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EVALUATION OF MESORHIZOBIAL STRAINS FOR ENHANCED GROWTH, NODULATION, AND YIELD OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

A field experiment was conducted during the rabi season of 2024–25 at the Zonal Agricultural Research Station, Kalaburagi (Karnataka), to evaluate the performance of different *Mesorhizobium* strains in enhancing nodulation, nitrogen fixation, plant growth, and yield of chickpea (*Cicer arietinum* L.). The experiment comprised eight treatments, including inoculation with different *Mesorhizobium* strains, an absolute control, and a fertilizer control (20 kg N/ha), laid out in a randomized complete block design (RCBD) with three replications. Data on plant growth, nodulation, yield attributes, and yield were recorded using standard procedures. The results revealed significant differences among treatments for growth parameters (plant height, number of branches per plant, nodulation and nodule dry weight per plant, and dry matter accumulation), as well as yield attributes (number of pods per plant, number of seeds per plant, test weight, biological yield, and grain yield). Among the tested strains, treatment T_6 (*Mesorhizobium* sp. NBAIM) recorded the highest values for growth traits and grain yield, and was superior to other treatments. Growth and yield attributing characters were comparatively higher in T_6, the maximum grain yield was achieved in T_6. It is concluded that inoculation with *Mesorhizobium* sp. NBAIM (T_6) is highly effective in improving chickpea growth, nodulation, and productivity,
***Keywords* :** Chickpea, Mesorhizobium inoculation, growth and yield attributes.

Introduction

Chickpea (*Cicer arietinum* L.) is a major cool-season legume crop cultivated extensively across semi-arid and sub-tropical regions of the world. It serves as an important dietary source of protein, carbohydrates, minerals, and vitamins, while also contributing significantly to nutritional security in predominantly vegetarian populations. In addition to its nutritional value, chickpea plays a pivotal role in sustainable agricultural systems due to its capacity to fix atmospheric nitrogen through a symbiotic association with *Mesorhizobium* spp. This biological nitrogen fixation (BNF) reduces reliance on synthetic nitrogen fertilizers, improves soil nitrogen status, and benefits subsequent crops in rotation.

Global increases in population and the corresponding surge in food demand have placed considerable pressure on agricultural productivity. Conventional strategies relying heavily on chemical fertilizers and pesticides have contributed to soil degradation, nutrient imbalances, and adverse ecological impacts, including the decline of beneficial soil microbiota and pollinators (Giri *et al.*, 2010). In this context, biofertilizers have emerged as a viable component of integrated nutrient management systems. Rhizobial inoculants, in particular, enhance nutrient availability, stimulate root development, and improve crop resilience under diverse environmental conditions (Henzell, 1988). Their application in chickpea cultivation offers an eco-friendly and cost-effective approach to enhancing both productivity and soil health.

The efficiency of rhizobial inoculants, however, is strongly influenced by strain–host compatibility, edaphic factors, and prevailing climatic conditions (Yanni *et al*., 1997). Field-based evaluations of *Mesorhizobium* strains are therefore essential to identify superior inoculants with high nodulation potential, nitrogen fixation efficiency, and yield-enhancing capacity under specific agro-ecological situations. The present investigation was undertaken to assess the performance of selected *Mesorhizobium* strains on nodulation, nitrogen fixation, and grain yield of chickpea under field conditions, with the objective of generating region-specific biofertilizer recommendations for sustainable pulse production.

Material and Methods

The field experiment was conducted at the Zonal Agricultural Research Station (ZARS), Kalaburagi, Karnataka during the rabi season of 2024–25 to evaluate the efficiency of different *Mesorhizobium* strains on the growth and yield of chickpea (*Cicer arietinum* L.). The experimental site is characterized by Vertisol soils, belonging to the semi-arid tropics, with low available nitrogen, medium phosphorus, and high potassium content. Soil analysis of the experimental site prior to sowing, soil samples were collected from the experimental field (0–15 cm depth) and analyzed for physico-chemical properties. The results are presented in Table 1.

Table 1 : Physico-chemical properties of the experimental soil (0–15 cm depth)

Parameter	Value	Rating	Method Used (Reference)
Soil texture	Clayey (Vertisol)	–	International Pipette Method
pH (1:2.5 soil:water)	7.8	Slightly alkaline	Jackson (1973)
Electrical conductivity (dS m^{-1})	0.35	Normal	Jackson (1973)
Organic carbon (%)	0.52	Medium	Walkley and Black (1934)
Available N (kg ha^{-1})	230	Low	Alkaline $KMnO_4$ method (Subbiah & Asija, 1956)
Available P_2O_5 (kg ha^{-1})	28	Medium	Olsen's method (1954)
Available K_2O (kg ha^{-1})	410	High	Flame photometer (Jackson, 1973)

Experimental details

The experiment was laid out in a Randomized Complete Block Design (RCBD) consisting of eight treatments with three replications. The recommended dose of fertilizers was applied as a basal dose at the time of sowing. The treatments were as follows:

- **T_1**: Absolute control (no nitrogenous fertilizer, no *Mesorhizobium* inoculation)
- **T_2**: 20 kg N ha^{-1}
- **T_3**: *Mesorhizobium* sp. 2022–NS 1 (New Delhi)
- **T_4**: *Mesorhizobium* sp. 2022–B15 (Kanpur)
- **T_5**: *Mesorhizobium* sp. LCP-23-33
- **T_6**: *Mesorhizobium* sp. NBAIM
- **T_7**: *Mesorhizobium* sp. RFC 117 (reference strain)
- **T_8**: Local best/commercial strain

The test crop was chickpea variety JG-11, and seeds were inoculated with respective *Mesorhizobium* strains as per treatment details prior to sowing. All recommended cultural practices and plant protection measures were uniformly followed throughout the experimental period to ensure healthy crop growth.

Observations were recorded at appropriate growth stages on plant height, number of nodules per plant, nodule dry weight, number of branches per plant, root dry weight, and shoot dry weight. The data were subjected to analysis of variance (ANOVA) following the RCBD procedure. Treatment means were compared at the 5% level of significance, and the standard error of mean (SEM) and degrees of freedom (df) were analyzed

Determination of Nitrogenase activity

The acetylene reduction assay (ARA) was used to estimate nitrogenase activity in *Rhizobium* by measuring the reduction of acetylene (C_2H_2) to ethylene (C_2H_4). Fresh and healthy root nodules were collected at 55 and 75 days after sowing (DAS) and were placed in glass vials, and sealed with Suba-Seal stoppers. The headspace air (2 mL) of each vial was replaced with an equal volume of acetylene gas, and the vials were incubated at 28 °C for 30 minutes to one hour. After incubation, a known volume of headspace gas was withdrawn and analyzed using a gas chromatograph (GC) equipped with a flame ionization detector (FID) and Porapak® N 80 column. The injector and column temperatures were maintained at 110 °C and 80 °C, respectively, while the flow rates of the carrier gas (N_2) and fuel gas (H_2) were set at 35 ml min^{-1} and 25 ml min^{-1}. From each vial, a 1 ml gas sample was injected to determine the amount of ethylene produced. The extent of acetylene reduction was quantified using a standard ethylene gas (105 mg kg^{-1}) obtained from EDT Research, London, England. Nitrogenase activity was expressed as µmol C_2H_4 produced g^{-1} fresh weight of nodules h^{-1} (Hardy *et al.*1968)

Estimation of legheamoglobin content

The leghaemoglobin content of root nodules was determined following the pyridine hemochrome method described by Dey *et al.* (2004) with slight modifications. Fresh, healthy, and pink nodules were collected, blotted dry, and weighed accurately. One g of nodules were homogenized in 5 mL of cold 0.1 M phosphate buffer (pH 7.0)(1 mM EDTA and 1% polyvinylpolypyrrolidone (PVPP)) using a pre-chilled mortar and pestle. The homogenate was centrifuged at 12,000 × g for 15 minutes at 4 °C, and the supernatant was collected for estimation. One ml of the clear supernatant was mixed with 1 ml of alkaline pyridine reagent (pyridine and 0.2 N NaOH in a 9:1 ratio) to form the pyridine–hemochrome complex. The mixture was then reduced by adding a few crystals of sodium dithionite, and the absorbance of the reduced solution was recorded at 556 nm using a UV–Visible spectrophotometer. The concentration of leghaemoglobin was calculated using the molar extinction coefficient of 34.5 mM^{-1} cm^{-1} and expressed as µmol g^{-1} fresh weight of nodules.

Results and Discussion

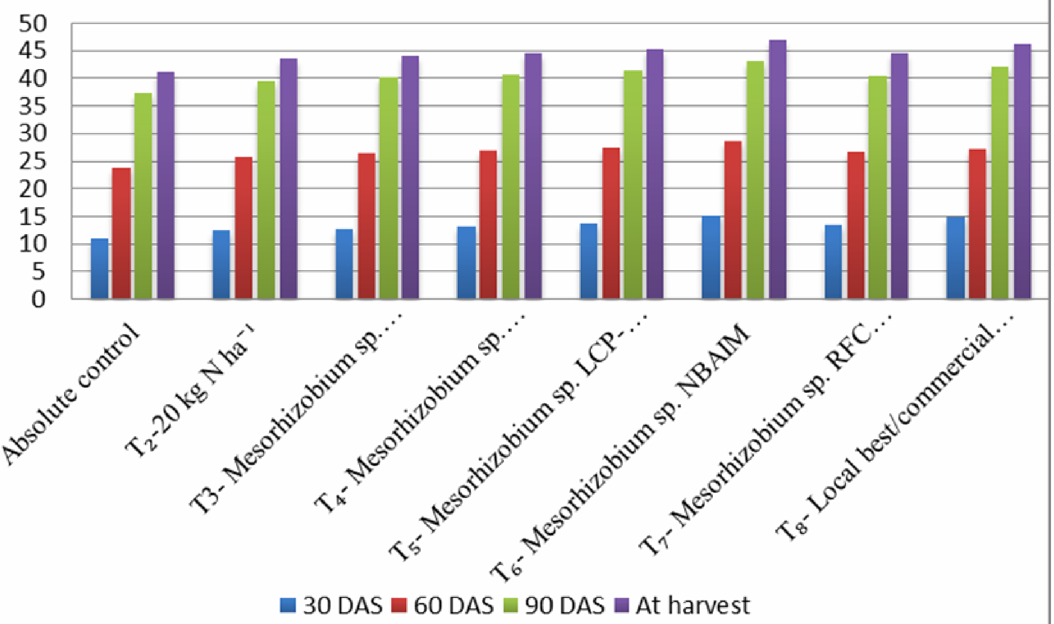
A total of 6 Mesorhizobium strains were tested in the experimental filed conducted at zonal agriculture research station Kalaburagi, with two control treatments i.e. without any Mesorhizobium inoculation and other is application of 20 kg nitrogen / ha.

Plant growth characters

Plant Height (cm)

Plant height was recorded at 30, 60, and 90 DAS as well as at harvest, and the data are presented in Fig 1. A progressive increase in plant height was observed with crop advancement, with maximum height recorded at harvest.

Fig.1. Effect of Mesorhizobium strains on plant height (cm) of chickpea at different growth stages



At 30 DAS, the height ranged from 11.03 cm in the uninoculated control (T_1) to 15.06 cm in Mesorhizobium NBAIM (T_6). The local best strain (T_8) also performed well (14.83 cm), while T_1 remained lowest. This trend continued at 60 and 90 DAS, where T_6 maintained its superiority (28.57 and 43.10 cm respectively), followed by T_8 (27.17 and 42.10 cm). Similar trend was observed at harvest, were T_6 recorded maximum height (47.0 cm), which was on par with T_8 (46.3 cm). The control treatment (T_1) remained significantly lower across all stages. The superior performance of *Mesorhizobium* NBAIM (T_6) and the local strain (T_8) can be attributed to their greater efficiency in root nodulation and nitrogen fixation,

leading to better vegetative growth. The increase in plant height under these treatments indicates improved nutrient uptake and enhanced photosynthetic activity, which promoted sustained growth up to harvest (Schimmel and Bennett (2004)

Nodulation Traits

The number of nodules and nodule dry weight recorded at 55 and 75 DAS were significantly influenced by inoculation with different *Mesorhizobium* strains (Table 2). At 55 DAS, T_6 recorded the maximum nodules (27.5 plant^{-1}) and nodule dry weight (146.3 mg), followed by T_8 (26.1 and 141.5 mg). Similar trends were maintained at 75 DAS, where T_6 recorded 29.8 nodules plant^{-1} with 152.4 mg nodule dry weight, closely followed by T_8 (28.4 nodules plant^{-1} and 148.7 mg plant^{-1}). Both treatments were significantly superior to the other strains as well as to the controls. In contrast, the uninoculated control (T_1) and chemical nitrogen treatment (T_2) showed least nodulation and at both the stages. The superior nodulation performance of *Mesorhizobium* NBAIM (T_6) and the local best strain (T_8) may be attributed to their higher symbiotic efficiency, rapid root colonization, and greater nitrogen-fixing ability. Enhanced nodulation and nodule biomass under these treatments provided a steady supply of biologically fixed nitrogen, which contributed to better crop growth and yield. Chickpea inoculated with efficient *Mesorhizobium* strains showed enhanced nodulation and nitrogen fixation at multiple growth stages (Kantar *et al.*, 2007). In contrast, the comparatively lower nodule formation in the chemical nitrogen treatment (T_2) may be due to the suppressive effect of readily available soil nitrogen on nodule initiation and activity (Aziz *et al.*, 2011)

Table 2 : Effect of *Mesorhizobium* strains on nodulation traits of chickpea at 55 and 75 DAS

Treatment	Number of nodules/plant		Nodule dry weight(mg/plant)	
	At 55 DAS	At 75 DAS	At 55 DAS	At 75 DAS
T_1- Absolute control	12.4^{e}	13.5^{e}	78.3^{e}	81.2^{e}
T_2-20 kg N ha^{-1}	15.6^{d}	16.7^{d}	92.5^{d}	95.4^{d}
T3- Mesorhizobium sp. 2022–NS 1 (New Delhi)	18.9^{cd}	20.1^{c}	105.7^{cd}	109.2^{c}
T_4- Mesorhizobium sp. 2022–B15 (Kanpur)	20.3^{c}	21.5^{c}	112.6^{c}	116.0^{c}
T_5- Mesorhizobium sp. LCP-23-33	22.7^{b}	24.1^{b}	124.8^{b}	129.2^{b}
T_6- Mesorhizobium sp. NBAIM	27.5^{a}	29.8^{a}	146.3^{a}	152.4^{a}
T_7- Mesorhizobium sp. RFC 117 (reference strain)	21.4^{bc}	22.7^{bc}	118.9^{c}	123.5^{c}
T_8- Local best/commercial strain	26.1^{a}	28.4^{a}	141.5^{a}	148.7^{a}

Letters in superscripts indicate mean separation by Duncan's multiple range test ($P \leq 0.05$).

Nitrogen fixation traits

Nitrogen fixation efficiency, assessed through Legheamoglobin content and nitrogenase activity, was significantly influenced by inoculation with different *Mesorhizobium* strains at both 55 and 75 DAS (Fig. 2 & Fig. 3). At 55 DAS, treatment T_6 recorded highest leghaemoglobin content (2.43 mg g^{-1} fresh nodule) and nitrogenase activity (88.5 µmol C_2H_4 g^{-1} h^{-1}) which was statistically on par with the local best strain (T_8: 2.36 mg g^{-1} and 85.7 µmol C_2H_4 g^{-1}h^{-1}). Similar trends were maintained at 75 DAS, where T_6 recorded 2.58 mg g^{-1} leghaemoglobin and 92.3 µmol C_2H_4 g^{-1} h^{-1}, and T_8 showing 2.51 mg g^{-1} and 89.8 µmol C_2H_4 g^{-1}h^{-1}, the treatments T_1 (uninoculated control) and T_2 chemical nitrogen treatment consistently exhibited the lowest values at both stages with 1.21 mg g^{-1} leghaemoglobin and 52.4 µmol C_2H_4 g^{-1}h^{-1} at 55 DAS, slightly increasing to 1.28 mg g^{-1} and 54.3 µmol C_2H_4 g^{-1} h^{-1} at 75 DAS. The higher nitrogen fixation in T_6 and T_8 at both stages can be attributed to increased nodulation, enhanced leghaemoglobin synthesis, and efficient nitrogenase activity, ensuring a continuous supply of biologically fixed nitrogen throughout the vegetative and early reproductive phases (Fatima *et al.*, 2008).

In contrast, nitrogen fixation in the chemical nitrogen treatment was relatively lower, likely due to the inhibitory effect of readily available nitrogen on nodule metabolism and nitrogenase activity (Tripathi *et al.*, 2015).

Fig.2. Effect of *Mesorhizobium* strains leghaemoglobin content of of chickpea root nodule at 55 and 75 DAS

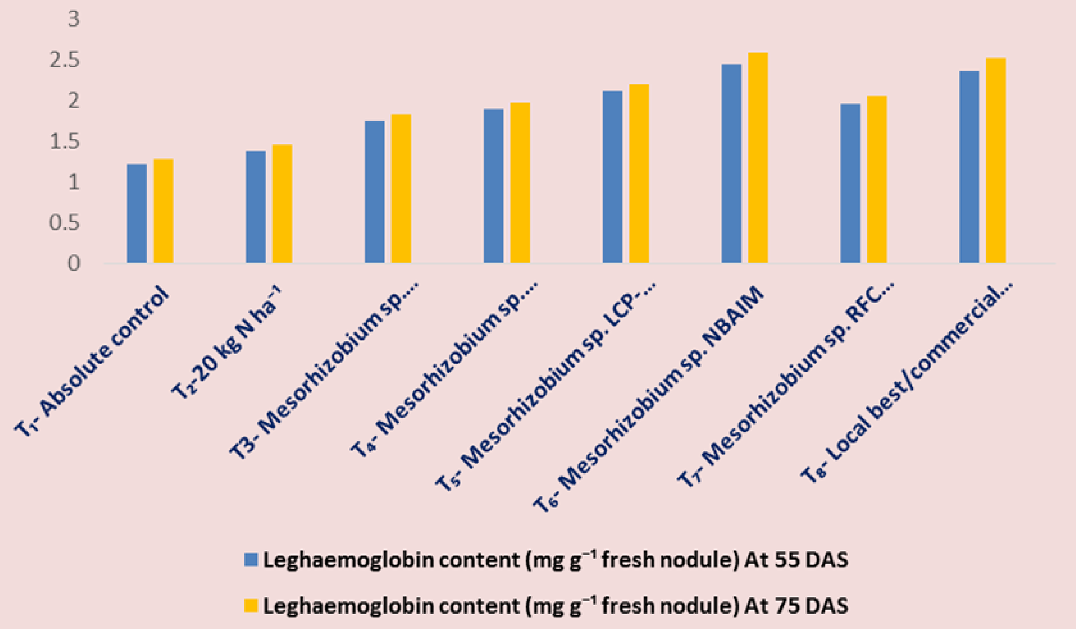
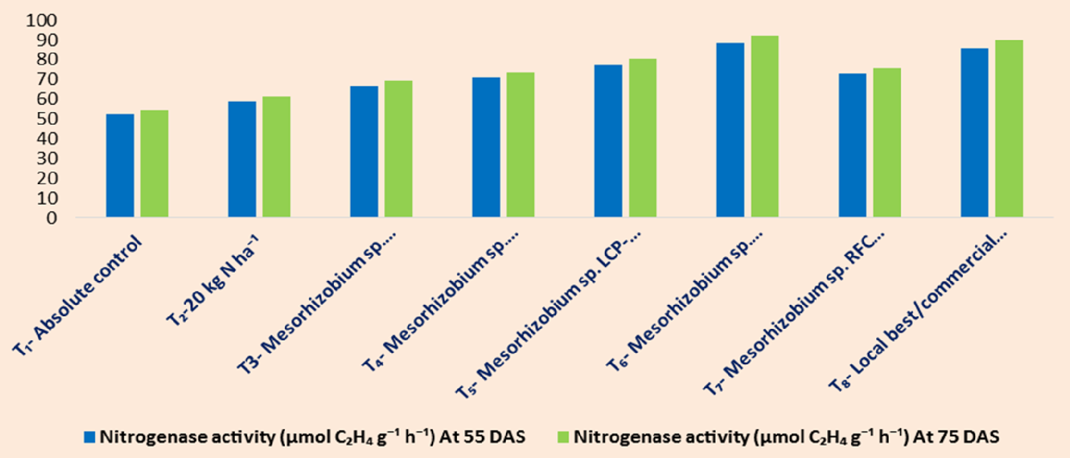


Fig.3. Effect of *Mesorhizobium* strains on nitrogenase activity of chickpea at 55 and 75 DAS



Yield and Yield Attributes

Yield and yield-attributing characters such as number of pods per plant, 100-seed weight, grain yield, and haulm yield were significantly influenced by inoculation with different *Mesorhizobium* strains (Table 3). Among the treatments, T_6 recorded the maximum values of 52.6 pods plant^{-1}, 20.7 g 100-seed weight, 1,620 kg ha^{-1} grain yield, and 2,670 kg ha^{-1} haulm yield. Which was on par with the local best strain (T_8) (50.9 pods plant^{-1}, 20.2 g 100-seed weight, 1,545 kg ha^{-1} grain yield, and 2,590 kg ha^{-1} haulm yield). The uninoculated control (T_1) recorded the lowest performance across all parameters, with 28.4 pods plant^{-1}, 17.4 g 100-seed weight, 1,020 kg ha^{-1} grain yield, and 1,880 kg ha^{-1} haulm yield. Application of 20 kg N ha^{-1} (T_2) slightly improved yield over the uninoculated control (32.7 pods plant^{-1} and 1,150 kg ha^{-1} grain yield. The superior performance of T_6 and T_8 can be attributed to their enhanced nodulation and nitrogen fixation efficiency (Tables 3 and 4), which provided a steady supply of biologically fixed nitrogen throughout the crop growth period. This, in turn, supported better vegetative development, higher pod setting, and improved seed filling (Drishty *et al,* 2020).

Table 3 : Effect of *Mesorhizobium* strains on yield and yield attributes of chickpea.

Treatment	Pods plant^{-1}	100 seed weight(g)	Grain yield (kg/ha)	Haulum yield (kg/ha)
T_1- Absolute control	28.4^{e}	17.4^{d}	1,020^{e}	1880^{e}
T_2-20 kg N ha^{-1}	32.7^{d}	18.2^{cd}	1,150^{d}	2,050^{d}
T_3- *Mesorhizobium* sp. 2022–NS 1 (New Delhi)	36.2^{cd}	18.7^{c}	1,240^{cd}	2,180^{c}
T_4- *Mesorhizobium* sp. 2022–B15 (Kanpur)	38.5^{c}	19.1^{c}	1,310^{c}	2,260^{c}
T_5- *Mesorhizobium* sp. LCP-23-33	2.11^{b}	77.2^{b}	1,420^{b}	2,410^{b}
T_6- *Mesorhizobium* sp. NBAIM	52.6^{a}	20.7^{a}	1,620^{a}	2,670^{a}
T_7- *Mesorhizobium* sp. RFC 117 (reference strain)	40.1^{c}	19.3^{c}	1,360^{c}	2,310^{c}
T_8- Local best/commercial strain	50.9^{a}	20.2^{a}	1,545^{a}	2,590^{a}

Letters in superscripts indicate mean separation by Duncan's multiple range test ($P \leq 0.05$).

Conclusion

The present study demonstrated that inoculation of chickpea with efficient *Mesorhizobium* strains markedly enhanced plant growth, nodulation, nitrogen fixation, and yield compared to uninoculated and fertilizer controls. Among the tested strains, NBAIM (T_6) and the local strain (T_8) consistently outperformed others, reflecting their superior symbiotic efficiency and adaptability to vertisols. These findings highlight the potential of effective *Mesorhizobium* inoculants as a sustainable alternative to chemical nitrogen fertilizers, contributing both to improved crop productivity and to environmentally friendly chickpea cultivation. Wider adoption of such bio-inoculants could play a pivotal role in enhancing chickpea production while promoting soil health and sustainability in pulse-based cropping systems

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