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Co – Inoculation of *Azospirillum lipoferum* and Phosphate Solubilizing Microorganisms on the growth of Rice (*Oryza sativa* L.)

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ABSTRACT

Co- inoculation with symbiotic microorganisms to create a successful system of biological nitrogen fixation in a crop can lead to many profits for plants. The present work was to evaluate the single and co-inoculation effects of Azospirillum lipoferum (Az 204) and Phosphate Solubilizing Microorganism, Bacillus megaterium (PB1) on rice variety of 'Co 47'. The biofertilizers are applied to rice seeds and pot experiments were performed to analyze the symbiotic relationship of these biofertilizers in individual and combined form. Plant Phytotoxicity and vigor index were calculated from root and shoot length and compared with one another. The results indicated that the co-inoculation of rice seeds with A. lipoferum and B.megaterium had significant effects on root and shoot length of plant in compared to either single inoculation or control plants. Hence it can be suggested that the co –inoculation of A. lipoferum and B. megateium can improves some growth indices in terms of root and shoot length.

Key words: *Co-Inoculation, Biofertilizers, Azospirillum lipoferum, Bacillus megaterium, Rice*

INTRODUCTION

Environmental pollution is mainly caused by excessive soil erosion and the associated transport of sediment, chemical fertilizer and pesticides to surface and ground water and improper treatment of human and animal waste has caused serious environmental pollution and social problems throughout the world.

Biofertilizer is defined as a substance, which contains living microorganisms and is known to help with expansion of the root system and better seed germination. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes. Conversely, in unhealthy soil, dominated by pathogenic microbes, optimum plant growth would not be possible. The production technology for biofertilizers is relatively simple and installation cost is very low compared to chemical fertilizer plants[8].

Many problems of environmental pollution have resulted from the excessive application of chemical fertilizers in the traditional farming system. Meanwhile, biofertilization has become more and important; it is an efficient and positive alternative to chemical fertilizers[14].

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These problems can be tackled by use of biofertilizers and organic fertilizers[7, 11]. Biofertilizers, more commonly known as microbial inoculants, include bacteria (Azotobacter), algae (Blue-Green Algae) and Mycorrhizal fungi; they are natural, beneficial and ecological, and they provide nutrients for the plants and maintain soil structure[1].

Increased attention is now being paid to developing an Integrated Plant Nutrition System (IPNS) that maintains or enhances soil productivity through balanced use of all sources of nutrients including chemical fertilizer, organic fertilizers and biofertilizers. The beneficial effects of combined application of chemical fertilizers with organic manures *viz.*, farmyard manure, vermicompost, biofertilizers, Green manure and many more of such materials are universally known. Application of organic manures in general improves the availability of micronutrients like zinc, iron, manganese and copper. A balanced application of both organic, inorganic and biofertilizers appear to be an ideal proposition to meet nutrient requirements of dry land crops rather than single application[8]. Application of organic matter positively affects the growth and development of plants roots and shoots.

Diazotrophic bacteria, belonging to *Azospirillum* genera, attract a lot of interest because of their capability of plant growth promotion, mineral and water nutrition improvement[3, 4, 6]. Experiments with cereal crops have demonstrated an evident benefit from Azospirillum inoculation[10, 12, 2, 5, 6]. The main purpose of this study was evaluation of the effects of biofertilizers on growth of rice for decreasing the effects of chemical fertilizers. Also, to analyze the symbiotic relationship of biofertilizers in plants to obtain higher productivity.

MATERIALS AND METHODS

The symbiotic relationship of biofertilizers like Phosphate Solubilizing Microorganisms (PSM) with Nitrogen fixers was analyzed in rice cultivation through pot experiment. The seeds are treated with biofertilizers alone and under combined form are sown to pots and analyzed for growth characteristics of plants in terms of root and shoot length.

Biofertilizers

The Nitrogen fixer and Phosphate Solubilizing Microorganism for rice are purchased from TNAU, Coimbatore. These are include PB1 (*Bacillus megaterium*) and Az 204 (*Azospirillum lipoferum*). These biofertilizers are applied through seed treatments. The symbiotic interactions of co-inoculation of PSM with Nitrogen fixers like *Azospirillum* provide better nutrient utilization[5].

Site Description

The soil sample for pot experiment was collected from paddy field, near herbal garden, S.T.E.T. Women's college, Mannargudi, Thiuvarur (Dt) and the trial was conducted in the college laboratory.

Experimental Design

The seeds are treated with biofertilizers individually and in combined form. The expeiment is lain out in randomized block design with 4 treatments and three replicates. The treatments include T1 = PB1 (*B. megaterium*), T2= Az 204 *(A. lipoferum*), T3= PB1 and Az 204 (*B. megaterium* and *A. lipoferum*), T4= Control. The treated seeds are sown to pots and observed for plant growth like root and shoot length and tiller number in every 3 day intervals.

Bioassay was carried out using healthy rice (*Oryza sativa* L.) seeds. The healthy seed variety of rice 'Co 47' was obtained from TNAU, Coimbatore and soaked in the water for overnight. The soaked seeds were removed and treated with biofertilizers alone (T1 and T2) and combined form (T3) and dried in air to get a uniform coating of the extract. The coated seeds were sown in pots containing soil. Seeds soaked in water sown in pot containing soil are served as control (T4). All the pots were irrigated immediately after sowing. Then the plant growth was observed carefully.

Root and shoot lengths were measured and recorded in 10^{th} and 15^{th} day. The phytotoxicity was calculated by using the following formula,

$$\text{Phytotoxicity (\%)} = \frac{\text{Root/Shoot length in control (cm)} - \text{Root/Shoot length in treatment (cm)}}{\text{Root / shoot length in control (cm)}} \times 100$$

Germination of the seeds were calculated in terms of vigor index by using the formula

Vigor Index = % Germination × Total length of the seedling

RESULTS AND DISCUSSION

In the present study, the symbiotic relationship of the biofertilizers like PSM and Nitrogen fixers were analyzed with four treatments in rice through pot experiments. The root and shoot length of plant were recorded and phytotoxicity were calculated to compare the effects of biofertilizers in rice. The phytotoxicity and vigor index of plant in 10^{th} and 15^{th} day were noted in table 1.

Table -1: Phytotoxicity and Vigor Index of Biofertilizers Treated Rice Seedlings

TREATMENTS	DAY	GERMINATION (%)	PHYTOTOXICITY (%)		VIGOR INDEX
			ROOT	SHOOT	
T1 (*A. lipoferum*)	10^{th}	100	-12.6	-14.9	2210
	15^{TH}	100	-16.7	-18.1	2650
T2 (*B.megaterium*)	10^{th}	100	-12.3	-13.3	2170
	15^{TH}	100	-15.9	-17.9	2750
T3 (*A. lipoferum* and *B. megaterium*)	10^{th}	100	-13.6	-15.3	2390
	15^{TH}	100	-17.5	-18.9	2890
T4 (Control)	10^{th}	100	0.00	0.00	1960
	15^{TH}	100	0.00	0.00	2560

Co – inoculation can benefit plant growth by different mechanisms. However one of the most commonly reported plant growth promotion mechanisms by bacteria is the changing of morphological and physiological changes in root systems. An increase in the number of lateral roots and root hairs cause addition of root surface available for nutrients and water uptake.

The results showed co –inoculation of biofertilizers showed positive effects in root and shoot length of rice both in 10^{th} and 15^{th} day (Table 1). The interaction between *Azospirillum lipoferum* and *Bacillus megaterium* was significant on root and shoot length of rice. There was no phytotoxicity observed after 10^{th} and 15^{th} days at all treatments both in root and shoot length as compared to control. The negative value of phytotoxicity showed that the bioinoculants enhances the plant root and shoot as compared to control. The results of this study have demonstrated a clear synergistic effect of *A. lipoferum* and *B. megaterium* and effects in plants co- inoculated with these biofertilizers.

Similar results were obtained by Mehray Askary *et al.*[9] for co-inoculation of *Triticum aestivum* with *Azospirillum brazilense* and *Rhizobium meliloti*, which enhance grain yield and Nutrient content of grains. The reports of Sindhu *et al.*[13] also correlated with the present work for co-inoculation of Chickpea with *Pseudomonas spp* and *Mesorhizobium spp*, which enhanced grain yield and N content of grain. Though the inoculation effects were analyzed at an early stage of plant growth, the results be conclusively suggest that PSB being compatible with other microorganisms in the rhizosphere can potentially be used as individual inoculants or co-inoculants with other plant growth promoting bacteria to increase the production in sustainable agricultural systems.

CONCLUSION

The co-inoculation of the bacterial strains increases the activity of nitrogenase, urease and phosphatase enzymes in soil when compared to uninoculated control or individual inoculants. The experimental results provided that the biofertilizes play a major role in improving the soil fertility and thereby increase the crop yield. In addition, it improves the soil biota and minimizes the sole use of inorganic fertilizers and the cost of cultivation.

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