ISOLATION OF PLANT GROWTH PROMOTING BACTERIA - *RHIZOBIUM* AND DEVELOPMENT OF BIO *RHIZOBIUM* FERTILIZER USING VERMICOMPOST CARRIER

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ABSTRACT:

In recent decades, agricultural production relies on the use of chemical inputs in order to achieve the highest yield, which causing major problems and environmental pollution and subsequently addressed as a major obstacle to achieve a stable yield. To fulfill the demand of increased population it is very essential to enhance crop productivity with precise inputs. Nowadays, people are very aware towards health risks of the use of food products from chemical fertilizers leading to transition for a generative progression on the demand for organic products. But it is quite difficult to produce adequate quantity of organic foods for theraised population in the developing countries that's why development of biofertilizers has arouseto supply nutrients to crops. Nitrogen is a nutrient that is required for plant growth anddevelopment. Most legumes can meet their physiological nitrogen requirements. Each majorlegume crop is nodulated by different species of *Rhizobium*. It is a rapidly- growing bacteria thatprovides a plentiful supply of nitrogen to the crop. Further, plant growth promoting rhizobacteria- *Rhizobium* has multifaceted advantages in crop nutrient uptake and soil quality improvement. Thus, this paper summarizes that Increasing and expanding the use ofbiofertilizers like *Rhizobium* would minimize the need for chemical fertilizers while also reducing negative environmentaleffects.

Keywords: Agriculture, biofertilizer, Legumes, Chemical fertilizers, *Rhizobium*

INTRODUCTION:

Fertilizers, whether natural or synthetic, are applied to soils or plant tissues to provide vital nutrients for plant growth or to overcome plant nutrient insufficiency. In recent decades, agricultural production relies on the use of chemical inputs in order to achieve the highest yield, which causing major problems and environmental pollution and subsequently addressed as a major obstacle to achieve a stable yield (Keeney*et al*., 1997).Chemical fertilizer can boost plant growth and vitality, contributing to global food security. However, it can also harm the plants. The plant lacks proper root, shoot, and nutritional characteristics, as well as enough growth and maturation time. Continuous use of soil additives can deteriorate soil health and quality, leading to pollution. inorganic fertilizers are known for their high cost

and their negative environmental effects if managed poorly. (Mahajan gupta*et al.*,2008). All these give rise to reduced crop yields as a result of soil degradation and nutrients imbalance. Therefore, in the recent years numerous organic fertilizers have been produced that work as natural stimulators for plant growth and development (Khan *et al.*, 2009).

Natural stimulators, also known as microbial inoculums, have been used in small-scale compost production for generations (Abdul Halim, 2009). Biofertilizers, also known as 'microbial inoculants', are a type of fertilizer that contains live or latent cells of efficient nitrogen-fixing, phosphate-solubilizing, or cellulytic microorganisms that promote plant growth. Biofertilizers play a significant role in integrated nutrient management. Potential biological fertilizers can significantly boost productivity. They are a cost-effective, environmentally friendly, and renewable source of plant nutrients that can supplement chemical fertilizers in a sustainable farming system.

Biological Nitrogen Fixation (BNF) is the cheapest and most environmentally friendly procedure in which nitrogen fixing micro-organisms interact with leguminous plants, fix aerobic nitrogen into the soil and are able to convert the nitrogen gas into a form that is usable for plant life (Franche*et al.,* 2009). *Rhizobium* spp. are a well- known group of bacteria that act as the primary symbiotic fixer of nitrogen.The best-known associations are the symbioses of *Rhizobium* bacteria with legumes. Nitrogen fixing leguminous plants not only support plant growth independent of mineral nitrogen in the soil but also improve soil nitrogen status for associated crops through the residues of these plants

Sample collection:

Root nodules:

Healthy groundnut plants were grown and uprooted along with adhering soil. The healthy nodules were transferred to a sterile polythene bag.

Isolation of *Rhizobium* sp:

The root nodules from *Arachis hypogaea* were surface sterilized and the crushed root nodules were spread plated on yeast extract mannitol agar using a sterile L- rod. The plates were incubated at 28-30^0 C for 3-5 days. Then these were streaked on YEMA plates containing congo red. The distinct colonies were picked up and transferred to the YEMA plate for purification and further characterization. Further streaking, spreading and visual characterization of colony morphology helped in isolation of pure culture. Pure isolates were streaked on YEMA slants for preservation and further analysis.

Identification and characterization of *Rhizobiumsp*:

Various test such as morphological, biochemical and confirmatory test was carried out to identify the isolate. the morphological identification methods were, gram staining and motility test. The biochemical identification includes indole test, MR test, VP test, catalase test, urease test, oxidase test.

Confirmatory test for *Rhizobia* isolates:

All the bacteria that grow on YEMA may not be *Rhizobia*. Thus, a presumptive and confirmatory study was done to check the purity of the *Rhizobia* by re culturing the purified isolates on different media types and examining their Gram stain reaction under microscopy (Somasegaran&Hoben, 1994)

Confirmatory test on YEMA containing Congo Red:

The purity of the *Rhizobia* isolate and their ability to absorb the dye were determined by adding congo red (CR) to YEMA media, as most *Rhizobia* absorb the dye weakly, but contaminants such as Agrobacterium absorb heavily.

Confirmatory test on peptone glucose agar medium:

The isolated broth culture was streaked and incubated for 5-7 days at 28 ± 2 °C. After 5-7 days of incubation, growth and color changes were seen throughout the colony. *Rhizobia* cells showed little or no growth on peptone glucose agar. This is a confirming test for *Rhizobia*, which cannot use peptone as a carbon source. Heavy growth in this media, especially if accompanied by a considerable change in media color, indicates contaminants (Kucuk*et al.*, 2006).

Confirmatory test on keto lactose medium:

In this test, *Rhizobia* isolate was streaked on sterile yeast extract lactose agar (YEMA), which replaces mannitol with the same amount of lactose. A loop of pure culture slant (5-7 days old) was streaked on yeast extract lactose agar medium and cultured for 5-7 days at 28 ± 2°C. After incubation, a shallow layer of Benedict's solution was added to the plate. The plates were then re-incubated at 28 ± 2 °C without disturbance. In contrast, no such yellow zone was observed around the growth of *Rhizobia* (Lupwayi and Haque 1994).

YEMA media with Bromothymol blue

The BTB agar medium was utilized to distinguish *BradyRhizobium*from *Rhizobia*. The cultures were streaked on BTB Agar plates. BTB agar was created by adding 5 ml of 0.5% BTB in ethanol to 1 liter of YEMA medium. The plates were incubated at 28±2ºC for 2–10 days.

The change in hue of the medium was noticed. The isolates were classified as slow growth

BradyRhizobium(medium goes blue) or rapid growers *Rhizobia* (medium turns yellow) based on their reaction to YEMA supplemented with BTB (Somasegaran and Hoben, 1994).

Mass cultivation of *Rhizobium* sp:

Starter culture:

The starter culture was inoculated into 100ml nutrient broth to prepare starter culture

Mass production:

For mass production of*Rhizobium*, starter culture was transferred to the 250ml of nutrient broth and continuous agitation for 7 days. When cell count reached 10^8-10^9cells/ml, the broth can be used as inoculant. for easy handling, packing, storing and transporting, the broth was mixed with vermicompost carrier material which contains sufficient number of cells.

Formulation of carrier -based inoculum:

1 kg of vermicompost carrier material was weighed, sterilized and the temperature was lowered by air drying. The pH was neutralized by adding calcium carbonate. 200ml of inoculum was mixed with 1kg of processed vermicompost carrier. The carrier -based inoculum was packed in sterile polythene bags and stored at room temperature and named as Bio-*Rhizobium*.

Quality control:

As per the bis specification for biofertilizer the count of *Rhizobia* is 10^7-10^8cells/ml.

Ten grams of Bio-*Rhizobium* was taken for estimating viable cells at the initial day and 10 days after storage using dilution plate technique on YEMA agar and incubated at 37^0C for 5-7 days. after incubation, colonies were counted and calculated the total viable count.

Determine the plant growth promoting ability of Bio-*Rhizobium:*

To determine the plant growth promoting ability of Bio-*Rhizobium*, it was applied to tomato seeds. Bio-*Rhizobium* was filled with garden soil and Bio-*Rhizobium*, control pot 1 was filled with garden soil, control pot 2 was filled with garden soil and vermicompost after that tomato seeds were sowed in all three pots and placed outdoor to provide a proper light and air conditions. Growth of the plants were continuously monitored.

RESULTS AND DISCUSSIONS

In this present work the plant growth promoting rhizobacteria (PGPR)*Rhizobium* sp was isolated, characterized and formulated into carrier - based inoculum in a vermicast carrier and its plant growth promoting efficiency was determined.

Isolation of *Rhizobium* sp on CRYEMA media:

In CRYEMA plates the colonies were circular, convex, entire, semi-translucent, gummy, creamy and approximately of 2-3 cm. the colonies had not absorbed congo red developing whitish colonies and confirmed to be *Rhizobium sp.*

Fig 1: Isolation of *Rhizobium* sp

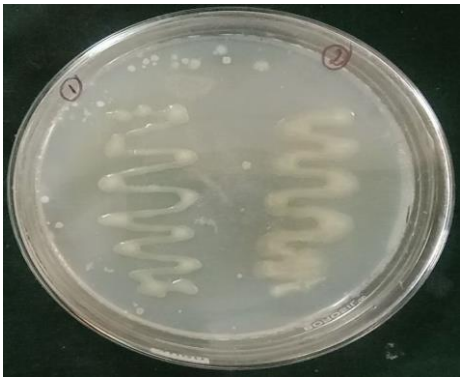


Table I: Colony morphology

Colony characters	**Observations**
Shape	Circular
Elevation	Convex
Margin	Entire
Opacity	Semi-translucent
Texture	Gummy
Color	Creamy
Size	2-3 cm

Morphological and biochemical identification of *Rhizobium* sp

The isolatewas identified as gram negative, rod shaped, aerobic nitrogen fixing bacteria. It can utilize citrate as sole source of carbon and it can produce an enzyme catalase that breakdown hydrogen peroxide to water and oxygen.The results were tabulated

Table II -Identification and characterization of *Rhizobium* sp

Characteristics	***Rhizobiumsp***
Morphological characteristics	
Gram staining	Gram -negative
Motility	motile
Biochemical characteristics	
Indole	Negative
Methyl red	Negative
Voges- Proskauer	Positive
Urease	Negative
Citrate	Positive
Catalase	Positive
Oxidase	Positive

Confirmatory test for *Rhizobium*

Congo red absorption test

The colonies did not absorb the red color of the dye indicating the isolate to be *Rhizobium sp*

Keto-lactose agar test

The isolate was being streaked on to keto-lactose agar plate and after incubation of 3-5 days the Benedict's reagent was flooded over the plate. Absence of yellowish zone was observed indicating the presence of *Rhizobium*.

YEMA with bromothymol blue

The YEMA plates inoculated with bromothymol indicator was incubated and after incubation the plates turned yellow colour indicating that their fast-growing *Rhizobia*.

Glucose peptone agar test

After incubation, the plates were observed where there was poor growth indicated the presence of *Rhizobium.*

FIG 2: *Rhizobium* on CRYEMAFig 3: *Rhizobium* on Glucose peptone agar

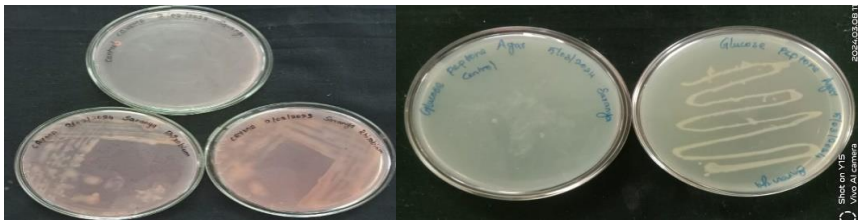
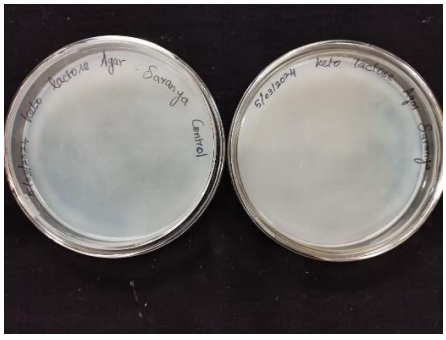
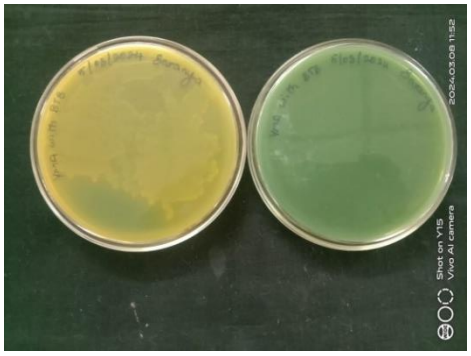


Fig 4: *Rhizobium* on keto lactose agarFig 5: *Rhizobium* on YEMA with BTB

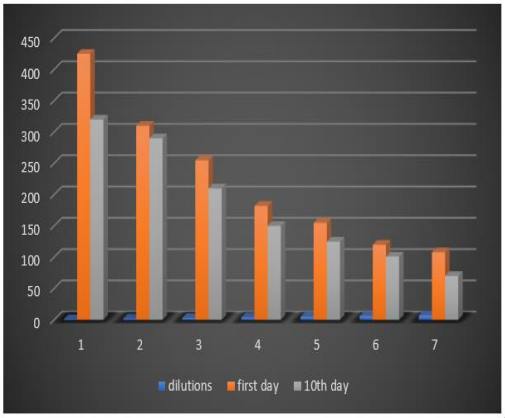




Quality control:

The quality control was determined using plate count technique for the initial and 10^{th} day

Graph I : **Determination of the initial day and 10 Day viable count in Bio-*Rhizobium***



Determination of plant growth promoting ability of Bio-*Rhizobium:*

The plant growth was determined in the first and second week such as the seed germination, Root and shoot length, plant height & number of leaves were observed and measured

Fig 6: Development of growth after first week

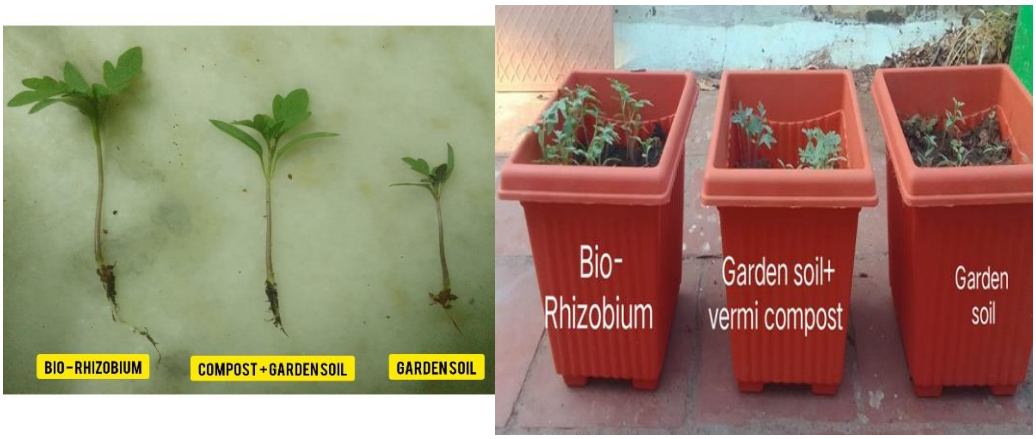


Fig 7: Development of growth after second week

In this study, the *Rhizobium*improved the growth of the non-leguminous tomato plant at different stages of growth from seed germination to root& shoot development.

Conclusion:

The present study focused on the isolation and identification of *Rhizobium*sp which was isolated from root nodules of *Arachis hypogaea using* YEMA media and isolate Confirmed morphological by gram staining and motility. The biochemical identification determined positive for Voges-Proskauer, citrate, catalase, oxidas*e* and confirmed using certain confirmatory test. In this study carrier-based inoculum of *Rhizobium* sp was named as Bio-*Rhizobium*. The plant growth promoting ability was determined and showed a better result in promoting plant growth in tomato.The Bio-*Rhizobium* reported excellent growth promoting ability. Tomato seeds treated with Bio-*Rhizobium* showed increased shoot length plant height, leaf length, number of leaves, root length and improved seed germination. The seeds treated with Bio-*Rhizobium* germinated within 4 days while the control took 6 and 8 days respectively. After two weeks of inoculation the shoot length of treated plant was2.2,3,4 in cm. the plant height of treated plant was 12cm but control was 5.3 and 8 respectively, whereas the root length was 4.5 cm in treated plant and 3.2 &1.5 respectively in control pots. Leaf height was to be of treated plant was 3.2cmand control pots had2&1.6 cm respectively. Number of leaves produced was literally higher than the uninoculated ones.Hereby, I conclude from this study that utilizing *Rhizobium* a PGPR can be efficiently used as a biofertilizer from tomato plant when applied as carrier-based inoculum. The use of Bio-*Rhizobium* -a formulated biofertilizer will be solution for eco-friendly environment reducing the utilization of chemical fertilizer and thereby preventing soil and ground water pollution and will be boon for farmers to have an organic agriculture and to increase to crop yield in agricultural crops.

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