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DISTRIBUTION AND ACTIVITY OF PHOSPHATE SOLUBILISING MICROORGANISMS IN THE RHIZOSPHERE OF COCONUT AND CACAO UNDER MIXED CROPPING

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ABSTRACT

Two efficient phosphate solubilising microorganisms, *Pseudomonas* sp. and *Aspergillus niger*, were isolated from the rhizosphere of coconut and cacao. They were capable of solubilising 49.0% and 49.7% inorganic phosphate. The incidence of phosphate solubilising microorganisms and available phosphorus in different rhizosphere soils were directly related.

INTRODUCTION

Phosphate is widely distributed in nature, both in organic and inorganic forms, but in a bound state not readily available to plants. It is generally applied to the soil in the form of phosphatic fertilizers. However, with the demand for this fertilizer exceeding its production in India, rock phosphate is being used as a substitute for phosphatic fertilizers. The objectives of the present study were to investigate the nature and distribution of phosphate solubilising microorganisms and the extent of solubilisation brought out by them in the rhizosphere of coconut and cacao under mixed cropping.

MATERIALS AND METHODS

1. Lay-out of the experimental plot.

Two sets of rhizosphere samples of coconut and cacao were collected in February 1974 and July 1974 from the Central Plantation Crops Research Institute, Kasaragod, Kerala, from an experimental plot of 1 ha area where cacao was planted as a mixed crop with coconut in 1970. The experiment has been laid out in a randomised block design with three treatments, (*a*) coconut alone without any mixed cropping of cacao; (*b*) coconut with single hedge of cacao; and (*c*) coconut with double hedge of cacao.

2. Isolation and characterisation of phosphate solubilising micro-organisms.

Five rhizosphere samples of coconut and cacao were collected at random from replications II and III of all the three treatments. Non-rhizoshpere samples were collected from an adjacent fallow land. Pure cultures of phosphate solubilising microorganisms were isolated on Pikovskaya's medium (Pikovskaya, 1948) using the soil dilution and plate count method of Timonin (1940). *In vitro* estimation of the solubilisation of tricalcium phosphate by different cultures was done by the method of Sethi and Subba Rao (1968).

Pseudomonas sp. was identified by its known cultural and biochemical properties. *Bergey's Manual of Determinative Bacterio-*

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logy (VII Edition, 1957) was used to compare and compile the characters of the bacterium studied. Fungi and actinomycetes were identified by the nature of their vegetative hyphae and spores. Available phosphorus and organic carbon of different soil samples were estimated by Olsen et al's (1954) and Walkley and Black's (1934) methods.

RESULTS AND DISCUSSION

The enhancement in the number of phosphate solubilising microorganisms in the rhizosphere soil as compared to the non-rhizosphere soil was very conspicous. The double hedge cultivation of cacao showed an increased proliferation of phosphate solubilisers both in the coconut and cacao rhizosphere as compared to single hedge cultivation and control plots (Table I). This may be due to the improved organic carbon content of the soil (cf., Table I). This increase in organic carbon may have been mainly due to periodic shedding of leaves by the cacao plants.

Twenty one different isolates of bacteria, actinomycetes, and fungi from the rhizosphere of coconut and cacao were found

Table I. *Distribution of phosphate solubilising microorganisms (Combined data for February & July 1974) and amount of available phosphorus and organic carbon in different rhizosphere soils*

Nature of isolates	Non rhizosphere soil	Coconut without cacao	Mixed cropping			
			Single Hedge		*Double Hedge*	
			Coconut	Cacao	Coconut	Cacao
Bacteria						
Isolate 1*	+	+	+	+	+	+
Pseudomonas sp.	—	—	+	—	+	—
Isolate 7*	—	—	+	+	+	+
Isolate 8*	—	+	—	+	—	+
Isolate 9*	—	—	—	+	—	+
Actinomycetes						
Streptomyces sp. (Iso. 4)	—	—	+	—	—	+
Streptomyces sp. (Iso. 5)	—	—	—	—	+	—
Streptomyces sp. (Iso. 6)	—	—	—	+	—	+
Streptomyces sp. (Iso. 7)	—	—	—	+	+	—
Streptomyces sp. (Iso. 8)	—	—	—	—	+	—
Fungi						
Fusarium sp. (Iso. 3)	—	—	+	+	—	+
Fusarium sp. (Iso. 4)	+	+	+	+	+	—
Fusarium sp. (Iso. 5)	—	+	+	+	+	—
Penicillium sp. (Iso. 1)	—	—	+	+	+	+
Penicillium sp. (Iso. 2)	—	—	—	+	+	+
Penicillium sp. (Iso. 3)	—	+	—	+	+	+
Penicillium sp. (Iso. 4)	—	+	—	—	+	+
Penicillium sp. (Iso. 5)	—	—	—	—	+	+
Aspergillus niger	—	+	+	+	+	+
Aspergillus terreus	—	—	+	+	+	—
Sterile mycelia (yellow)	—	—	+	—	—	—
Available phosphorus (ppm/g soil)	10.0	20.0	41.0	38.0	65.0	55.0
Organic carbon (%)	0.39	0.42	0.48	0.50	0.53	0.60

*Bacterial isolates unidentified

capable of solubilising tricalcium phosphate to varying extent (Table II). Among these, one isolate of *Pseudomonas sp.* and one of *Aspergillus niger* were found to solubilise respectively 49.0% and 49.7% of the total $Ca_3(PO_4)_2$ incorporated to their respective media. Microbial solubilisation of inorganic phosphate is brought about by the production of organic acids (Mayer and Konig, 1960; Chhonkar and Subba Rao, 1967; Mehta and Bhide, 1970). In the present study also, a negative correlation was obtained between the pH of the culture filtrate and amount of phosphate solubilised by different organisms ($r = -0.809$; r at $d.f. = 19$ and 1% significance $= 0.549$). This was more evident from the specific cases of *Pseudomonas* sp. and *A. niger*. This lowering in pH could primarily be due to the production of 2-ketogluconic acid by *Pseudomonas* sp. and citric acid by *A. niger* (Louw and Webley, 1959; Bardiya, 1970).

Concurrent with the above observation, a direct relation was also noticed between the incidence of phosphate solubilising microorganisms and the amount of available phosphorus in different soils. The maximum amount of available phosphorus, 65 ppm per gram soil, was obtained in the coconut rhizosphere under double hedge cultivation as compared to 41 ppm in single hedge, 20 ppm in control, and 10 ppm in non-rhizo-

Table II. *Quantitative studies on the solubilisation of tricalcium phosphate*

Organism studied	P_2O_5 in mg/50 ml medium* (Average of triplicate)**	Percentage $Ca_3(PO_4)_2$ solubilised	pH of the culture filtrate @	Mycelial mat wt. (g)
Bacteria				
Isolate 1	0.764	6.67	6.5	—
Pseudomonas sp.	5.577	48.95	3.5	—
Isolate 7	0.496	4.39	6.7	—
Isolate 8	0.496	4.39	6.5	—
Isolate 9	0.496	4.39	6.5	—
Actinomycetes				
Streptomyces sp. (Iso. 4)	1.604	6.99	6.0	1.14
Streptomyces sp. (Iso. 5)	0.764	3.32	6.0	1.13
Streptomyces sp. (Iso. 6)	0.496	2.18	6.0	1.13
Streptomyces sp. (Iso. 7)	0.229	1.00	6.5	1.12
Streptomyces sp. (Iso. 8)	0.229	1.00	6.5	1.10
Fungi				
Fusarium sp. (Iso. 3)	1.299	5.68	6.5	1.19
Fusarium sp. (Iso. 4)	0.229	1.00	6.5	1.17
Fusarium sp. (Iso. 5)	0.229	1.00	6.5	1.16
Penicillium sp. (Iso. 1)	5.271	23.01	5.7	1.28
Penicillium sp. (Iso. 2)	5.271	23.01	5.5	1.26
Penicillium sp. (Iso. 3)	2.445	10.66	6.2	1.24
Penicillium sp. (Iso. 4)	4.278	18.69	6.5	1.15
Penicillium sp. (Iso. 5)	2.216	9.69	6.4	1.19
Aspergillus niger	11.384	49.69	3.0	1.25
Aspergillus terreus	3.056	13.36	6.5	1.21
Sterile mycelia (Yellow)	0.229	1.00	6.5	1.29

*25 mg of tricalcium phosphate was incorporated intially in 50 ml of the medium for bacteria and 50 mg for actinomycetes and fungi

**Value are expressed after substracting the control values.

@ pH of the culture medium after sterilization was 6.5.

shpere soil. The corresponding values for available phosphorus in the cacao rhizosphere were 55 and 38 ppm, respectively (Table I). The higher amount of available phosphorus in the coconut rhizosphere as compared to the cacao rhizosphere must be due to the top dressing of rock phosphate given to individual coconut palms at the time of fertilization. The beneficial role of microorganisms in solubilising bound phosphate has been repeatedly observed (Johnston, 1954; Sen and Paul, 1957; Mickovski, 1964; Myskow, 1966).

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