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Effect of efficient *Rhizobium* isolates on seed germination, seedling vigour, nitrogen fixation (*in vitro*) and field performance (*in vivo*) of lentil (*Lens Culinaris* L.) in comparison with a commercial *Rhizobium* strain

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ABSTRACT

Rhizobium inoculation is a low-cost, eco-friendly means of improving nodulation and productivity of lentil (*Lens culinaris* L.). In continuation of an earlier study in which four native *Rhizobium* isolates, designated RLGB1 (Bhadgoun), RLCH3 (Halkarni), RLAU5 (Uttur) and RLBK7 (Kadgaon), were isolated and characterized from lentil root nodules of Kolhapur district, the present investigation evaluated the efficiency of these isolates first under *in vitro* conditions and then in the field, in comparison with the commercial MPKV *Rhizobium* strain. Under *in vitro* conditions, germination and seedling vigour index (SVI) were recorded by the roll-towel paper method at 3, 5 and 7 days after sowing (DAS), and nitrogen fixation was estimated in broth culture seven days after inoculation. Isolate RLGB1 recorded the highest germination (98.00%), root + shoot length (8.73 cm at 7 DAS) and seedling vigour index (855.54 at 7 DAS), followed by RLBK7 (97.00% germination; SVI 695.49), and also recorded the highest nitrogen fixation among the native isolates (8.6 mg/100 ml), next only to the commercial strain (9.1 mg/100 ml). Based on these results, RLGB1 was selected for field evaluation against the commercial strain under three levels of recommended nutrient dose. In the field trial, conducted during the winter season of 2026 at the Plant Pathology and Microbiology Section Farm, Govt. College of Agriculture, Pune, seed treatment with the commercial strain combined with 100% RDF (20:40:40 NPK kg/ha) (T₆) recorded the maximum germination (96.33%), plant population (1.30 lakh/ha), plant height (29.73 cm at 45 DAS), number of branches (9.10 at 45 DAS), root nodules (17.70 at harvest) and grain yield (11.36 q/ha), statistically at par with 75% RDN + 100% RDPK combined with the commercial strain (T₅) (10.88 q/ha). Seed treatment with the efficient isolate RLGB1 at 100% RDF (T₃) also performed well (10.74 q/ha), while treatments without *Rhizobium* seed treatment recorded the lowest germination, growth and yield throughout. These results indicate that integrating *Rhizobium* seed inoculation, whether with an efficient native isolate or a commercial strain, with an adequate nutrient dose can maximize lentil crop performance and support sustainable productivity.

Keywords: *Rhizobium*, Lentil, *Lens culinaris*, Germination, Seedling vigour index, Nitrogen fixation, Commercial strain, RDF, Nodulation, Yield

Introduction

Lens culinaris L., commonly known as lentil or Masur, is an important cool-season pulse valued for its protein-rich seeds and its ability to fix atmospheric nitrogen in symbiosis with *Rhizobium* bacteria, with fixation reported in the range of 10 to 129 kg N/ha depending on environmental conditions, and up to 57-100 kg N/ha under favourable conditions (ICARDA, 1989; Wagner, 2012) [1, 2]. However, effective native *Rhizobium* strains are not present in all soils, which limits nodulation and nitrogen fixation efficiency, and increasing concern over the environmental and health hazards of excessive chemical fertilizer use has intensified the search for eco-friendly biofertilizer alternatives. Since the efficiency of *Rhizobium* strains varies with the source strain, host genotype and agro-climatic conditions, native isolates need to be screened *in vitro* and subsequently validated in the field before they can be recommended as biofertilizer inoculants.

In an earlier phase of this study, four native *Rhizobium* isolates were obtained from root nodules of lentil collected from different locations of Kolhapur district and were characterized on the basis of their morphological, cultural and biochemical properties. Building on that work, the present investigation was undertaken in two stages: first, to assess the efficiency of these isolates in promoting seed germination, seedling vigour index and nitrogen fixation of lentil under laboratory (*in vitro*) conditions and to identify the most efficient isolate; and second, to evaluate this efficient isolate against the commercial MPKV *Rhizobium* strain, seed-

treated at different levels of recommended nutrient dose, on the germination, growth, nodulation and yield of lentil under actual field conditions.

Materials and Methods

The *in Vitro* evaluation was carried out in the laboratory, and the field trial was conducted during the winter season of 2026, both at the Plant Pathology and Agricultural Microbiology Section, Govt. College of Agriculture, Pune, using the four characterized native *Rhizobium* isolates (RLGB₁, RLCH₃, RLAU₅ and RLBK₇), an uninoculated control, and the commercial MPKV *Rhizobium* strain.

Seed germination and seedling vigour index (in vitro)

Healthy, uniform lentil seeds were surface-sterilized and treated with 48-hour-old broth cultures of each isolate before being placed on moist germination paper by the roll-towel method under controlled laboratory conditions. Germination percentage and root + shoot length of seedlings were recorded at 3, 5 and 7 days after sowing (DAS). The seedling vigour index (SVI) was calculated as the product of germination percentage and total seedling (root + shoot) length, following the standard procedure. Each treatment was replicated three times in a Completely Randomized Design (CRD).

Nitrogen fixation assay (in vitro)

The nitrogen-fixing ability of each isolate and of the commercial strain was assessed by growing pure cultures in nitrogen-free broth medium under standard incubation conditions (28 ± 2 °C). The amount of nitrogen fixed in the broth was estimated seven days after inoculation and expressed as mg/100 ml. The experiment was laid out in a Completely Randomized Design (CRD) with three replications.

Field trial

Based on the *in Vitro* results, nine field treatments, comprising three levels of recommended dose of nitrogen and phosphorus-potassium (RDN/RDPK: 50%, 75% and 100% of 20:40:40 NPK kg/ha) combined with seed treatment using the efficient isolate RLGB₁, the com-

mercial MPKV *Rhizobium* strain, or no seed treatment (T₁-T₉, as detailed in Table 3), were evaluated in a Randomized Block Design with three replications. Seed treatment was applied at the rate of 25 ml culture/kg seed prior to sowing. Germination percentage and plant population (lakh/ha) were recorded at establishment; plant height (cm) and number of branches per plant were recorded at 15, 30 and 45 days after sowing (DAS); the number of root nodules per plant was recorded at 50% flowering and at harvest; and grain yield was recorded at harvest and expressed as kg/plot and q/ha.

Statistical analysis

Data on germination, seedling vigour index and nitrogen fixation (in vitro) were analysed by ANOVA for a Completely Randomized Design, while field data were analysed by ANOVA for a Randomized Block Design, following the standard statistical procedure of Panse *et al.* (1985) [3].

Results and Discussion

A. in vitro evaluation

A.1 Effect of efficient *Rhizobium* isolates on seed germination and seedling vigour index of lentil

Data presented in Table 1 revealed that RLGB₁ recorded the highest germination (98.00%) and the maximum root + shoot length at 3 DAS (5.03 cm), 5 DAS (6.40 cm) and 7 DAS (8.73 cm). RLGB₁ also recorded the highest seedling vigour index at 3 DAS (492.94), 5 DAS (627.20) and 7 DAS (855.54), indicating superior seedling growth, followed by RLBK₇ (97.00% germination; SVI of 414.19, 615.95 and 695.49 at 3, 5 and 7 DAS, respectively) and RLAU₅ (93.33% germination; SVI of 460.11, 590.64 and 578.64 at 3, 5 and 7 DAS). RLCH₃ recorded the lowest germination among the isolates (92.33%) with SVI of 237.28, 366.50 and 535.51 at 3, 5 and 7 DAS. All isolates performed significantly better than the uninoculated control, which recorded only 80.33% germination and the lowest seedling vigour index (243.39, 299.37 and 444.22 at 3, 5 and 7 DAS, respectively).

Table 1. Effect of efficient *Rhizobium* isolates on germination and seedling vigour index of lentil (data are mean of three replications)

Sr. No.	Isolate	Germination (%)	Root+shoot length 3 DAS (cm)	Root+shoot length 5 DAS (cm)	Root+shoot length 7 DAS (cm)	SVI 3 DAS	SVI 5 DAS	SVI 7 DAS
1	RLGB ₁	98.00	5.03	6.40	8.73	492.94	627.20	855.54
2	RLCH ₃	92.33	2.57	3.97	5.80	237.28	366.50	535.51
3	RLAU ₅	93.33	4.93	6.33	6.20	460.11	590.64	578.64
4	RLBK ₇	97.00	4.27	6.35	7.17	414.19	615.95	695.49
5	Control	80.33	3.03	3.73	5.53	243.39	299.37	444.22
	SE(m)±	1.15	0.33	0.38	0.52	0.51	0.58	0.61
	CD at 1%	3.64	1.04	1.21	1.63	1.61	1.82	1.93





Plate 1. Effect of Efficient *Rhizobium* isolates on Seedling Vigour Index and Germination of lentil (Towel paper method).

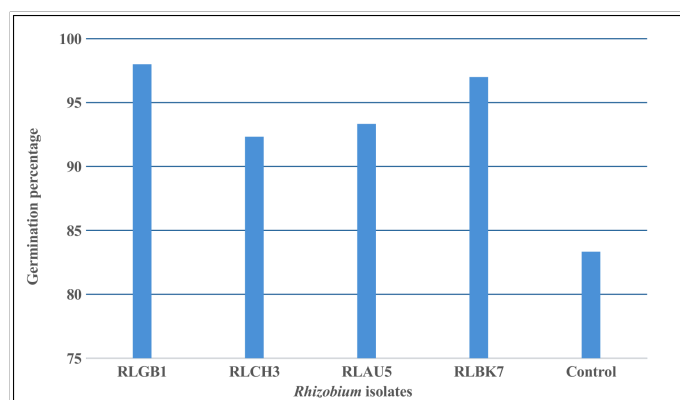


Figure 1. Effect of efficient *Rhizobium* isolates on germination percent of lentil (*in Vitro*)

after inoculation of isolates (*in Vitro*)

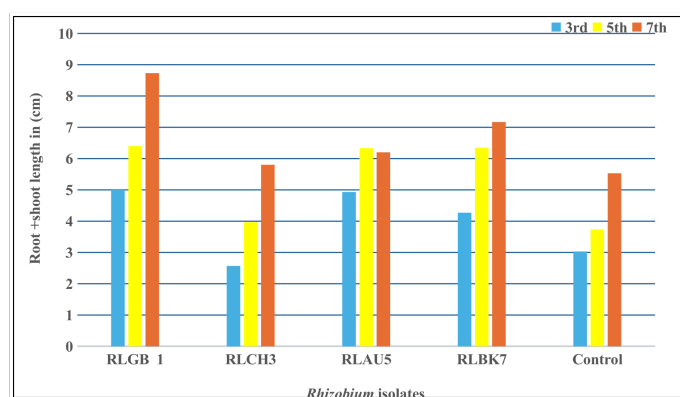


Figure 2. Effect of efficient *Rhizobium* isolates on seedling height of lentil 3rd, 5th and 7th days



Figure 3. Effect of efficient *Rhizobium* isolates on seedling Vigour index of lentil 3rd, 5th and 7th days

7th days after inoculation of isolates (*in Vitro*)

Overall, on the basis of germination, seedling vigour index and root + shoot length, RLGB₁ was identified as the most efficient isolate compared to the other isolates tested. These findings are in agreement with Meena *et al.* (2015), who demonstrated the beneficial effect of *Rhizobium* inoculation on seed germination and seedling vigour in legumes.

A.2 Nitrogen-fixing ability of *Rhizobium* isolates

Table 2. Nitrogen fixation ability of different *Rhizobium* isolates and the commercial strain

Sr. No.	Isolate / strain	N ₂ fixation, 7 days after inoculation (mg/100 ml)
1	RLGB ₁	8.6
2	RLCH ₃	6.6

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Sr. No.	Isolate / strain	N ₂ fixation, 7 days after inoculation (mg/100 ml)
3	RLAU ₅	7.2
4	RLBK ₇	7.4
5	MPKV <i>Rhizobium</i> strain (commercial)	9.1
	SE(m)±	0.32
	CD at 1%	1.02

commercial strain

The nitrogen fixation ability of the isolates ranged from 6.6 to 8.6 mg/100 ml at seven days after inoculation. Among the tested isolates, the highest nitrogen fixation was recorded by the commercial MPKV *Rhizobium* strain (9.1 mg/100 ml), followed by RLGB₁ (8.6 mg/100 ml). RLBK₇ (7.4 mg/100 ml) and RLAU₅ (7.2 mg/100 ml) showed moderate nitrogen fixation, while RLCH₃ recorded the lowest value (6.6 mg/100 ml). These findings are in agreement with Bhite (2021), who evaluated the nitrogen-fixing efficacy of indigenous *Rhizobium* isolates in comparison with commercial strains on fenugreek (*Trigonella foenum-graecum* L.). On the basis of germination, seedling vigour and nitrogen-fixing efficiency, RLGB₁ was identified as the most efficient native isolate and was carried forward, along with the commercial strain, for field evaluation.

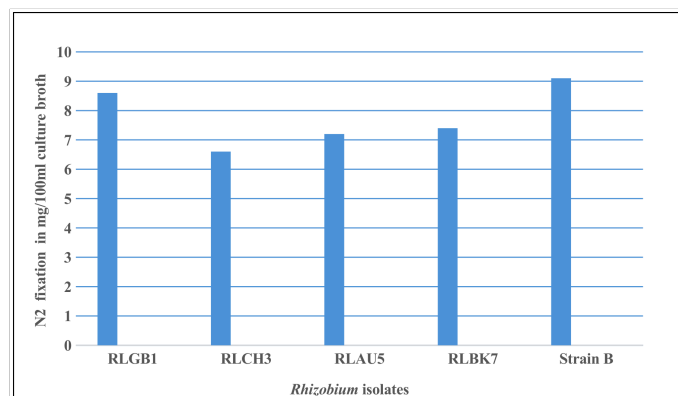


Figure 4. Nitrogen fixation ability (mg/100 ml) of efficient *Rhizobium* isolates in comparison with the commercial strain

B. Field evaluation

B.1 Germination percentage and plant population

The maximum germination percentage was recorded in T₆ (100% RDF + seed treatment with the commercial strain) (96.33%), followed by T₅ (95.00%) and T₃ (94.67%); T₆ was statistically at par

Table 3. Effect of the efficient *Rhizobium* isolate and the commercial *Rhizobium* strain on germination percentage and plant population of lentil

Treatment	Treatment details	Mean germination (%)	Plant population (lakh/ha)
T ₁	50% RDN + 100% RDPK (10:40:40 kg/ha) + seed treatment with efficient isolate of <i>Rhizobium</i> (@25 ml/kg seed)	90.33	1.22
T ₂	75% RDN + 100% RDPK (15:40:40 kg/ha) + seed treatment with efficient isolate of <i>Rhizobium</i> (@25 ml/kg seed)	93.00	1.26
T ₃	100% RDF (20:40:40 kg/ha) + seed treatment with efficient isolate of <i>Rhizobium</i> (@25 ml/kg seed)	94.67	1.28
T ₄	50% RDN + 100% RDPK (10:40:40 kg/ha) + seed treatment with commercial strain of <i>Rhizobium</i> (25 ml/kg seed)	90.67	1.27
T ₅	75% RDN + 100% RDPK (15:40:40 kg/ha) + seed treatment with commercial strain of <i>Rhizobium</i> (25 ml/kg seed)	95.00	1.29
T ₆	100% RDF (20:40:40 kg/ha) + seed treatment with commercial strain of <i>Rhizobium</i> (25 ml/kg seed)	96.33	1.30
T ₇	50% RDN + 100% RDPK (10:40:40 kg/ha), no seed treatment	85.00	1.15

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with T₂, T₃ and T₅. The lowest germination was recorded in T₇ (85.00%), at par with T₈ (88.67%). Similarly, the maximum plant population was recorded in T₆ (1.30 lakh/ha), followed by T₅ (1.29 lakh/ha), T₃ (1.28 lakh/ha) and T₄ (1.27 lakh/ha); T₆ was statistically at par with T₁, T₂, T₃, T₄, T₅ and T₉. The lowest plant population was recorded in T₇ (1.15 lakh/ha). These results are in agreement with Nagananda *et al.* (2010) [4], who reported that *Rhizobium* inoculation had a positive effect on the germination and growth of fenugreek (*Trigonella foenum-graecum* L.).

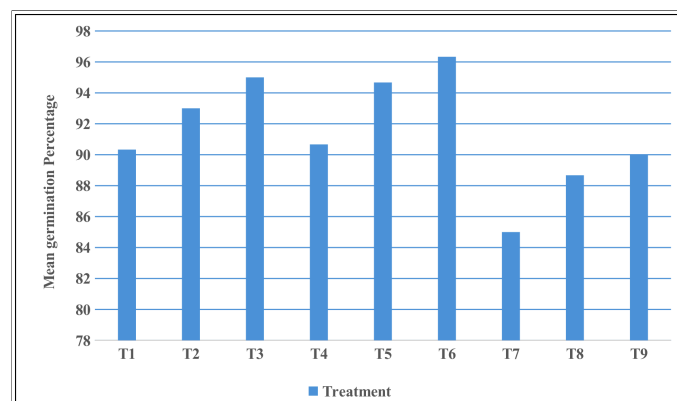


Figure 5. Effect of efficient *Rhizobium* isolate and commercial *Rhizobium* isolate on the germination of lentil (*in Vivo*).

Plant population of lentil (*in Vivo*).

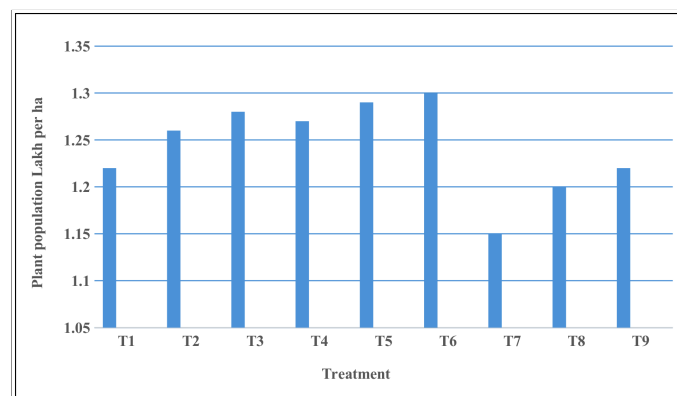


Figure 6. Effect of efficient *Rhizobium* isolate and commercial *Rhizobium* isolate on the plant population of lentil (*in Vivo*).

Treatment	Treatment details	Mean germination (%)	Plant population (lakhs/ha)
T ₈	75% RDN + 100% RDPK (15:40:40 kg/ha), no seed treatment	88.67	1.20
T ₉	100% RDF (20:40:40 kg/ha), no seed treatment	90.00	1.22
	SE(m)±	1.82	0.028
	CD at 5%	5.29	0.086

B.2 Plant height

Table 4. Effect of the efficient *Rhizobium* isolate and the commercial *Rhizobium* strain on plant height of lentil at 15, 30 and 45 DAS

Treatment	Plant height 15 DAS (cm)	30 DAS (cm)	45 DAS (cm)	Mean (cm)
T ₁	5.42	12.73	25.30	13.76
T ₂	6.90	14.50	26.93	16.11
T ₃	7.05	15.20	27.27	16.51
T ₄	6.32	14.47	25.30	15.36
T ₅	7.87	15.60	28.67	17.38
T ₆	8.34	17.33	29.73	18.47
T ₇	4.87	12.00	21.60	12.82
T ₈	5.01	12.70	21.90	13.20
T ₉	6.59	14.67	26.63	16.11
SE(m)±	0.67	0.89	1.49	1.44
CD at 5%	1.96	2.60	4.33	2.95

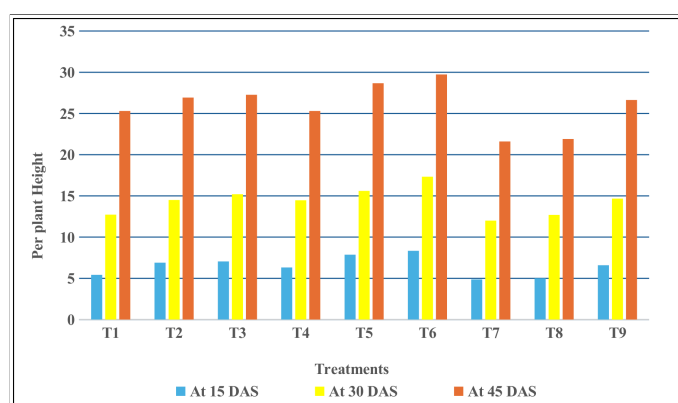


Figure 8. Effect of efficient *Rhizobium* isolate and commercial *Rhizobium* isolates on plant

height of lentil at 15th, 30th and 45th DAS.

Plant height was significantly influenced by the nutrient levels and *Rhizobium* seed treatments at all three growth stages. At 45 DAS, the maximum plant height was recorded in T₆ (29.73 cm), followed by T₅

(28.67 cm) and T₃ (27.27 cm), whereas the minimum was recorded in T₇ (21.60 cm). Overall, T₆ (100% RDF + commercial strain) recorded superior plant growth throughout the crop growth period, statistically at par with T₅ (75% RDN + 100% RDPK + commercial strain). These results agree with Singh *et al.* (2016) [5], who reported that seed inoculation with a suitable *Rhizobium* species is highly recommended to boost early lentil growth, and with Chaudhary (1999) [6], who reported no significant difference in plant height up to a 20% replacement of chemical nitrogen with *Rhizobium* biofertilizer treatment.

B.3 Number of branches per plant

The number of branches per plant was also significantly influenced by the treatments. At 45 DAS, the maximum number of branches was recorded in T₆ (9.10), followed by T₅ (8.90) and T₃ (8.23), whereas the minimum was recorded in T₇ (5.47). On a mean basis, T₆ recorded the highest branching (7.06 branches/plant), at par with T₅ (6.46), followed by T₃ (6.16), while the lowest was recorded in T₇ (3.92 branches/plant). These results are in line with Singh *et al.* (2014) [3], who reported that combining balanced chemical NPKS with dual biofertilizers (*Rhizobium* + PSB) significantly augmented the branch count and leaf area index of lentil.

Table 5. Effect of the efficient *Rhizobium* isolate and the commercial *Rhizobium* strain on number of branches per plant of lentil at 15, 30 and 45 DAS

Treatment	Branches 15 DAS	30 DAS	45 DAS	Mean
T ₁	3.40	4.37	6.77	4.85
T ₂	3.73	6.07	7.87	5.89
T ₃	4.07	6.17	8.23	6.16
T ₄	3.63	4.70	7.60	5.31
T ₅	4.27	6.20	8.90	6.46
T ₆	5.07	7.00	9.10	7.06
T ₇	2.73	3.57	5.47	3.92
T ₈	3.07	3.73	5.93	4.24
T ₉	3.13	4.43	6.33	4.63
SE(m)±	0.57	0.64	0.81	0.82
CD at 5%	1.17	1.86	2.34	1.68

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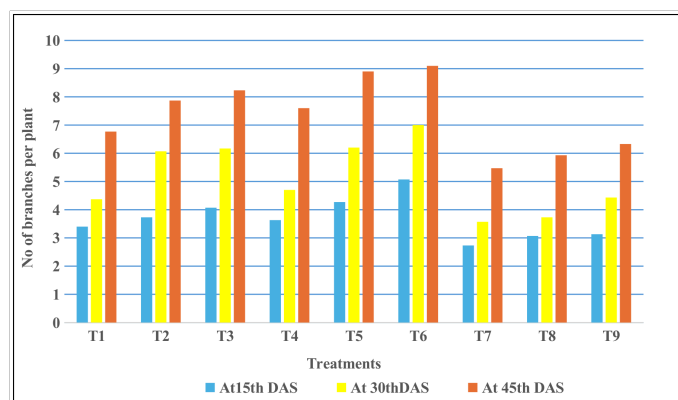


Figure 9. Effect of efficient *Rhizobium* isolate and commercial *Rhizobium* isolate on

number of branches of lentil at 15th, 30th and 45th DAS.

B.4 Root nodulation

Table 6. Effect of the efficient *Rhizobium* isolate and the commercial *Rhizobium* strain on number of root nodules per plant of lentil

Treatment	Nodules at 50% flowering	Nodules at harvest	Mean
T ₁	5.87	13.33	9.60
T ₂	6.27	13.40	10.34
T ₃	6.40	15.00	10.70
T ₄	6.00	13.67	9.84
T ₅	8.33	15.30	11.82
T ₆	8.43	17.70	13.07
T ₇	4.93	9.23	7.08
T ₈	5.20	11.00	8.10
T ₉	5.40	13.28	9.34
SE(m)±	0.77	1.26	0.74
CD at 5%	2.23	3.68	2.14

The number of root nodules per plant was significantly affected by the treatments at both 50% flowering and harvest. At harvest, T₆ recorded the maximum number of nodules (17.70), followed by T₅ (15.30) and T₃ (15.00), while T₇ recorded the minimum (9.23). On a mean basis, T₆ recorded the highest nodulation (13.07 nodules/plant), followed by T₅ (11.82) and T₃ (10.70), whereas the lowest mean was recorded in T₇ (7.08). This agrees with Mehta and Patel (2011), who reported that *Rhizobium* inoculation leads to better root development, nodulation and nutrient availability, resulting in vigorous plant growth, and with Mishra *et al.* (2009) [7], who found that co-inoculation improved nodulation by 86.60 per cent over the uninoculated control.

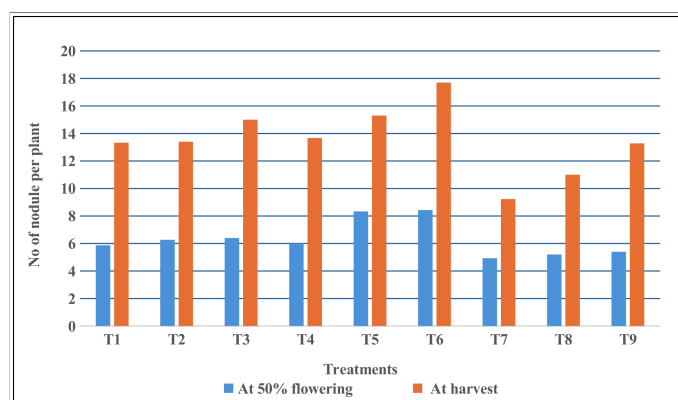


Figure 10. Effect of efficient *Rhizobium* isolate and commercial *Rhizobium* isolate on

nodule per plant of lentil.

B.5 Yield

Table 7. Effect of the efficient *Rhizobium* isolate and the commercial *Rhizobium* strain on the yield of lentil (kg/plot and q/ha)

Treatment	Yield (kg/plot)	Yield (q/ha)
T ₁	0.222	6.84
T ₂	0.334	10.30
T ₃	0.348	10.74
T ₄	0.243	7.51
T ₅	0.352	10.88
T ₆	0.368	11.36
T ₇	0.195	6.01
T ₈	0.201	6.21
T ₉	0.335	10.34
SE(m)±	0.04	1.35
CD at 5%	0.13	3.93

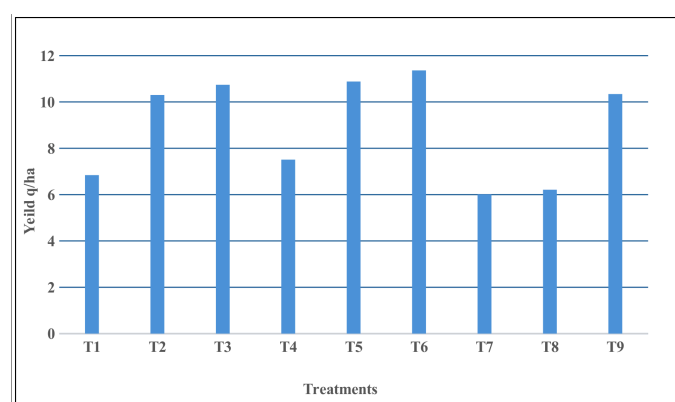


Figure 4. Grain yield (q/ha) of lentil as influenced by the efficient *Rhizobium* isolate and the

commercial *Rhizobium* strain across nutrient levels

The maximum yield was recorded in T₆ (100% RDF + seed treatment with the commercial strain) (0.368 kg/plot and 11.36 q/ha), followed by T₅ (0.352 kg/plot and 10.88 q/ha) and T₃ (0.348 kg/plot and 10.74 q/ha); the lowest yield was recorded in T₇ (0.195 kg/plot and 6.01 q/ha). Overall, T₆ recorded the highest yield, indicating the beneficial effect of 100% RDF combined with commercial *Rhizobium* seed treatment, statistically at par with T₅. These findings are consistent with Tiwari *et al.* (2018) [8], who reported that integrated nutrient management with dual inoculation (*Rhizobium* + PSB) generated significant increases in lentil yield attributes.

Conclusion

Under *in vitro* conditions, isolate RLGB₁ recorded the highest seed germination (98.00%), seedling vigour index (855.54 at 7 DAS) and nitrogen-fixing ability among the native isolates (8.6 mg/100 ml), next only to the commercial MPKV *Rhizobium* strain (9.1 mg/100 ml), and was therefore identified as the most efficient native isolate. Under field conditions, seed treatment with the commercial strain combined with 100% RDF (T₆) resulted in the maximum germination, plant population, plant height, branching, root nodulation and grain yield of lentil, statistically at par with 75% RDN + 100% RDPK combined with the commercial strain (T₅). Seed treatment with the efficient native isolate RLGB₁ at 100% RDF (T₃) also produced growth and yield comparable to the commercial strain, indicating its potential as an effective, locally adapted alternative biofertilizer for lentil. Treatments without *Rhizobium* seed treatment recorded the lowest performance both *in vitro* and, in the field, reaf

firming the value of *Rhizobium* seed inoculation, combined with an adequate nutrient dose, for maximizing lentil productivity.

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