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# Impact of Rhizobium on seed germination of selected leguminous crop plants

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## Abstract

*Rhizobium* species are the primary symbiotic nitrogen fixing bacteria. These bacteria infect the roots of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. Rhizobium was isolated from the root nodules of selected leguminous crop plants Soybean (*Glycine max*) Rh1, Methi (*Trigonella foenum graecum*)Rh2, Gram (*Cicer arietinum*) Rh3, Black gram (*Vigna mungo*) Rh4, Green gram(*Vigna radiate*) Rh5, Pea (*Pisum sativum*) Rh6, Groundnut (*Arachis hypogea*) Rh7 and Moth bean (*Vigna aconitifolia*) Rh8 of Nanded district of Maharashtra, India on Yeast extract mannitol agar (YEMA) medium. Morphological and biochemical tests were carried out to characterize and identify the Rhizobium. There were produced gummy colonies on YEMA plates after 2-3 days of incubation at 28$^{o}$C. All strains were rod shaped, gram-negative and motile. The experiment was conducted to observe the effect of Rhizobial inoculation on seed germination, seedling emergence, growth and development under laboratory condition using filter paper in Petri dish Seeds inoculated with *Rhizobium* strains and the Petri dish were kept in an incubator at 28 ± 2°C for 72 hrs. The results suggested that maximum seed germination was observed in inoculated seeds compared to the control (non-treated seeds).The Rhizobium can be used as bioinoculant to enhance the yield of crops.

**Keywords:** *Rhizobium*, root nodule, nitrogen fixation, seed germination, PGPR

## Introduction

Rhizobia are soil bacteria that fix N2 (diazotroph) after becoming established inside root nodules of legumes (Fabaceae) ( Ankur Tyagi. *et al* 2017). These bacteria in association with legumes can fix atmospheric Nitrogen and through this feature, they are introduced into agricultural systems to improve soil fertility, plant growth and limit the use of chemical fertilizers (Ouma EW *et al*., 2016). Leguminous species of economic importance are grown in India under different agroclimatic conditions and therefore, the presence of native rhizobia has been anticipated. Root nodulating bacteria are important in legumes due to their host specificity. They provide with the advantageous factor in relation to nitrogen fixation along with their antagonistic activity against certain plant pathogens (Kumar *et al* 2011). The Rhizobia-legume symbiosis benefits not only the host crop but also the subsequent crops in that field. Bacteria present in root

nodules of legumes are mainly species of *Rhizobium* (*Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium* and *Sinorhizobium*). Rhizobacteria that benefit plant growth by producing plant growth regulators, enhancing the nutrient(s) availability, inducing root exudation and controlling phytopathogens are termed as PGP bacteria (Kloepper and Schroth, 1980). *Rhizobium* strains secrete growth hormones like indole acetic acid (IAA), which shows positive influence on plant growth and also plays an important role in the formation and development of root nodules (Al-Mujahidy *et al*.2013). Nitrogen (N) is required for synthesis of nucleic acids, enzymes, proteins and chlorophyll and hence it is a vital element for plant growth ((Bhattacharyya and Jha 2012,Gopalkrishnan 2015).Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. The microbe first enters the root and later on form nodules in which nitrogen fixation occurs. Rhizobia are a vast group of rhizobacteria that have the ability to lay symbiotic interactions by the colonization and formation of root nodules with leguminous plants, where nitrogen is fixed to ammonia and make it available for the plant (Ahemad. M 2014). Several studies have shown that *Rhizobium*, a Gram negative N-fixing soil bacterium, has a positive impact on legumes (Gouda *et al*. 2018). The main purpose of this study was to isolate and characterized *Rhizobium* strains from different selected leguminous crop plants and assessed their abilities of plant growth promoting rhizobacteria attributes in seed germination under laboratory condition. Plant-growth promoting traits could improve plant growth and yield of the crops.

## Materials and Methods

**Collection of plant samples:** Soybean (*Glycine max*), Methi *(Trigonela foenum gracium)*, Gram (*Cicer arietinum*), Black gram (*Vigna mungo*), Green gram (*Vigna radiata*), Pea plant(*Pisum sativum*), Groundnut (*Arachis hypogea*) and Moth bean (*Vigna aconitifolia*), these plants were uprooted carefully from the field and the samples were collected aseptically in plastic bags and brought to the laboratory healthy nodules were detached from the root and further isolation of root nodulating rhizobia was carried out (Deshwal *et al*., 2014).

**Isolation of Rhizobium from root nodules of the selected leguminous Crop plants:** The nodules were washed under tap water to remove adhering mud and soil particles. After washing, treated carefully with 95% ethanol for surface sterilization. The nodules were crushed according to standard protocol. Ten fold serial dilution of nodular extract was prepared by taking 1g of nodular extract into 10 ml of sterile distilled water and mixed well to get nodular extract suspension. One ml of nodular extract suspension was diluted with 9 ml of sterile distilled water making the dilution to 10-2. Similarly dilution up to 10-8 was made separately for each nodular extract. Suspension (0.1 ml) of nodular extract was prepared from 10-3 to 10-8 dilutions (*Rajesh, et al., 2019)*. The crushed nodules were streaked on yeast extract mannitol agar [(YEMA) Mannitol 10.00 g, $MgSO_4 7H_2O$ 0.20 g, NaCl 0.10 g, $K_2HPO_4$ 0.50 g, $CaCl_2 \cdot 2\ H_2O$ 0.20 g, $FeCl_3 \cdot 6\ H_2O$ 0.01 g, yeast extract 1.00 g, agar20.00 g and distilled water 1000 ml) with the right calibration of pH (6.8-7)] medium and incubated at 28 ± 2ºC for 24 hrs (Shahzad *et al*., 2012)

**Morphological characterization**

Morphological characters such as size, shape, colour, elevation, margin, opacity, bacterium shape and gram staining were performed for identification of the bacteria (Gachande and Khansole, 2011, Paudyal *et al*., 2021).

**Biochemical characterization**

Biochemical characteristics namely Indole test, Catalase test, Oxidase test, Gelatin liquefaction, Starch hydrolysis, Urease production, Growth on Glucose Peptone Agar (GPA) test, Citrate utilization and $H_2S$ production of different isolates were done for the identification of the isolates following standard procedure (Singh et al., 2008 , Gauri *et al* ., 2011, Ankur Tyagi *et al*., 2017, Kumari *et al*.,2018 and Paudyal *et al*., 2021).

**Effect of Rhizobium on seed germination**: Isolated rhizobial colonies were inoculated in nutrient broth and allowed to grow overnight. Different seeds namely, Soybean (*Glycine max*), Black gram(*Vigna mungo*), Green gram (*Vigna radiata*), Groundnut (*Arachis hypogea*) and Moth bean (*Vigna aconitifolia* were surface sterilized by 70% ethanol and then treated with 1% sodium hypochlorite for 2 min followed by repeated washing with sterile water. After this, the seeds were soaked in the rhizobial culture broth, while seeds which were soaked in normal nutrient broth kept as a control. Ten seeds of each treatment were kept equidistance in sterilized petriplates containing moist filter paper and the petriplates were incubated at 28°C. Seed germination and percent seedling emergence was calculated using following formula (Pawar V.*et al*., 2014):

$$\%\ Seed\ Emergence = \frac{\text{Number of emerged seedling}}{\text{Number of seeds sown}} \text{ X } 100$$

## Results and Discussion

### Collection and Isolation of Rhizobium

Root nodules samples of selected leguminous crop plants Soybean (*Glycine max*) Rh1, Methi (*Trigonella foenum graecum*) Rh2, Gram (*Cicer arietinum*) Rh3, Black gram (*Vigna mungo*) Rh4, Green gram (*Vigna radiata*) Rh5, Pea (*Pisum sativum*) Rh6, Groundnut (*Arachis hypogea*) Rh7 and Moth bean (*Vigna aconitifolia*) Rh8 were collected from the fields of crop plants of Nanded district of Maharashtra, India. A total of eight rhizobacterial strains were successfully isolated from the root nodules of the selected crop plants on YEMA medium (Fig.-1).

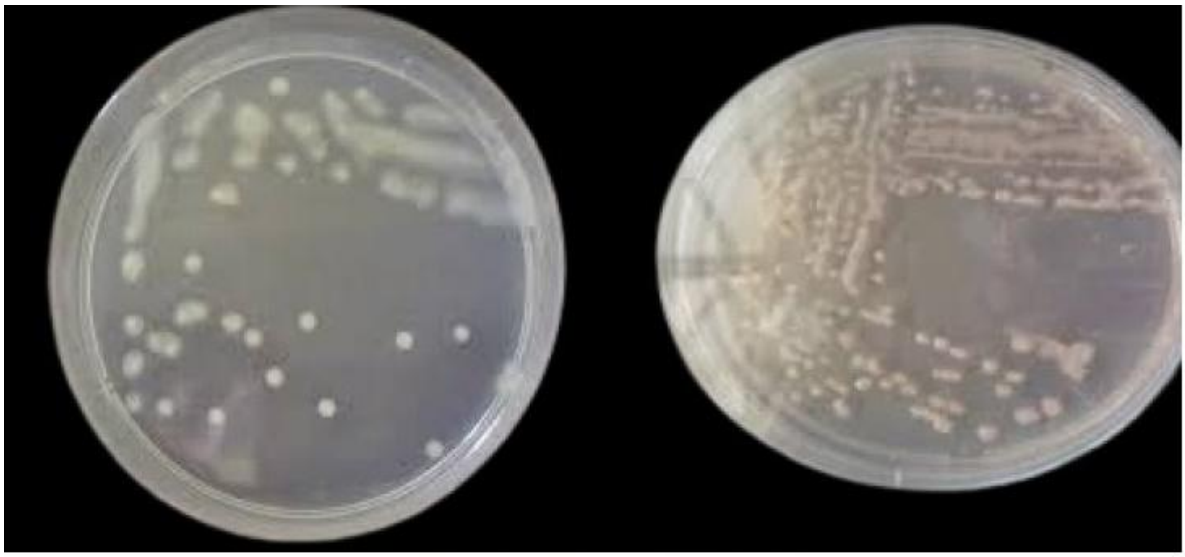


Fig.1 Isolation of Rhizobium on YEMA Plate

### Morphological characteristics

All the strains isolated on YEM agar medium each from the eight different leguminous crop plant species were cultured and showed the best positive growth at 28±2°C in YEMA medium and morphologically tested. All the strains were found to be motile, Gram negative (Fig.-2), rod shaped. The colonies on YEM agar were circular, non-spreading, translucent, convex and smooth after 48 h of incubation at 28±1°C (Table.- 1). Morphological characteristics resembles with the study carried out by different researchers (Gachande and Khansole, 2011, and Paudyal *et al.* 2021**)**

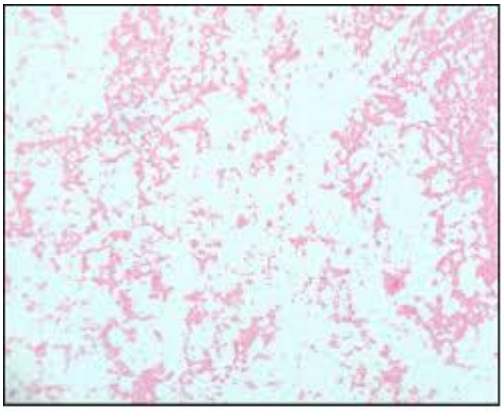


**Fig. 2 Gram Staining**

**Table.-1 Morphological Characteristics *Rhizobium***

| Morphological Characters | Strains of *Rhizobium* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Soybean (*Glycin max*) Rh1 | Methi (*Trigonella foenum-graecum*) Rh2 | Gram (*Cicer areitinum*) Rh3 | Black Gram (*Vigna mungo*) Rh4 | Green gram (*Vigna radiata*) Rh5 | Pea (*Pisum sativum*) Rh6 | Groundnut (*Arachis hypogea*) Rh7 | Moth bean (*Vigna aconitifolia*) Rh8 |
| Gram Staining | -ve | -ve | ve | -ve | -ve | -ve | -ve | -ve |
| Shape of Colony | Circular | Circular | Circular | Circular | Circular | Circular | Circular | Circular |
| Colour of Colony | White | White | Whitish pink | White | White | White | White | White |
| Elevation | Convex | Convex | Convex | Convex | Convex | Convex | Convex | Convex |
| Opacity | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque |
| Motility | Motile | Motile | Motile | Motile | Motile | Motile | Motile | Motile |
| Shape of Bacterium | Rod Shaped | Rod Shaped | Rod Shaped | Rod Shaped | Rod Shaped | Rod Shaped | Rod Shaped | Rod Shaped |

## Biochemical characterization

The various biochemical tests of the isolated strains like Indole test, Catalase test, Oxidase test, Gelatin liquefaction test, Starch hydrolysis, Glucose-Peptone Agar (GPA), were positive for all isolates while Citrate utilization and $H_2S$ production test were found to be negative for all isolates (Table.-2) Similar results were also reported by various workers 100% of the strains showed the positive test for the catalase activity and negative test for citrate utilization which for the *Rhizobium* isolated from root nodules of cultivated leguminous plants (Paudyal *et al*. 2021), and Similar results were also reported in *Rhizobium* isolates from French Bean ( *Phaseolus Vulgris*) Kumari *et al* .,2018 , *Rhizobium* strains from *Vigna mungo*, *Cicer arietinum* and *Vigna radiate* Ankur Tyagi *et al*., 2017 resembled with result found . Abrar T.Hamza *et al.*, (2017) also reported catalase activity in isolates of Rhizobium from nodules of Cowpea, Elephant and Lab Lab plants rhizosphere were Catalase positive and Citrate utilization negative.

**Table 2: Biochemical Characteristics Rhizobium**

| Isolates | Biochemical Characteristics | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Indole test | Catalase test | Oxidase test | Gelatin Liqui-faction test | Starch Hydro-lysis | Urease Produc-tion | Growth on Glucose Peptone Agar | Citrate Utiliz-tion test | $H_2S$ produc -tion |
| **Rh1** | + | + | + | + | + | + | + | - | - |
| **Rh2** | + | + | + | + | + | + | + | - | - |
| **Rh3** | + | + | + | + | + | + | + | - | - |
| **Rh4** | + | + | + | + | + | + | + | - | - |
| **Rh5** | + | + | + | + | + | + | + | - | - |
| **Rh6** | + | + | + | + | + | + | + | - | - |
| **Rh7** | + | + | + | + | + | + | + | - | - |
| **Rh8** | + | + | + | + | + | + | + | - | - |

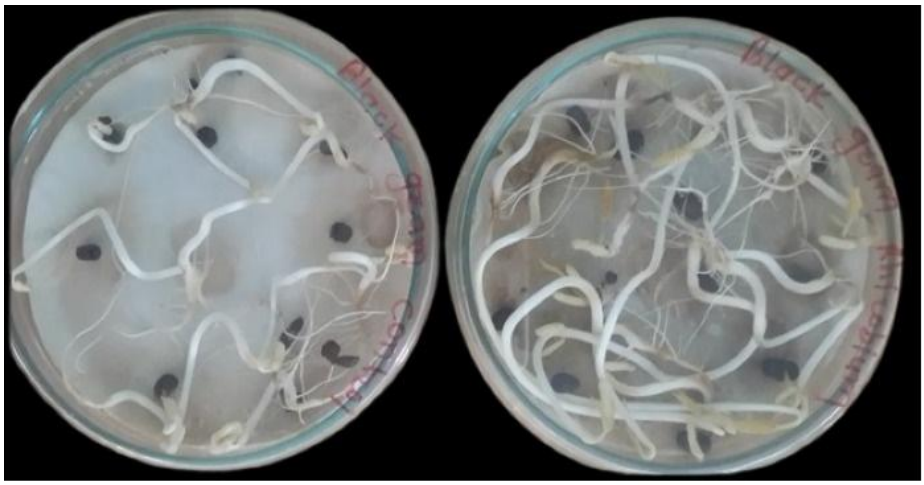
**Effect of Rhizobium on seed germination**:

Maximum germination of all seeds observed after 48 h. In comparison of the control (non-inoculted seeds) and treated (seeds coated with *Rhizobium* culture), highest seed germination was obtained in the treated. The number of germinating seeds and length of the sprouts were significant in the treated as compared to the control (non-inoculated) PawarV *et al*., (2014) similar result found. Soybean (*Glycine max )* inoculated seeds with Rhizobium 60% germintion while control (non-inoculated ) showed 30% seed germination, Black gram (*Vigna mungo)* inoculted with Rhizobium 100% seed germination observed while control (non- inoulated) showed 70% seed germination, Green gram (*Vigna radiata*) 100 % seed germinated inoculated with Rhizobium 80% seed germinated in control (non –inoculated), Groundnut(*Arachis hypogea)* 40% seed germinated inocuted with *Rhizobium* and only 10% seed germinated in control (non- inoculated) and in Moth Bean (*Vigna aconitifolia)* 100% seed germination was observed in inoculated with *Rhizobium* while 70% seed gemination was observed in control (non-inoculated) Table -3 and Fig.-3. Cross inoculation test showed positive test for the *Rhizobium* isolated from *Cicer aerientunum* and *Vicia faba* most of the legumes of the samples while most other legumes did not nodulate with the strains isolated from other hosts (Paudyal et.al, 2021). Seeds of black gram and groundnut were inoculated with *Rhizobium* species performed better germination (Rajesh *et al* 2019).

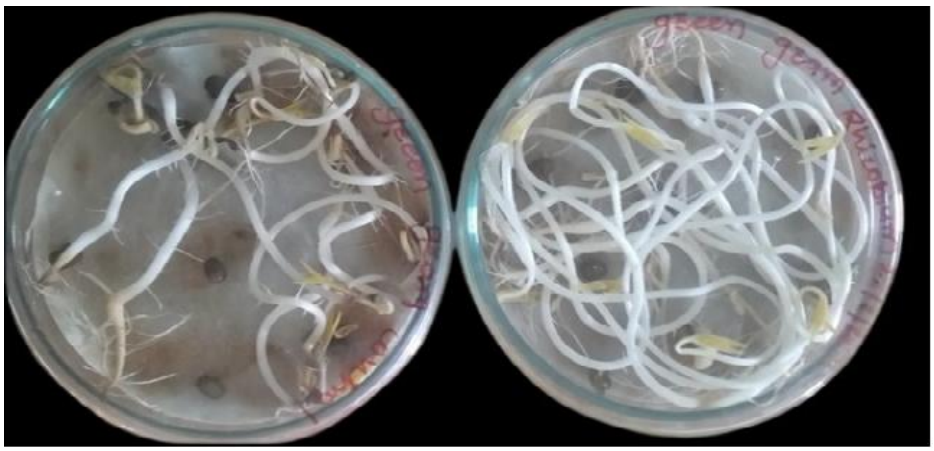
**Table 3. Effect of *Rhizobium* on Seed germination**

| Sr. No. | Plants | Test | Germinating seeds | Non germinating Seeds | Germination Percentage ( % ) |
|---|---|---|---|---|---|
| 1 | Soybean (*Glycine max )* | Rhizobium Treated | 6 | 4 | 60% |
| | | Control | 3 | 7 | 30% |
| 2 | Black gram(*Vigna mungo)* | **R**hizobium **Treated** | 10 | 0 | 100% |
| | | **Control** | 7 | 3 | 70% |
| 3 | Green gram (*Vigna radiata*) | **R**hizobium **Treated** | 10 | 0 | 100% |
| | | **Control** | 8 | 2 | 80% |
| 4 | Groundnut(*Arachis hypogea)* | **R**hizobium **Treated** | 4 | 6 | 40% |
| | | **Control** | 1 | 9 | 10% |
| 5 | Moth Bean (*Vigna aconitifolia)* | **R**hizobium Treated | 10 | 0 | 100% |
| | | **Control** | 7 | 3 | 70% |

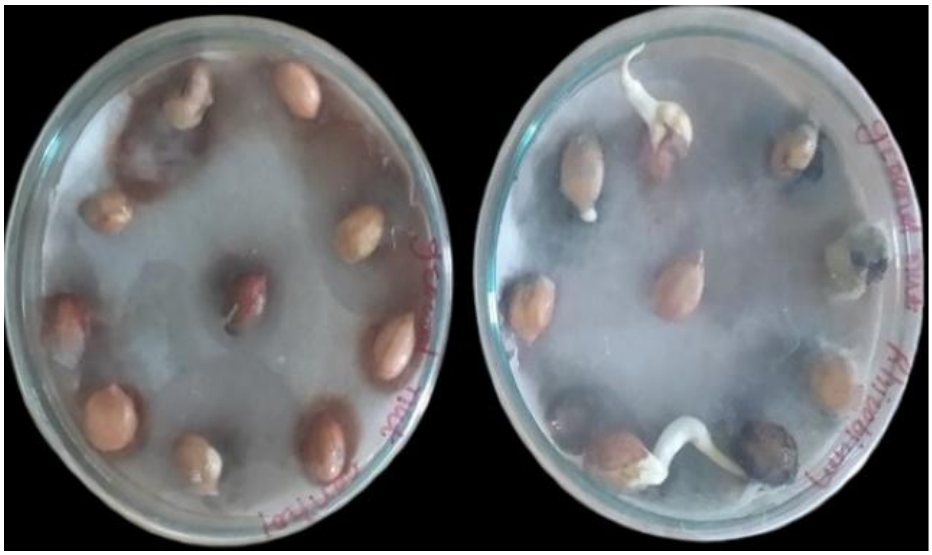
A. Soybean (Glycin max) seeds control and treated with Rhizobium culture



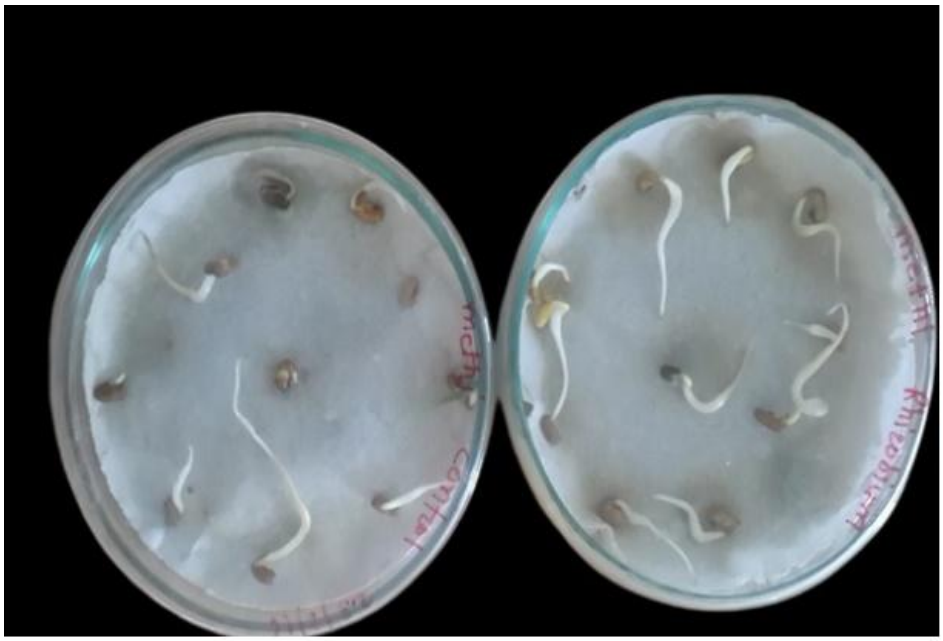
B. Black gram (*Vigna mungo*) seeds control and treated with Rhizobium



C. Green gram (*Vigna radiata*) seeds control and treated with Rhizobium



D. Groundnut (*Archis hypogea*) seeds control and treated with Rhizobium



E. Moth bean (*Vigna aconitifolia*) seeds control and treated with Rhizobium

**Fig.- 3 Effect Of Rhizobium Seed Germination**

## Conclusion

The conclusion of the present study, the isolated *Rhizobium* species from the root nodules of leguminous crop plants enhance the plant growth and yield of crop. Seed quality improvement is positively affected upon Rhizobium inoculation.

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