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Effect of Mangnous Oxide, Zinc Sulphate on a plant growth promoting rhizobacteria - Azotobacter chrococcum.

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Abstract:

The nitrogen assimilating bacterium *Azotobacter chrococcum* is a non symbiotic nodule forming bacterium which grows in the Jenson media which is devoid of nitrogen ingredients, here saturated solution of Mangnous oxide (MnO), Zinc sulphate ($ZnSo_4$) were separately used. The bacterium *Azotobacter chrococcum* was inoculated in the Jenson broth and incubated for 48 hrs. 1ml of this incubated broth was serially diluted to 10^9 dilution and 0.1 ml of 10^9 dilution was spread inoculated on Jenson 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^OC for 5 minutes for diffusion. The petri plate was incubated at 32^OC, room temperature for 72 hours. In vitro experimental observations for MnO showed no bacterial growth upto 7 mm radial zone from centre of the filter paper disc whereas in case of $ZnSo_4$ no bacterial growth was seen upto 9mm radial zone from the centre of the filter paper disc.

Keywords : Jenson Broth Media, Jenson Agar Media, PGPR (Plant Growth Promoting Rhizobacteria). Serial Dilution, Zone Of Inhibition, Agriculturally Important Bacteria, Saturated Solution.

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 (Doran and Zeiss, 2000). 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 non living).

Fertiliser 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 concludes 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). 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 upto 50 day from the date of sowing gave maximum crop yield per hectare (Khin S. Aye et al, World Academy of Science, Engineering and Technology, Vol. 5, 2011-03-26, p-233-235).

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, 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 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, N2O, NO2) 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 optimum concentration of a-biotic entities and N_2-fixing microbes are directly influenced by “rhizospheric” agriculturally important microorganisms (Jonas *et al*., 2011). 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 through manually or machines by farmers is always more than scientifically recommended. During irrigation such granules in soil gets solubilised which directly affect the microbial population in its vicinity. The study of fate of *Azotobacter chrococcum* a potential PGPR (Plant Growth Promoting Rhizobacteria) is therefore experimented in-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 (Gomare, *et al*., 2013). The present study is made to find the effect of saturated compound of plant micronutrient on *Azotobacter chrococum* bio-inoculant which is used for all monocot crop.

Materials and Methods

Sample Collection

Bacterial Strain: *Azotobacter chrococcum* are the native non nodule forming bacteria found in nature. The bacterium used in the present study is *Azotobacter chrococcum* 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 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 *Azotobacter chrococcum* are inoculated in Jenson 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 1.0×10^9 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 Jenson agar plate medium, inoculums is spreaded evenly 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 of Saturated Concentration of Zinc sulphate / Mangnous oxide on Bacterial strain :-

Aseptically pre-sterilised whatman filter paper grade 1 disc were dipped in saturated solution and placed in the centre of freshly inoculated *Azotobacter chrococcum* and spread plated on petri plate of the Jenson agar medium. Separate experiments were performed for Zinc sulphate and Mangnous oxide. The plates were kept in $4^{O}C$ in refrigerator for diffusion and then uprightly incubated at $32^{O}C$, room temperature for 72 hours.

Results and Discussion.

Effect of Saturated solution of $ZnSO_4$ / MnO on *Azotobacter chrococcum* :-

Zone of inhibition of growth of Azotobacter chrococcum is observed in both the experiment conducted for Zinc sulphate and Mangnous oxide. The experiment with Zinc sulphate showed no growth of bacterium at 7mm as zone radius, whereas no growth zone of 5mm was 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 *Azotobacter chrococcum* 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 fertiliser on the population of agriculturally important plant growth promoting rhizobacteria *Azotobacter chrocrccum*, therefore such inorganic Zinc sulphate / Mangnous oxide instead of granular form may be used in other appropriate forms.

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Tables

Table 1:- Effect of saturated solution of compounds on *Azotobacter chrococcum*.

S.no	Saturated solution	Zone of no growth of Azotobacter chrococcum	
		Radius of no growth	Diameter of no growth
1	**MnO**	7 mm.	14 mm.
2	**$ZnSO_4$**	9 mm.	18 mm.

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