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## **MODERN TRENDS IN GREEN CHEMISTRY, MEDICINAL CHEMISTRY AND ENVIRONMENTAL SCIENCE**

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## Effect of Saturated Solution of Ferrous Sulphate and Boric Acid on a Plant Growth Promoting Rhizobacteria–*Rhizobium species* – Host –*Cajanuscajan* (Arhar/ tur).

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### Abstract:

The nitrogen assimilating bacterium *Rhizobium species* is naturally existing symbiotic nodule forming bacterium in the roots of *Cajanuscajan*. In-vitro conditions it grows in the Yeast Extract Mannitol media. In this study saturated solution of Ferrous sulphate ( $\text{FeSO}_4$ ) and Boric acid ( $\text{H}_3\text{BO}_3$ ) were separately experimented. The bacterium *Rhizobium species* was aseptically inoculated in the Yeast Extract Mannitol broth and shake incubated for 48 hrs at R.T. One ml of this cultured bacterium was serially diluted to  $10^9$  dilution further from it 0.1 ml of  $10^9$  dilution was spread inoculated on Congo Red Yeast Extract Mannitol (Cr.Y.E.M.A) Agar media plate with thickness of 5mm. Single steam pre-sterilized Whatman filter paper disc grade 1 was dipped in pre-sterile saturated solution and placed in the centre of Cr.Y.E.M.A petri plate already inoculated with *Rhizobium species* culture through spread plate technique. The petri plate was kept in fridge at  $4^\circ\text{C}$  for 5 minutes for diffusion. The petri plate was incubated at  $32^\circ\text{C}$ , for 72 hours. In vitro experimental observations for  $\text{FeSO}_4$  no bacterial growth upto 1mm radial zone was observed from the periphery of the filter paper disc and for  $\text{H}_3\text{BO}_3$  no bacterial growth upto 8mm radial zone was observed from the periphery of the filter paper disc. These compounds of Ferrous and Boron of commercial grade are generally produced by companies for farmers to use in their farms.

### Keywords :

Yeast Extract Mannitol Broth Media, Congo Red Yeast Extract Mannitol Agar Media, PGPR (Plant Growth Promoting Rhizobacteria, Serial Dilution, Zone Of Inhibition, Agriculturally Important Bacteria, Saturated Solution.

### Introduction

Usually all agricultural crop yield depends totally upon its soil fertility by biotic and abiotic entities, enabling the survival and extinction for most land-based life on the thin layer of soil covering earth's surface (Doran and Zeiss, 2000). Soil is the mixture of live organism (biotic) and minerals (abiotic) that provides vital nutrient and a hence a favorable environmental condition for optimum crops growth, thus farm soil is composed of two crop favorable parts as biotic and abiotic (living and non living).

Fertiliser is man made factory product agricultural input which provides inorganic type nutrient to the farming soil resulting in enhancement of soil fertility leading to increased crop yield. A good Soil health is the judicial balance of micronutrients, macronutrient, plant growth promoting microbial masses and organisms favoring such activities. The scientists conclude that 50mM of ionic strength is required for adherence of microbial mass to the sand surface Saeed Torkzaban et al, Shiva S. Tazehkhand et al, Sharon L. Walker et al and Scott A. Bradford et al, 5 April 2008 (Transport and fate of bacterial in porous media coupled effects of chemical conditions and pore space geometry, Water resources research, Vol 44, W04403, doi: 10.1029/2007, WR006541, 2008).

Organic and inorganic fertilizers continuously applied for 59 years on farm soil (clay loam, orthicluvisol) there studies were carried on through soil application treatment as per the combinations of manure + NPK, compost + NPK, cattle manure + straw + NPK and its result were compared with the results obtained through soil application treatment as per combination of cattle slurry + straw + NPK. The conclusion of the study revealed that, the cattle slurry + straw was most favorable to increase the soils total C, N, hot water soluble C, microbial biomass C, and dehydrogenase activity. (Influence of long term application of organic and inorganic fertilizer on soil properties, T. Simon et al, A.Czako et al, Plant soil environment. Vol. 60. 2014, No. 7: 314-319). Plant use 50% of nitrogenous fertilizer whereas 2 – 20% lost through evaporation, 15 -25% react with organic compounds and 2-10% interfere surface and ground water, apart decrease in pH of soil to critical acidic level also the air pollution by nitrogen oxide ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}_2$ ) which increases from 0.2-0.3% each year, leading in nitrate content threatening human life (Serpil Savei et al "An agricultural pollutant: Chemical fertilizer", International journal of environmental science and development, Vol. 3, No. 1, Feb 2012).



The well balanced concentration of a-biotic entities and biotic entities as  $N_2$ -fixing microbes are directly influenced by "rhizospheric soil" consisting agriculturally important microorganisms (Jonas *et al.*, 2011). Therefore ideal microcosms through rhizospheric soil is needed for agriculture crop yield which help such microorganisms to dwell in harmony in the nature, and this favor the commercial crop yields through fulfilling their own nutrient requirements through symbiotic or commensalism associations. It is well known that the farmers use inorganic fertilizers in a quantity which do not adhere to the scientifically recommended proven doses. On broadcasting such inorganic form of chemical fertilizer granules in soil gets solubilised which directly impart certain stress on the microbial population in its vicinity either through its efficacy or its survival. Thus here the study of fate of *Rhizobium species*- Host – *Cajanuscajan* arhar / tur a potential PGPR (Plant Growth Promoting Rhizobacteria) is therefore experimented in-vitro for Ferrous and Boron attained from its compound form as  $FeSO_4$  and  $H_3BO_3$  respectively.

The production of bio-inoculants i.e. PGPR (Plant Growth Promoting Rhizobacteria) are grown in aseptic conditions in desired standard media which are then mixed with suitable inert carriers such as peat, lignite now also available in liquid form. These bio-inoculants so produced provide nutritional support to commercial crops (Gomare, *et al.*, 2013). The present study is made to find the effect of saturated compound of plant micronutrient on *Rhizobium species* – Host – *Cajanuscajan* – i.e. Arhar / tur bio-inoculant which is wide-spread used for dicot / leguminous crop.

## Materials and Methods

### Sample Collection

**Bacterial Strain:** *Rhizobium species* are the native nodule forming bacteria naturally found in roots of *Cajanuscajan* (Arhar/ tur). The bacterium used in the present study is *Rhizobium species* which is isolated from Biofertilizer Packet produced by M.P Agro Ind., Bhopal, these strains are said to have high nitrogen fixing efficiency.

#### a) Preparation of saturated solution –

The saturated solution of  $FeSO_4$  and  $H_3BO_3$  is prepared by gradually adding small quantities of it in 100 ml of double distilled water in 500 ml beaker. The stirring is facilitated by using magnetic stirrer till no more compound gets solubilised. The solution is poured in 250 ml conical flask and stoppered with cotton plug and steam sterilized.

#### b) Preparation of broth culture of Bacterial strain :-

The pure culture of *Rhizobium species* are inoculated in pre-sterilised Yeast Extract Mannitol broth media and incubated at room temperature for 48 hours to 72 hours till the cell concentration exceeds the optical density (OD) 1 at 620 nm and a viable cell count of  $1.0 \times 10^9$  per ml of matured (stationary phase bacteria) broth. This matured broth is then diluted to  $10^9$  and its 0.1 ml is used as inoculum on experimental Congo Red Yeast Extract Mannitol agar plate medium, inoculum is spread evenly with the help of sterile glass spreader.

#### c) Preparation of filter paper discs :-

Whatman filter paper grade 1 is evenly punched with help of punching machine and several uniform discs were prepared. These discs were wrapped in brown paper and then steam sterilized.

#### d) Studies of Saturated Concentration of $FeSO_4$ and $H_3BO_3$ on *Rhizobium species*:-

Aseptically pre-sterilised whatman filter paper grade 1 disc were dipped in saturated solution and placed in the centre of freshly spread plate inoculated *Rhizobium species* which is spread plated with the help of sterile glass spreader on petri plate of Congo Red Yeast Extract Mannitol agar medium. Separate experiments were performed for Ferrous sulphate and Boric acid. The plates were kept uprightly at  $4^\circ C$  in refrigerator for diffusion and then plates were upside down incubated at  $32^\circ C$  for 72 hours.

**Table 1:-** Effect of saturated solution of compounds on *Rhizobium species* – Host *Cajanuscajan* (arhar/ tur).

S.no	Saturated solution (compound)	Zone of inhibition of <i>Rhizobium species</i> – Host- <i>Cajanuscajan</i> (arhar/ tur)	
		Radius of no growth	Diameter of no growth
1	$FeSO_4$	1mm	2mm
2	$H_3BO_3$	8mm	16mm

Note: - The thickness of Yeast extract mannitol agar media in petri plate is 5 mm.

## Results and Discussion.

**Effect of Saturated solution of  $FeSO_4$  and  $H_3BO_3$  on *Rhizobium species* – Host *Cajanuscajan* (arhar/ tur):-**



Zone of inhibition measuring to 1mm of growth of *Rhizobium species* is observed from the disc till the periphery of the zone in the experiment conducted for  $\text{FeSO}_4$  whereas for  $\text{H}_3\text{BO}_3$  Zone of inhibition measuring to 8mm of no growth of *Rhizobium species* is observed from the disc till the periphery (Table 1). Trace elements/metal function as co-factors in enzymatic reactions, stabilizing structure of enzyme itself. (Zhuoer Lin *et al.*, 2009).

The chief objective of the present investigation is to know the individual effect of concentration of compound of such plant micronutrients on the bacterium *Rhizobium species* enabling the fate of agriculturally useful microorganism by widespread use of granular inorganic fertilizer on farm soil.

### Conclusions.

The compounds of micronutrients are the prime requirement (trace elements) for various vital metabolic / catabolic activities of plants. As such the usual practice adopted by most farmers about usages of inorganic micronutrient is always on higher side than standard doses as recommended by agriculture scientists. The in-vitro experimental results concludes the ill effect of such inorganic fertiliser on the population of agriculturally important plant growth promoting *Rhizobacteria* that is *Rhizobium species* its natural host being *Cajanus cajan* (arhar/ tur) as this study suggests that inorganic  $\text{FeSO}_4$  or  $\text{H}_3\text{BO}_3$  of granular or in saturated liquid form may be used in other appropriate forms in the farming process during the cultivation of *Cajanus cajan* - Arhar/ tur.

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