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EFFECT OF SATURATED SOLUTION OF MANGNOUS OXIDE, ZINC SULPHATE ON A PLANT GROWTH PROMOTING RHIZOBACTERIA - *Rhizobium species*- HOST - *Cajanus cajan*(ARHAR)

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ABSTRACT

The nitrogen assimilating bacterium*Rhizobium species* is a symbiotic nodule forming bacterium which grows in the Yeast Extract Mannitol. In this study saturated solution of Mangnous oxide (MnO), Zinc sulphate ($ZnSO_4$) were separately used. The bacterium *Rhizobium species* was inoculated in the Yeast Extract Mannitol broth and shake incubated for 48 hrs. 1ml of this incubated broth was serially diluted to 10^9 dilutions and 0.1 ml of 10^9 dilutions was spread inoculated on congo red yeast extract mannitol agar media plate with thickness of 5mm, single sterile Whatman filter paper disc grade 1 was dipped in sterile saturated solution and placed in the centre of spread inoculate media in petri plate. The petri plate was kept in fridge at 4^0C for 5 minutes for diffusion. The petri plate was incubated at 32^0C, room temperature for 72 hrs. In vitro experimental observations for MnO showed no bacterial growth upto 10 mm radial zone from centre of the filter paper disc where as in case of $ZnSO_4$ no bacterial growth was seen upto 2mm radial zone from the centre of the filter paper disc.

Keywords: Yeast Extract Mannitol Broth Media, Congo red Yeast Extract mannitol Agar Media, PGPR (Plant Growth Promoting Rhizobacteria). Shake incubated, Serial Dilution, Zone of Inhibition, Agriculturally Important Bacteria, Saturated Solution.

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INTRODUCTION

Agricultural crops yields are dependent on soil fertility, thus the difference between survival and extinction for most land-based life is characterized by the thin layer of soil covering earth's surface[4]. Soil is the living mixture of minerals and organisms that provides vital nutrient and a healthy environment that nurtures crops growth, therefore the soil is divided into two parts as biotic and abiotic (living and nonliving).

Fertilizer is any substance to add nutrient to the soil to promote soil fertility and increase plant growth. Soil health relies on the balance of macronutrient as well as microbial health. The scientists conclude that 50mM of ionic strength is required for adherence of microbial mass to the sand surface [10-12].The importance of ionic concentration along with biofertiliser in agriculture was also supported by the study on combination of biofertiliser along with zinc sulphate (20 lit/ ha and 50 gm/ ha) on mustard crop at every 10 days of interval up to 50 day from the date of sowing gave maximum crop yield per hectare[8]

Organic and inorganic fertilizers continuously applied for 59 years on farm soil (clay loam, orthic luvisol) studies where the soil applications treatments consisting the combinations as manure + NPK, compost + NPK, cattle manure + straw + NPK were compared with cattle slurry + straw + NPK revealed that, the cattle slurry + straw was most favorable in increasing 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,[9-10]Plant use 50% of nitrogenous fertilizer whereas 2–20% lost on evaporation, 15-25% react with organic compounds and 2-10% interfere surface and ground water, apart decrease in pH of soil to critical level along with air pollution by nitrogen oxide (NO, N_2O, NO_2) which increases from 0.2-0.3% each year, leading in nitrate content threatening human life.[11]

The optimum concentration of a-biotic entities and N_2-fixing microbes are directly influenced by "Rhizospheric" agriculturally important microorganisms[12]

Therefore ideal microcosms is needed for agriculture helping microorganisms to dwell, and this favor the commercial crop yields through fulfilling their own nutrient requirements.The practice of broadcasting of inorganic fertilizers throughmanually or machines by farmers

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isalways more than scientifically recommended.During irrigation such granules in soil gets solubilized which directly affect the microbial population in its vicinity. The study of fate of *Rhizobium species*- Host - *Cajanus cajan* (Arhar/ Tur)a potential PGPR (Plant Growth Promoting Rhizobacteria) is therefore experimentedin-vitro for Zinc and Manganese through Zinc sulphate and Mangnous oxide.

The production of bio-inoculants, PGPR (Plant Growth Promoting Rhizobacteria) are grown in aseptic conditions in desired media which are then mixed with suitable inert carriers such as lignite. These bio-inoculants so produced provide nutritional support to commercial crops[13]The present study is made to find the effect of saturated compound of plant micronutrient on *Rhizobium sps* - Host - *Cajanus cajan (*Arhar/ tur*)* bio-inoculant which is used for dicot / leguminious crop.

MATERIALS AND METHODS

Sample Collection

Bacterial Strain: *Rhizobium sps* are the native nodule forming bacteria found in roots of *Cajanus cajan* (Arhar/ Tur) nature. The bacterium used in the present study is *Rhizobium sps* 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 Zinc sulphate / Mangnous oxide is prepared by gradually adding small quantities of compound in 100 ml of double distill water in 500 ml beaker. The stirring is facilitated by using magnetic stirrer till no more compounds gets solubilized. 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 sps* are inoculated in pre-sterilized 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

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1.0×10^9per ml of matured (stationary phase bacteria) broth. This matured broth is then diluted to 10^9 and its 0.1 ml isused as inoculumonexperimental Congo Red Yeast Extract Mannitol agar plate medium, inoculums is spreadevenly with the help of sterile 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 ofSaturated Concentration of Zinc sulphate / Mangnousoxide on Bacterial strain:-

Aseptically pre-sterilized whatman filter paper grade 1 disc were dipped in saturated solution and placed in the centre of freshly inoculated *Rhizobium species*and spread plated on petri plate of the Congo Red Yeast Extract Mannitol agar selective medium.Separate experiments were performed for Zinc sulphate and Mangnous oxide. The plates were kept in 4^0C in refrigerator for diffusion and then uprightly incubated at 32^0C, room temperature for 72 hours.

RESULTS AND DISCUSSION

Effect of Saturatedsolution of $ZnSO_4$/MnO on *Azotobacter chrococcum*:

Zone of inhibitionof growth of *Rhizobium species*is observed in both the experiment conducted for Zinc sulphate and Mangnous oxide. The experiment with Zinc sulphate showed no growth of bacterium at2mm as zone radius, whereas no growth zone of10mmwas seen in experiment using Mangnous oxide. (Table 1). Trace elements/metal function as co-factors in enzymatic reactions, stabilizing structure of enzyme itself. (Zhuoer Lin *et al*., 2009). Zinc is a multi-functional element found in almost 300 enzymes, and is involved in catalytic, co-catalytic, and/or structural functions; enzymes containing zinc in the reactive center are widespread in nature (Tubek*et al*., 2008). Manganese are vital components of biological redox reactions (Zhuoer Lin *et al*., 2009).

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

CONCLUSION

The compounds of micronutrients are well known to impart healthy effect on the plant growth as they are the prime requirement (trace elements) for various vital activities of plants. As such the usual practice adopted by most farmers during broadcasting of inorganic micronutrient is always more than per hectare recommended doses by agriculture scientists. The in-vitro experimental results concludes the ill effect of such inorganic fertilizer on the population of agriculturally important plant growth promoting Rhizobacteria -*Rhizobium species*, therefore such inorganic Zinc sulphate / Mangnous oxide instead of granular concentrated form may be used in other appropriate forms.

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Tables

Table 1:- Effect of saturated solution of compounds on *Rhizobium species* - Host - *Cajanus cajan* (Arhar / Tur)

S.no	Saturated solution	Zone of no growth of *Rhizobium species*	
		Radius of no growth	Diameter of no growth
1	MnO	10 mm	20 mm
2	$ZnSO_4$	2 mm	4 mm

Note: - The thickness of Jenson agar media in petri plate is 5 mm.