Once the symbiosis is established, the *rhizobia* convert atmospheric N_2 into ammonia to their legume host plant. These bacteria may be considered as Plant Growth Promoting Bacteria (PGPB), since they directly affect plant growth development.
Plants never live alone in soil. They release chemical signals that allow them to interact with other organisms, forming a complex micro ecosystem. Within this system, different plant tissues and surrounding areas are inhabited by diverse communities of endophytic and rhizospheric bacteria. These bacteria can positively influence host plant growth (Long *et al.* 2008) [13].

Rhizobium

Rhizobia are a special class of bacteria that live as symbionts with legumes and fix inert air nitrogen. The major contributions of fixed nitrogen to farming systems come from symbiotic partnerships between legumes and *rhizobia*, which are among the microorganisms that fix N_2. *Rhizobium* is found in soil and aids in the nitrogen fixation process in leguminous plants. It grows nodules and affixes to the leguminous plant's roots. These nodules capture nitrogen from the atmosphere and transform it into ammonia, which the plant can use to thrive and expand. BNF is the biochemical mechanism where *rhizobia* bacterial symbiont of legumes fixes inert atmospheric nitrogen into a plant usable from under the presence of enzyme nitrogenise (Mohammadi and Sohrabi, 2012) [7].

2. Materials and Methods

The investigation was conducted at Instructional farm, Barrister Thakur Chhedilal College of Agriculture and Research Station, Bilaspur (C.G.), it aimed to assess the selection of effective *Rhizobium* isolates of chickpea under complete randomized design (CRD) with three replications and twelve treatments. To assess the performance of selected indigenous *Rhizobium* inoculants seed priming on chickpea growth, no. of nodulation, fresh and dry weight of nodules and straw nitrogen uptake at 55 DAS under pot culture with unsterilized soil conditions. Salt tolerance tests investigating 1%, 2% and 3% sodium chloride concentrations (w/v), and characterization of *rhizobial* isolates under different pH levels (5-9) and temperature regimes (15-50 °C) were performed to select efficient *rhizobia* for inoculation. The selected efficient *rhizobial* isolates were used to inoculate chickpea seeds in the experiment.

Isolation of *Rhizobium* from chickpea growing soil.

Chickpea *rhizobia* were isolated from rhizosphere soil of various chickpea growing area at Bilaspur and Mungeli Districts, (Chhattisgarh).

Serial dilution Method

After the extraction of bacteroids solution from the chickpea serial dilution was done. To get the growth of the *Rhizobium* bacteria about 1 gram of serially diluted root rhizosphere soil were diluted to 9 ml of water in a test tube which served as stock solution. Remaining 9 test tubes were filled with 9 ml of water. Transferring of 1 ml of water from the stock solution to 9 ml of sterilized distilled water with the help of pipettes yielded 10^{-1} dilutions and the series continued upto 10^{-9} dilutions. Sterility is the hallmark of any bacteriological isolation so the entire process was carried in the laminar air flow.

Salt tolerance

The salinity tolerance was assessed by culturing the bacteria on YEMA medium containing different salt concentration 1%, 2%, 3% (w/v) NaCl.

pH tolerance

The ability of *Rhizobial* isolate to grow at different pH was tested in YEMA medium by adjusting the pH to 5.0, 6.0, 7.0, 8.0 and 9.0 with NaOH and HCl.

Temperature tolerance

Temperature tolerance was investigated by assaying the growth of bacterial cultures in YEMA medium at different temperature *viz*. 15 °C, 25 °C, 35 °C, 45 °C and 50 °C.

3. Results

Pot soil conditions (Unsterilized soil).

The data pertaining to effect of seed priming by indigenous isolates of *Rhizobium* against plant height, *Rhizobium* population, Fresh shoot weight (g/plant), Dry shoot weight (g/plant), No. of nodules/plant, Fresh weight of nodules (mg/plant), Dry weight of nodules (mg/plant), Straw N-content (%) and Straw N-uptake. (mg/ plant) of chickpea under pot condition are presented in Table 4.1

Plant height (cm) at 30, 45, and 55 DAS

At 30 days after sowing the significantly highest plant height (18.20 cm) was noticed in treatment T_2. Which was at par with the treatment T_6 (17.50 cm), T_5 (17.33 cm) and $T_{7.}$ (17.23 cm). The significantly lowest plant height (15.43 cm) was observed in treatment T_{12} (Control).

At 45 days after sowing the significantly highest plant height (26.67 cm) was noticed in treatment T_2. Which was at par with the treatment T_6 (26.23 cm), T_5 (25.37 cm) and $T_{7.}$ (25.17 cm). The significantly lowest plant height (23.47 cm) was observed in treatment T_{12} (Control).

At 55 days after sowing the significantly highest plant height (34.10 cm) was noticed in treatment T_2. Which was at par with the treatment T_6 (33.27 cm) and T_5 (33.10 cm), T_1 (32.87 cm), T_3 (32.73 cm), T_{10} (32.73 cm), T_4 (32.70 cm), $T_{7.}$ (32.67 cm), T_8 (32.43 cm) and T_{11} (32.27 cm). The significantly lowest plant height (28.97 cm) was observed in treatment T_{12} (Control).

At all time periods, priming significantly improved plant height over the unprimed control. Isolate 4 gave the tallest plants at 30 and 45 DAS while Isolate 15 facilitated superior growth at 55 DAS, demonstrating *rhizobial* priming promoted early vigor and development under pot conditions as well. The results obtained in the present study is in accordance with the results of Dinesh Kumar *et al.* (2015) [4].

***Rhizobium* population at 30 and 55 DAS**

Based on the data presented in table shown that there was significant highest *Rhizobium* population per gram of soil was observed in treatment T_2 (5.29 X 10^4) at 30 days after sowing. Which was at par with the treatment T_6 (5.18 X 10^4) and T_5 (4.76 X 10^4). While the significantly lowest *Rhizobium* population per gram of soil was observed in treatment T_{12} (control) (1.98 X 10^4).

As per the data presented in table showed that the significant highest *Rhizobium* population per gram of soil was observed in treatment T_2 (6.19 X 10^4) at 55 days after sowing. Which was at par with the treatment T_6 (6.16 X 10^4), T_5 (5.73 X 10^4) and T_7 (5.60 X 10^4). While the significantly lowest *Rhizobium* population per gram of soil was observed in treatment T_{12} (control) (2.35 X 10^4).

Priming with isolates 4, 21 and 15 significantly increased *rhizobial* populations in soil at 30 and 55 DAS compared to the control. Higher *rhizobia* in the root zone indicates priming aided their competitiveness and survival. This favored improved nodulation and symbiotic function, resulting in superior early plant growth under pot conditions. Similar result was also found by Kamithi *et al.* (2009) [5].

Fresh shoot weight (g/plant)

As par the data showed that the significant highest fresh shoot weight (g/plant) was observed in treatment T_2 (23.55 g/plant). Which was at par with the treatment T_6 (23.14 g/plant) T_7 (22.02 g/plant), T_4 (21.56 g/plant), T_5 (21.11 g/plant), T_{11} (21.34

g/plant) T_{10} (21.31 g/plant), T_1 (21.01 g/plant), T_8 (20.69 g/plant) and T_3 (20.60 g/plant). While the significantly lowest fresh shoot weight (g/plant) was observed in treatment T_{12} (control) (16.62 g/plant).

Under pot conditions, priming with isolates 4, 21 and 28 significantly improved fresh shoot weight per plant compared to the unprimed control. Higher biomass accumulation in shoots indicates enhanced symbiotic nitrogen fixation and assimilation when primed. The results demonstrate *rhizobial* priming aided superior growth and photosynthetic production in chickpea shoots. Similar results were also observed by Rajpoot and Panwar (2013) [9].

Dry shoot weight (g/plant)

Based on the data presented in table observed that the significant highest dry shoot weight (g/plant) was observed in treatment T_2 (6.01 g/plant). Which was at par with the treatment T_6 (5.60 g/plant), T_5 (5.49 g/plant) and T_7 (5.32 g/plant). In contrast, the significantly lowest dry shoot weight (g/plant) was observed in treatment T_{12} (control) (3.99 g/plant).

Priming with isolates 4, 21 and 15 significantly improved dry shoot weight per plant under pot conditions compared to the control. Higher biomass conversion in shoots indicates enhanced nitrogen fixation and assimilation when primed. The results demonstrate *rhizobial* priming optimized photosynthetic translocation and promoted superior aboveground growth in chickpea. These outcomes are consistent with findings of Thite *et al.* (2023) [11].

No. of nodules/plant

It is clearly showed from the table the significant highest No. of nodules/plant was observed in treatment T_2 (17.66). Which was at par with the treatment T_6 (17.23), T_5 (16.41), T_7 (16.33), T_4 (16.28), T_1 (15.68), T_3 (15.54), T_8 (15.45) and T_{11} (15.43). While the significantly lowest No. of nodules/plant was observed in treatment T_{12} (control) (11.67).

Priming with isolates 4, 21 and 15 significantly increased the number of nodules per plant compared to the control under pot conditions. More nodule formation indicates enhanced *rhizobial* infection and nodulation facilitated by priming. This favored optimized symbiotic functioning and nitrogen assimilation in chickpea. Similar result was also reported by Singh *et al.* (2014) [10].

Fresh weight of nodules (mg/plant)

It is clearly showed that the significant highest fresh weight of nodules (mg/plant) was observed in treatment T_2 (285.17 mg/plant). Which was at par with the treatment T_6 (282.79 mg/plant), T_5 (277.24 mg/plant) and T_7 (274.95 mg/plant). While the significantly lowest fresh weight of nodules (mg/plant) was observed in treatment T_{12} (control) (161.71 mg/plant).

The fresh weight of nodules per plant was highest for isolate 4, showing it helped develop larger nodules that aid biological nitrogen fixation. Isolate 4 thus helped chickpea plants acquire more nitrogen through its effectiveness in colonizing roots and stimulating nodule formation for maximum growth benefits under pot condition. These outcomes are consistent with findings of Zaman *et al.* (2011) [15].

Dry weight of nodules (mg/plant)

As par the data presented in table showed that the significant highest dry weight of nodules (mg/plant) was observed in treatment T_2 (113.12 mg/plant). Which was at par with the treatment T_6 (110.85 mg/plant) and T_5 (105.32 mg/plant). While the significantly lowest dry weight of nodules (mg/plant) was observed in treatment T_{12} (control) (36.72 mg/plant).

Treatment with isolate 4 resulted in the maximum dry nodule weight per plant compared to other isolates and the unprimed control. Heavier dry nodules indicate isolate 4 enhanced nitrogen-fixing nodule formation. This led to higher availability of fixed nitrogen for chickpea growth under potted soil conditions. Similar results were also observed by Vala *et al.* (2002) [14].

Table 1.1: Effect of seed priming by indigenous isolates of *Rhizobium* against pot soil conditions of chickpea.

Tr. No	Treatments	Plant height (cm)			*Rhizobium* population X 10^4 per gram soil		Fresh shoot weight (g/plant)	Dry shoot weight (g/plant)	No. of nodules/plant	Fresh weight of nodules (mg/plant)	Dry weight of nodules (mg/plant)	Straw N-content (%)	Straw N-uptake (mg/plant)
		30 DAS	45 DAS	55 DAS	30 DAS	55 DAS							
T_1	Isolate 1	16.60	24.50	32.87	4.22	4.61	21.01	4.80	15.68	267.12	97.63	1.30	6.15
T_2	Isolate 4	18.20	26.67	34.10	5.29	6.19	23.55	6.01	17.66	285.17	113.12	1.35	8.11
T_3	Isolate 6	16.67	24.67	32.73	4.37	4.62	20.60	4.67	15.54	264.94	96.09	1.32	6.17
T_4	Isolate 7	16.87	25.03	32.70	4.58	4.65	21.56	4.76	16.28	272.95	102.93	1.32	6.26
T_5	Isolate 15	17.33	25.37	33.10	4.76	5.73	21.11	5.49	16.41	277.24	105.32	1.33	7.31
T_6	Isolate 21	17.50	26.23	33.27	5.18	6.16	23.14	5.60	17.23	282.79	110.85	1.34	7.70
T_7	Isolate 28	17.23	25.17	32.67	4.60	5.60	22.02	5.32	16.33	274.95	104.06	1.32	7.05
T_8	Isolate 36	16.70	24.70	32.43	4.21	4.61	20.69	4.52	15.45	263.96	95.20	1.31	6.12
T_9	Isolate 42	16.33	24.37	31.17	4.01	4.58	18.25	4.47	14.10	257.59	86.85	1.29	5.65
T_{10}	State check	16.80	24.57	32.73	3.99	4.51	21.31	4.62	14.51	259.10	88.89	1.26	5.51
T_{11}	RDF (20:50:30 NPK kg ha^{-1})	17.13	24.70	32.27	2.50	4.36	21.34	4.72	15.43	261.97	93.46	1.27	5.98
T_{12}	Control (Uninoculated)	15.43	23.47	28.97	1.98	2.35	16.62	3.99	11.67	161.71	36.72	0.88	3.52
	C.D. (5%)	1.29	1.61	2.16	0.60	0.76	3.23	0.72	1.63	9.05	3.96	0.03	1.03
	S.E. (m)±	0.44	0.55	0.73	0.20	0.26	1.10	0.24	0.55	3.08	1.35	0.01	0.35

Straw N-content per plant (%)

The data displayed in table clearly indicate that the significant highest straw N-content per plant was observed in treatment T_2 (1.35%). Which was at par with the treatment T_6 (1.34%) and T_5 (1.33%). While the significantly lowest straw N-content per plant was observed in treatment T_{12} (control) (0.88%).

Seed priming with isolate 4 resulted in the maximum straw nitrogen content per plant compared to other treatments and the control. The heavier nodule formation promoted by isolate 4 aided greater biological nitrogen fixation, thereby increasing the nitrogen absorbed and accumulated in the straw for enhanced plant growth and yield. Also, similar results were reported by Chinnaswamy and Dhar (2005) [3].

Straw N-uptake. (mg/plant)

The data depicted in table clearly showed that the significant highest straw N-uptake per plant was observed in treatment T_2 (8.11 mg/plant). Which was at par with the treatment T_6 (7.70 mg/plant) and T_5 (7.31 mg/plant). While the significantly lowest straw N-uptake per plant was observed in treatment T_{12} (control) (3.52 mg/plant).

Treatment with isolate 4 facilitated maximum straw nitrogen uptake per plant compared to other isolates and control. The heavy nodule formation and fixation of nitrogen resulted in more availability and absorption of nitrogen into straw. This indicates isolate 4 has better potential to enhance nitrogen nutrition of chickpea. The finding of present study is in accordance with those of Rokhzadi *et al.* (2011) [8].

4. Conclusion

The study concluded that treatment T_2 involving priming of chickpea seeds with *Rhizobium* isolate 4 consistently exhibited superior performance under unsterilized soil conditions. It registered the tallest plants, highest shoot weights, maximum *rhizobial* population, nodule numbers and weights, as well as highest straw nitrogen content and uptake. Whereas, treatment T_{12} (uninoculated control) showed the poorest results. Thus, isolate 4 can thus be selected as the most effective inoculant for improving chickpea growth and nitrogen nutrition in natural soils.

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