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Effect of root exudates of cotton on phorate degradation by PGPR organism *Azotobacter chroococcum*

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Root exudates are substances released by roots and many affect growth and activity of soil organisms including PGPR microorganisms in the rhizosphere. Amino acids, sugars and vitamins have been indentified in the exudates of wild varieties of the plants. Other substances include organic acids, nucleotides, flavnones, enzymes, hydrocyanic acid, glycosides, auxins and saponins. They found that the exudates of pea, maize, oat and wheat stimulated *A. chroococcum* more than that of other soil bactereia including *Rhizobium*. In the present investigation efforts were made to find out whether the root exudates of cotton affect the phorate degradation.

Azotobacter isolates were obtained from different soil and rhizosphere of different crop plants. Isolation was done as per soil dilution technique described by Timonin (1941). In addition soil enrichment method using Thompson and Skerman medium (1979) was also used. Tolerence of *A. chroococcum* was studied on organophosphate insecticide, phorate. Isolate showing high tolerance (200µg/ml) towards phorate determined by replica plate technique was used for degradation studies *in vitro*.

Root exudates of cotton varieties NHH-44 were obtained following the method of Rovira (1956). Seeds were germinated on the nutrient agar medium. The germinated seeds were transferred to sterile tube containing washed sand and plant nutrient solution. Culture solution was prepared by dissolving 1 5 g of mixture in 1 lit of distilled water. The tubes were placed in racks with the tops exposed to light and the root region covered with blank paper. The root exudates were collected after 75 days by removing the plants and placing in sterile distilled water. The sand was also washed with sterile distilled water and all the roots and sands washings were pooled together. It was concentrated in vaccum desiccator to reduce the volume to 5ml for every 10 plants.

The effect of various constituents of root exudates and degradation of phorate by *Azotobacter chroococcum* isolates was done *in vitro*. Tubes of nitrogen free medium with phorate (200 µg/ml) were inoculated with *A. chroococcum* isolate. Root exudate was added in different amounts to the above inoculated tubes and incubated at 30°C for seven days. Tubes were analysed at regular interval for degradation of phorate. Inoculated tubes without the addition of root exudates were considered as control.

All the 29 isolates of *Azotobacter* showed their efficacy on cotton varieties NHH-44. All the isolates enhanced shoot and root lengths and dry weoght of the plants Nitrogen content and dry matter was also increased. Isolate ACI-28 was highly effective when compared with other isolates. Acetylene reduction test for nitrogenase activity also showed that the other isolates were effective. Gangawane (1992) and Subbarao (1992)suggested use of *Azotobacter* as biofertilizer for various cereals.

Tabel 1. Effect of root exudates of cotton variety NHH-44 on phorate degradation by ACI-28

Azotobacter chroococcum isolate	Root exudates (ml)	Phorate added (µg/ml)	Phorate remaining broth (days)(µg/ml)			% Degradation of phorate	S.E.	C.D. P=0.01
			0	2	5	on 5th day		
ACI-28	---	200	194 (0)#	150 (44)	22 (112)	57.73		
	1.0	200	196 (0)	141 (55)	74 (122)	62.24	11.19	0.947*
	2.0	200	196 (0)	134 (62)	68 (128)	65.30	11.03	1.756*
	3.0	200	196 (0)	128 (68)	57 (139)	70.91	11.36	2.165*

* Significant

- indicates the amount of phorate (µg/ml) degraded.

There are many reports indicating the degradation of various pesticidal compounds (Alexander, 1981; Deo *et. al.,* 1994). Earlier studies by various workeers also showed that *Azotobacter,* the free PGPR microbe can be used as biofertilizer for non legume crops (Gangawane, 1987).

Commonly the first step in the study of biological degradation of pesticide is to establish the tolerance of organism against pesticidal compound. In the present study tolerance of 29 *A. choococcum* isolates against phrorate was evaluated. There was quite a large variation among the isolates for the tolerance of these insecticides Cypermenthrin, dimecran and endosulfan was tolerated from 75 to 100 µg/ml by certain isolates while dimethoate and phorate tolerated upto 150 µg/ml by some isolates. The results are in conformity with the workers like Sylvester and Fournier (1979) and Lal and Sexena (1982)who have well revised the interaction of insecticide with soil microorganisms. The variation in the tolerance may be attributed to alteration of permeability of cell membrane, respiration and other metabolic processes in the cell (Fisher *et. al.,* 1978).

There is much evidence where healthy plant shoots release soluble organic compounds and influence the rhizosphere microflora in various ways (Ravira, 1956; Gangawane and Deshpande, 1973), In this investigation phorate degradation by *A. chroococcum* was improved in the presences of root exudates of cotton variety NHH-44.The increase of degradation was seen from 57.73% to 70.91% (Table 1). The analysis of toot exudates also indicated the presence of glutamic acid, threonine, alanine, aspartic acid, valine and Leucine. Increase in the proportion of root exudates in the medium was still favourable for the degradation of phorate. Amino acids and vitamins are known to act as growth regulating factors for various microorganisms in the rhizosphere and hence the phorate degradation might have been enhanced due to *A. chrococcum* strains in this study. Moreover presence of root exudates in soil are well known to overcome toxic effects of pesticides on microorganisms (Leelavathy, 1969).

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