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Studies on *Azotobacter chroococcum* Isolated from Rice Fields

By

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ABSTRACT

The ability of different types of water samples to support the population of inoculant strains of *Azotobacter* vary considerably. The nitrogen fixing ability is influenced by the type of water used for irrigation and the fertilizers applied.

INTRODUCTION

Microflora of rice soils and effects of various factors like moisture, pH, nutrients, manuring etc. on microbial number have been studied widely. Microflora of water samples of soils studied over last several years showed wide fluctuation (Rangaswami and Narayanaswami, 1965).

Different types of water are used for irrigating rice fields. Apart from rainwater in rainfed areas, subsoil water, river water, tank water etc. are used in various places. In the deltaic region water is flowing from one field to another. Under laboratory condition distilled water, deionized water and protected drinking water are sometimes used for pot culture studies. Bacterial inoculants are added to soil in rice fields and their effects on crop production are studied. But the type and nature of water used for irrigation is rarely considered. With a view to understand how the water samples affect the inoculated bacteria, the present study was made.

MATERIALS AND METHODS

Water samples were collected from channels feeding rice fields, from rice fields and also from drinking water source. Equal quantities of the samples were taken in glass jars. One set of jars with water samples were sterilized. Freshly isolated culture of *Azotobacter chroococcum* was used for inoculation of Waksman's 77 broth medium and equal quantities of this broth culture were added to the water sample. The number of *Azotobacter* cells was estimated immediately after inoculation by the standard dilution plate method. This population represents the initial population and further estimations were made at 15 day intervals.

In the second experiment soil was collected from rice field and placed in rectangular cement pots. Urea, ammonium sulphate and N P K mixture were added to the soil at the recommended rate to supply 75 kg N per acre and the pots were irrigated with the three types of water. The bac-

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terium was inoculated and the population was estimated immediately after inoculation and at 15 day intervals. Pots without fertilizer were maintained as controls. The survival of inoculant strain of *Azotobacter* was observed up to 105 days.

RESULTS AND DISCUSSION

The three water samples used supported the organism differently but in all cases the initial population of 1.6 million per ml was drastically reduced to few thousands in 15 days (Table I). Such a reduction of added culture is already reported (Batacharya, 1958.) Since rain water samples contain only small amounts of nutrients they are unable to support large populations of bacterial cells compared to soil. Further in a standing column of water the undissolved organic matter settles quickly and they too are utilized by a variety of organisms in an unsterilized sample (Narayanaswami, 1969). The reduction was more pronounced in unsterile water samples than in sterile ones. This may be attributed to the competition for nutrients and to the phenomenon of antibiosis.

The numbers of *Azotobacter* cells remained more or less constant after 60 days indicating that the ability of a particular water sample to support the growth depended upon the nutrients and salt concentrations. Such a phenomenon was reported by Narayanaswami *et al.* (1969).

Survival of *A. chroococcum* in channel and field waters with fertilizers was better than in soil irrigated with drinking water with fertilizer (Table II). Bleaching powder treatment may be responsible for the poor survival in drinking water.

Soil applied with N P K mixture supported the bacteria better than application of single fertilizers. This

TABLE I. Survival of *Azotobacter chroococcum* in channel, field and drinking waters
(Population expressed in 1×10^3 per ml.)

Days after inoculation	Sterilized water samples			Unsterilized water samples		
	Channel water	Field water	Drinking water	Channel water	Field water	Drinking water
0	1600.0	1600.0	1600.0	1600.0	1600.0	1600.0
15	13.1	28.1	7.1	2.8	6.7	0.8
30	27.3	35.5	5.7	2.8	7.0	0.8
45	20.2	25.2	3.4	2.6	6.2	0.7
60	11.3	14.3	3.3	2.5	3.8	0.3
75	7.1	8.4	2.8	2.4	2.6	0.4
90	7.2	5.5	2.8	2.4	2.2	0.3
105	6.8	5.6	2.6	2.3	2.1	0.2

TABLE II. Survival of *Azotobacter* in paddy soil irrigated with different water samples with and without fertilizers (Population expressed in 1×10^3/g soil)

Days after inoculation	Channel water				Field water				Drinking water			
	No fert.	Urea	Am. sul.	NPK	No fert.	Urea	Am. sul.	NPK	No fert.	Urea	Am. sul.	NPK
0	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
15	52	22	12	45.2	48.2	24.1	18.2	42.4	45.1	21.1	13.1	40.1
30	24	12	5.2	32.5	30.8	13.2	12.1	31.1	22.2	11.2	6.2	30.7
45	12.1	6.0	7.1	26.2	22.4	8.4	28.7	27.7	10.1	5.2	5.2	21.2
60	11	7.2	7.3	15.4	16.2	8.6	6.2	16.1	8.2	6.8	6.1	13.1
75	8.1	7.8	7.2	12.4	12.8	8.7	6.8	12.2	7.3	7.2	7.1	10.7
90	8.2	8.3	7.3	9.2	10.2	8.9	7.1	9.8	7.1	7.5	7.1	8.2
105	8.2	8.4	7.4	8.7	9.4	9.1	7.3	8.3	7.2	7.6	7.2	7.8

TABLE III. Effect of Urea, Ammonium sulphate and mixed fertilizers on nitrogen fixation by *Azotobacter* (Expressed as percentage over control)

Days after inoculation	Urea			Ammonium Sulphate			NPK Mixture (75-35-35)		
	20 ppm	40 ppm	80 ppm	20 ppm	40 ppm	80 ppm	20 ppm	40 ppm	80 ppm
0	—	—	—	—	—	—	2.01	—	—
15	—	—	—	—	—	—	2.01	0.05	—
30	2.03	—	—	0.55	—	—	3.04	1.01	—
45	2.23	—	—	1.07	—	—	3.35	2.01	1.02
60	3.04	1.05	—	1.08	0.06	—	3.95	3.02	1.22
75	3.18	1.06	—	2.38	1.10	—	4.06	3.23	1.71
90	4.26	2.12	—	2.36	1.08	—	4.08	3.21	1.82
105	4.28	2.01	—	2.23	1.12	—	4.08	3.22	1.85

explains the need for a balanced type of manuring rather than simple salts.

Higher doses of nitrogenous fertilizers suppressed the fixation of nitrogen by *Azotobacter* (Table III). Pramanik and Misra (1955) and Ebert (1959) noted poor fixation of N in the presence of added N but the rate of N fixed by the bacterium was found

to be more after the fertilizer nitrogen was exhausted. This indicates that under conditions where there is no nitrogen or less nitrogen the bacterium is able to fix nitrogen.

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