



COMPARITIVE STUDY ON THE EFFECT OF RHOZBIUM INOCULUM ON IMBIBED SEEDS AND GERMINATED SEEDS OF VIGNO MUNGO L.

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ABSTRACT

The Rhizobium was isolated from the plant root and purified. The isolated rhizobium sample was identified based on morphological and bio chemical characterization. The result of biochemical tests showed positive result in indole test, citrate utilization test, starch hydrolysis test, urea hydrolysis test and catalase test. The isolated culture was mass multiplied and used by seed treatment like imbibed treatment and germinated treatment. After treatment the seedlings per planted separately by part culture method. The result showed that the rhizobium inoculation on the emerging radicals of one day old seedling is highly effective in nodulation and biomass production respectively.

Key words: YEMA medium; *Vigno mungo*; Germinated seed; biochemical test.



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INTRODCUTION

Nitrogen is one of the major important nutrients very essential for crop growth. But the availability of this element in soil is naturally deficient (Bras *et al.*, 2000).so the nitrogen requirements of crop plants met by the addition of fertilizer nitrogen. Fertilizer nitrogen application often commonly increase agricultural yields but these fertilizers also associated with the population of water resources declining yields and greenhouse gas emission. Our challenge is to develop and disseminate technologies which conserve natural resources while they enhance agricultural productivity and human welfare.

In soil, a number of microorganisms are at work in fixing atmospheric nitrogen. Microbial fixation of nitrogen is carried out by free living and symbiotic groups of microorganisms play an important role in agricultural soil as well as forest soil. Leguminous crops or leguminous forest plants can obtain most of nitrogen they need from the vast supply of gaseous nitrogen in the air. Legume plants fix and utilize this nitrogen by working symbiotically with special bacteria called *Rhizobium* in nodules on their roots (Chandra,1991).

Non-legumes are known to develop nodules and to accommodate symbiotic nitrogen fixation. While the potential for nitrogen fixation is directly related to the rhizobial survival, the extent of effective nodulation and the plant growth factors (Gandhi and Godbole, 1990). However, some strains of the native soil population infect the roots but are not able to fix nitrogen, while other native strains fix nitrogen but often not as efficiently as the specially selected strains used in commercial inoculants or through the natural selection (Ghalab *et al.*, 2000). The present study was undertaken with the view to comparative study on the effect of *Rhizobium* inoculation on imbedded seeds and germinated seeds of Black gram (*Vigno mungo* L.).

MATERIALS AND METHODS

Plant material

Vigno mungo coming under the family Fabaceae, is a short duration commercial pulse crop. The relevance of this plant is used as multipurpose cooking item and the leaves have medicinal properties. The plant involved in the nitrogen fixation process (Kulhare *et al.*, 1996)) and used as delicious fodder.

Collection of nodules

Around the Devaram nagar in Thanjavur (*Vigno mungo*) plants were dug out at flowering stage. Large sized pink nodules were collected by uprooting the plants with intact root system. The nodules were brought to the laboratory for the isolation of rhizobia.

Isolation of *Rhizobium*

For the isolation of *Rhizobium* from the root nodules, the method described by Vincent (1970) has been followed.

Characterization of *Rhizobium* isolate

The isolates of rhizobia were confirmed for their identify by growth of congored yeast extract, staining of polyhydroxyl butyrate and growth of YEMA medium containing bromo phenol blue.

Cultural characteristics of *Rhizobium* (Lazzo and Wopereis, 2000)

The rhizobial isolates were grown on YEMA medium, after 24 hrs the colony shape and size (diameter) of the rhizobial isolates were measured and recorded.

Mass cultivation of *Rhizobium*

The isolated rhizobial strain was cultures in YEMA medium for about 3 days in order to establish better growth of bacteria. Three to six days old cultures of *Rhizobium* was examined for purity and the bacterial colonies were scraped with the help of an inoculation needle and was transferred to 250 ml conical flask containing YEMA broth.The subculture flasks were incubated at 28°C on rotary shaker for 2-3 days.

Experimental design (Poonam Chaudhary *et al.*, 2002)

Garden soil was initially sieved and then sterilized in an autoclave at 15lb pressure for 15-20min. Then the soil was sprinkled with water and mixed to field capacity. Then the pots were filled with the soil and labeled. For culture comprised of one control sterilized soil and one imbeded seed one geminating seed with three replications were conducted.



Parameters analysis

Total number of root nodules

Total number of nodules, total biomass and total root biomass were analyzed and expressed in number and gram respectively.

RESULT AND DISCUSSION

In the present study rhizobium was isolated by root nodules of legume plant *Vigna mungo*. The isolates were purified and showed positive result in PHB test and failed to congo red in YEMA coloration was not found in lactose agar test, 3- keto lactose test and no growth was found in Hofer's alkaline agar and litmus milk reaction test of rhizobial isolates.

Many workers surveyed (Poornina Yada, 2004) many strains of rhizobial and used many cultural test, pointed out that the rhizobia typically show slow growth on peptone glucose agar, form little or no H₂S from bismuth sulphite and no precipitate in glycerol phosphate agar, not absorb in congo red from YEMA medium, unable to grow.

The isolated culture were identified by various morphological and biochemical method. The result clearly indicate all the rhizobium were gram-negative, rod shaped and motile in nature the cell and diameter of rhizobial isolates ranged from 0.5 to 3µm respectively. The result of biochemical test such as indole test, citrate utilization test, starch hydrolysis test, urea hydrolysis test and catalase test showed all the positive result in all the rhizobial isolates, were all the isolates showed the negative result in methyl red test, VP test, triple sugar iron test and oxidase (Table-1).

Rhizobium inoculums on emerging radical of *Phaseolus mungo* is highly effective in nodulation and biomass production when compared to inoculation on imbedded seeds. Application of Rhizobium culture directly on the seed surface before sowing can improve the nodule development and biomass production (Yousif and Sprent, 1983).

The isolated culture was mass multiplied using YEMA broth. The culture was used by seed treatment. Two type of seed treatment was carried out after treatment the imbibed and young seedlings were planted separately in pot under uniform conditions of soil mixture and temperature.

Rhizobium inoculated plants showed nodules on 20th, 30th, 40th and 50th day of planting (table-2) indicate the total biomass production in control and rhizobium inoculated *Vigna mungo* seeds. The rhizobium inoculation on the emerging radicals of one day old seedlings is highly effective in nodulation and biomass production. When compared to the plant developed from rhizobium imbibed seeds, plant from rhizobium inoculated seedlings recorded about 100% increase in nodule development at different growth stages. Result obtained from the present study indicate that the influence of environmental and soil factors on nodulation can be reduced by inoculating thee young seedlings with rhizobial before planting.

The study conclude that the influence of environmental and soil factors on nodulation can be reduced by inoculating the young seedlings with rhizobial before planting.

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TABLE-1: Biochemical characteristics of Rhizobial isolates for *Vigna mungo* L.

S.NO.	Biochemical tests	Result
1	Mac Conkey agar	+
2	Indole	+
3	Voges-proskauer	-
4	Citrate utilization	+
5	Methyl red	-
6	Triple sugar ion	+
7	Starch	+
8	Urea hydrolysis	+
9	Catalase	+
10	Oxidase	+

+ denote positive result

- Denotes negative result

Table-2: Mass production of *Rhizobium*

Total number of nodules			
Total number of root nodules			
Day after planting	Control plants	Rhizobium inoculated plants	
		Seed inoculated	Radical inoculated
20	0	0	2
30	4	4	6
40	8	11	13
50	12	18	25
Total biomass production			
Day after planting	Control plants	Seed inoculated	Radical inoculated
20	20	20	25
30	55	62	78
40	70	78	108
50	85	96	115
Root biomass in control			
Day after planting	Control plants	Seed inoculated	Radical inoculated
20	10	05	10.5
30	15	10	18
40	20	18	28
50	40	25	36