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Effects of Micronutrient amendment on Growth and Efficacy of Biofertilizers

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ABSTRACT

Micronutrients play an important role in growth and efficiency of plant growth promoting rhizobia. Rhizobial growth is ceases during scarcity of micronutrient elements like iron, molybdenum, boron and zinc. Now due to depletion of soil quality the nutrient content of soil is decreasing rapidly. In nutrient deficiency biofertilizers were not working efficiently. Therefore, there is urgent need of assessment of quantity and type of micronutrient require in growth of rhizobia. In the present research investigation, effect of different concentrations of micronutrients (boron and molybdenum)on the growth of rhizobial isolate MRH 59 was evaluated. It was found that small change in micronutrient concentration leads to drastically decrease in rhizobial population.

KEYWORDS: Maintenance, Performance Management, Optimal Maintenance

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INTRODUCTION

Due to overexploitation of chemical fertilizers and pesticides the quality of agriculture soil is decreases continuously. Use of biofertilizers in the form of bioinoculants gained worldwide importance due to its ecofriendly nature. Rhizobia are root nodulating bacteria which reside inside the plant and help in biological nitogen fixation. Moreover, rhizobia play important role in plant growth promotion due to its diverse efficacy like hormones production, reduces biotic stress through siderophore production and hydrogen cyanide production, mitigate abiotic stress via amelioration of soil salinity[1]. The rhizobial inoculants markedly increased nodule number, nodule mass, shoot height and crop yield in comparison to chemically treated crops. In legume crops rhizobial inoculation enhanced crop yield to 30-47% [2].

In soil the efficacy of rhizobia is affected by number of factors like competition to native bacteria, nutrient unavailability or deficiency of nutrient in soil. In present day, Indian soil is become deficient not only on major nutrients (Nitrogen, phosphorus but also in some micronutrients like iron (Fe), molybdenum (Mo), boron (B), copper (Cu) and zinc also. Therefore, a balanced micronutrient concentration is necessary for higher crop productivity. The quantum and quality Rhizobium is very basic need to increase production of food legumes in India. Micronutrient deficiency in soil results in decrease in crop productivity as well as increased malnutrition condition on consumption [3].

The common medium used for culturing rhizobia is yeast extract mannitol medium, which is made up of yeast extract powder or paste and is found to be best suiting nutrient medium for Rhizobium growth. The ideal medium contains mannitol as the source of nitrogen, growth factors and mineral salts. Other than these elements for the appropriate growth of rhizobia, micronutrients are also essential. The small quanity of micronutrients can trigger the growth enhancement in production. Micronutrients play an important role in increasing yield of pulses and oilseed legumes through their effects on the plant itself and on the nitrogen fixing symbiotic process[4]. Boron involved in hormone synthesis and translocation, increases nodule and flowers number, zinc is involved in auxin formation; activation of dehydrogenase enzymes and stabilization of ribosomal fractions. Therefore to evaluate the effect of micronutrient concentration efficiency of rhizobia a pot house experiment was conducted in which soil different concentration of micronutrient is added with coinoculation of rhizobia

MATERIAL AND METHODS

For inoculum preparation, a loopful of culture from freshly prepared slants of rhizobial isolates was transferred to YEM broth. The rhizobial isolates were grown in YEM broth for three days on shaker at 30°C.

After three days of incubation on shaker, one ml of the inoculum was transferred to the pre-sterilized yeast extract mannitol broth (YEMB) containing different concentration (0.05, 0.1, 0.5, 1.0 and 1.5 %) of micronutrients (Fe, Mo, B and Zn), flasks were incubated at 28-30^{0}C and initial population was estimated by using standard plate count technique Vincent et al [5].

RESULT AND DISCUSSION

Boron deficiency affects legume root colonization, nodulation, nitrogen fixation, and induction of nodules in legumes. Moreover, a requirement for boron has been reported for rhizobial infection and the nodule invasion process. Boron is essential for symbiosome development and bacteroid maturationand for early events in plant–bacteria signalling. In boron-deficient plants, the number of rhizobia infecting the host cells and the number of infection threads are reduced and the infection threads develop morphological aberrations[6]. Boron deficiency affects legume root colonization, nodulation, nitrogen fixation, and induction of nodules in legumes. Therefore, effect of boron on rhizobial growth was studied using different concentrations (0.05, 0.5, 1.0 and 1.5%) of boron as a supplement in YEMB. Data given in the table showed that viable count of rhizobial isolate MRH59 increased from first to 5th day in YEMB as well as on YEMB supplemented with different concentrations sodium tetraborate (borax) at the rate 0.05, 0.5, 1 and 1.5%. Maximum log no. cfu/ml 9.951 was reported when YEMB was supplemented with 0.5 % sodium tetraborate followed by supplementation of 0.05% sodium tetraborate (9.005 log no of cfu/ml). On supplementation of 0.5% boron in YEMB, 19% higher log no. of cfu/ml was recorded than the control. Supplementation of 0.05% sodium tetraborate, although increased growth rate of 8 percent over the control, but this growth was neither at par or superior over 0.5% supplementation of sodium tetraborate in YEM broth. Supplementation of 1.0 % sodium tetraborate had approximately similar log no. of cfu/ml as that of control. But further increase in borax concentration caused decrease in log no of cfu/ml over control (Table 1). On supplementation of 0.5% sodium tetraborate in the YEM broth 19% higher log no of cfu/ml were achieved. This study is in line with the previous study carried out by Zerpa et al. [7]that boron effectively stimulates the rhizobial growth at low concentration of 0.5, 0.6 and 0.7%.

Table I Effect of different concentrations of borax on growth of isolate MRH59

Incubation time (Days)	Log no. of cfu ml^{-1}				
	$Na_2B_4O_7$concentration (%)				
	*Control	0.05	0.5	1.0	1.5
1	2.267	3.477	3.806	2.477	2.431
2	3.298	4.869	4.690	4.623	4.301
3	5.368	6.602	7.819	5.544	5.477
4	6.431	8.457	8.587	6.480	6.602
5	8.380	9.005	9.951	8.381	7.778
6	7.260	7.832	7.812	7.707	5.301
7	7.079	7.557	7.619	6.956	4.462

Table II Effect of different concentrations of zinc sulphate on growth of isolateMRH59

Incubation time (Days)	Log no. of cfu ml^{-1}				
	$ZnSO_4$concentration (%)				
	*Control	0.05	0.5	1.0	1.5
1	2.267	3.638	3.795	2.707	2.707
2	3.298	5.851	4.929	3.643	3.643
3	5.368	6.530	6.534	4.245	2.245
4	6.431	7.931	7.787	6.397	-
5	8.380	9.077	8.798	5.158	-
6	7.260	8.579	7.029	4.954	-
7	7.079	7.728	7.011	4.913	-

Zinc acts as micronutrient and is known to affect nodulation and dinitrogen fixation. Zinc plays important role in development and maintenance of cell surface components. Besides this, zinc is involved in auxin formation; activation of dehydrogenase enzymes and stabilization of ribosomal fractions[8]. Thus, zinc improves nodulation and nitrogen fixation. Zinc is required for indole acetic production which is important for nodulation in legumes[9]. Effect of zinc on growth of rhizobal isolates was studied from 1st to 7th day using zinc sulphate as a nutrient supplement in YEMB. Growth represented as log no of cfu/ml increased from 1st to 5th day on supplementation of ZnSO4 but after that it started declining. Maximum 9.077 log no of cfu/ml was reported on 5th day in YEM broth supplemented with 0.05% ferrous sulphate and it was 8.3% higher as compared to control. Further increase in concentration of zinc sulphate to 0.5% and more was not effective in terms of growth enhancement. Supplementation of 1.5% zinc sulphate in YEM broth was found to inhibitory for the growth and drastic decline in growth of rhizobial isolate MRH 59 was observed. No rhizobial growth was observed after 3 days of incubation in flask supplemented with 1.5% zinc sulphate Maximum log no of cfu/ml was obtained on supplementing 0.05% zinc sulphate in YEM broth

which was 8.3% higher than obtained in unsupplemented YEM broth. With increase in $ZnSO_4$ concentration thereafter, there was drastic decrease in *Rhizobium* count (Table 2). These results agreed with the study of Gauri*et al*[10]that zinc sulphate stimulated *Rhizobium* growth at low concentration; at higher concentration zinc sulphate become inhibitory zinc being a metal is toxic for rhizobia at higher concentration.Bansodand Upadhaya[11]also reported decrease in viable count of rhizobia at higher concentration of zinc.

From the above results it was concluded that micronutrients were very essential for rhizobial growth. A small amount of micronutrient change viable count of rhizobial population. Therefore, it is very essential to check the effect of various micronutrient on rhizobial growth

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