

MICROBIOLOGY OF THE ROOT REGION OF COCONUT AND CACAO UNDER MIXED CROPPING

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SUMMARY

Mixed cropping of cacao in coconut plantations improved the microbial activity in the rhizosphere of coconut which may be attributed to an increase in organic matter content of soil due to periodic shedding of cacao leaves. When compared to coconut cultivation without cacao, mixed cropping of coconut with cacao appeared to stimulate the population of bacteria and fungi in the rhizosphere of coconut, including the nitrogen fixing and phosphate solubilising bacteria. The occurrence of indole acetic acid producing *Escherichia* sp. on the root surface of coconut and the appearance of gibberellin(like-substance) producing *Aspergillus flavus* and *A. fumigatus* in the rhizosphere were other salient observations made in a pioneering study on the microbiology of the root region of plantation crops under mixed cropping.

INTRODUCTION

In recent years, the practice of mixed cropping is being advocated in plantation crops to bring more income to the farmer by utilizing the vacant space between rows of standing crops. In a long duration experiment at the Central Plantation Crops Research Institute, Kasargod, Kerala State, India, it was observed that the yield of coconut (*Cocos nucifera*) increased when cacao (*Theobroma cacao*) was grown as a mixed crop. The increase in yield was consistent in succeeding years and was 95% with double hedge of cacao and 65% with single hedge of cacao, over corresponding controls. While there may be several reasons for the observed increase in coconut yield, the objective of this investigation was to find out the number, nature and specific biological attributes of bacteria, fungi and actinomycetes in the rhizosphere of coconut under mixed cropping with cacao. Such studies were also considered worthwhile since there has

been no work on the microflora of the root region of perennial crops under mixed cropping.

METHODS AND MATERIALS

General layout of the experiment

The experiment was started in 1970 and was of randomised block design, with the following three treatments:

1. Cultivation of coconut (Tall × dwarf hybrids) without any mixed cropping.
2. Cultivation of coconut with single hedge of cacao as a mixed crop.
3. Cultivation of coconut with double hedge of cacao as a mixed crop (Fig. 1).

The average size of each plot was 465 square meters. Coconut palms were planted at a distance of 7.5 meters and cacao at a distance of 3.5 meters from the coconut palm. The distance between individual cacao plants in a row was 3.5 meters and the distance between two rows of cacao plants in double hedge cultivation was 2 meters.

Collection of rhizosphere soil samples

Two sets of samples were collected – one in February and the other in July, 1974. Blocks of soil containing roots were cut out and rhizosphere samples



Fig. 1. Coconut plantation with double-hedge mixed cropping of cacao.

were collected at a depth of 3 to 5 inches. Non-rhizosphere soil samples were collected from an adjacent fallow land.

Total counts of microorganisms

An estimate of the total number of bacteria, actinomycetes and fungi per gram of dry rhizosphere soil was made by the well known soil-dilution and plate count method⁵ using the following media: Thornton's media for bacteria and actinomycetes⁹, Martin's Rose Bengal agar for fungi⁶, nitrogen-free medium for nitrogen fixing bacteria² and Pikovskaya's medium for P-solubilizers⁷.

Quantitative estimation of nitrogen-fixed by Beijerinckia sp.

Two weak old bacterial cultures grown on nitrogen-free liquid medium were subjected to Kjeldahl's digestion and the amount of nitrogen was estimated using a 'Technicon' auto-analyser with reference to a standard curve prepared with ammonium chloride.

Qualitative and quantitative estimation of phosphate solubilization

The method followed by Chhonkar and Subba Rao⁴ was used for the quantitative determination of phosphate solubilization in liquid media containing tricalcium phosphate.

Quantitative estimation of IAA

Aliquots of sterilized tryptone broth (100 ml) were inoculated with a loopful of 24 hour old bacterial culture and incubated for 48 hours at $28 \pm 2^\circ\text{C}$. The contents were centrifuged at 17,000 rpm for ten minutes and the supernatant treated with peroxide-free ether to extract IAA. The ether extract was subjected to Salkowski reaction⁸ and the intensity of the pink colour developed after incubation at 27°C for 30 minutes in dark was measured in a Hilger's absorptiometer using filter no. 52. The amount of IAA produced was then calculated by comparing the optical density values of experimental samples with a standard curve prepared earlier with pure IAA (indole-3-acetic acid, Merck-Germany).

The pellets of bacteria were washed and transferred to tarred miniature glass vials, dried to a constant weight at 60°C for 48 hours and weighed in a balance (Mettler model H-16) for calculating the amount of IAA in terms of $\mu\text{g}/\text{mg}$ dry weight of bacterial cells.

Chromatographic and bioassay methods for detecting the presence of gibberellin-like substances (GLS) in the culture filtrates of Aspergillus flavus and A. fumigatus

The fungi were grown on Czapek-Dox broth for 14 days and GLS were extracted¹ from the culture filtrate with ethyl acetate after adjusting the pH to 3.0. The extract was evaporated to dryness at 40°C in dark, the residus re-dissolved in 5 ml of double distilled water and used for chromatography and bioassay.

One μ l of the extract was spotted on Whatman no. 1 chromatographic paper (22 × 40 cms) and compared with standard gibberellic acid (Gibberellic acid, serial No. 691104, Phylaxia, Budapest – Hungary) using the technique of ascending chromatography³. Both the samples were run simultaneously at 24°C using benzene–acetic acid–water (10: 2.5: 5) as the solvent system for 8 hours. The presence of GLS and gibberellic acid was indicated by yellow spots on a reddish purple background on spraying with 0.5% aqueous solution of potassium permanganate. The spots were quickly sprayed with more of permanganate solution and the chromatogram was immediately washed in running water to remove the unreacted reagent. The locations of GLS and gibberellic acid were then revealed as permanent brown spots on an almost white background. The RF values of the spots were then determined.

Forty eight hour old rice seedlings grown aseptically in Erlenmeyer flasks were used in a bioassay method¹¹ to compare the extent of elongation of the seedlings by the application of standard gibberellic acid (0.2 μ g), gibberellin-like substances extracted from the culture filtrate (2.0 ml) and the crude culture filtrate (10 ml).

Determination of pH and organic carbon of soil samples

Fifty ml of double distilled water was added to each 10-g soil sample, stirred well and allowed to stand for 3 hours before the pH was determined by a pH meter (Model LI-10 'Elico'). Organic carbon was determined by a rapid titration method¹⁰.

RESULTS AND DISCUSSION

From the data presented in Table 1, it could be inferred that microbial activity in the rhizosphere of coconut under double hedge cultivation of cacao was intense as revealed by the population of bacteria and fungi in different treatments under mixed cropping of cacao in a coconut plantation. When organic carbon content of rhizosphere soils was determined, the per cent organic carbon in different treatments was as follows: non-rhizosphere – 0.39; coconut rhizosphere – 0.42; coconut rhizosphere (single hedge) – 0.48; cacao rhizosphere (single hedge) – 0.50; coconut rhizosphere (double hedge) – 0.53; cacao rhizosphere (double hedge) – 0.60. The observed increase in the organic carbon content of rhizosphere soil under double hedge cultivation of cacao can be attributed to the periodic shedding of cacao leaves which might have contributed to the enhanced microbial activity in the rhizosphere under mixed cropping.

TABLE 1
 Number and nature of microorganisms (per gram dry rhizosphere soil) in the rhizosphere of coconut under mixed cropping with cacao

Nature of isolates	Non-rhizosphere soil	Mixed cropping with cacao								
		February - 1974				July - 1974				
		Single hedge		Double hedge		Single hedge		Double hedge		
Coconut without cacao	Cacao	Coconut Cacao	Cacao	Coconut without cacao	Cacao	Coconut Cacao	Cacao			
Total bacteria (10^6)	48	160	92	212	128	140	152	80	200	104
Total <i>Beijerinckia</i> (10^3)	0	3	7	6	14	3	3	5	4	9
Total phosphate-solubilising bacteria (10^6)	0	2	4	3	5	3	4	2	4	8
Total actinomycetes (10^6)	1	3	2	3	4	2	2	3	4	5
Total fungi (10^4)	10	32	16	39	18	22	76	11	90	14

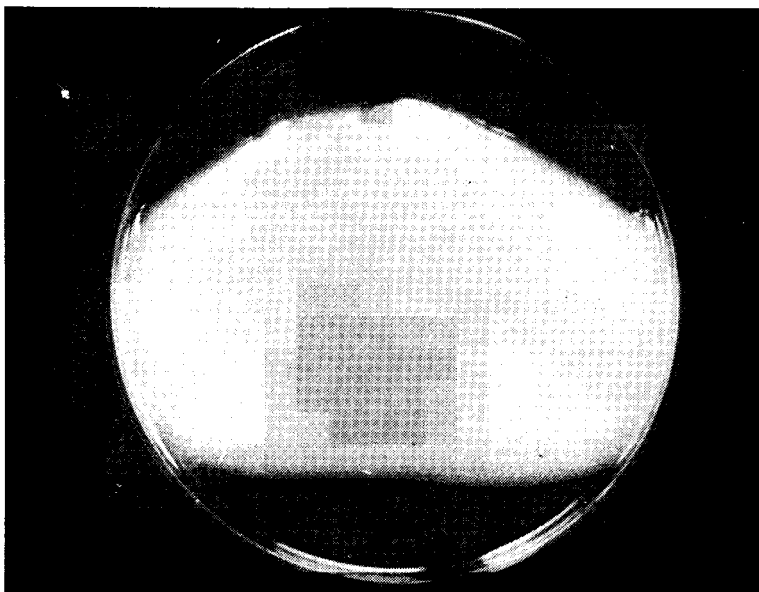


Fig. 2. A 10-day-old colony of *Beijerinckia* sp. isolated from the rhizosphere of coconut, producing abundant gum.

Nitrogen fixation

The dominant nitrogen fixing bacterium in the rhizospheres of coconut and cacao was a species of *Beijerinckia* (Fig. 2) which upon testing on nitrogen-free liquid medium produced abundant gum and fixed 9.83 mg N/g sucrose. *Beijerinckia* was also dominant on the root surface of coconut under double hedge cultivation in more than 80% of the root samples plated on nitrogen-free agar medium.

Phosphate solubilisation

Several micro-organisms isolated from the rhizosphere of coconut and cacao had the ability to solubilize $\text{Ca}_3(\text{PO}_4)_2$ to varying degrees. Among them, an isolate of *Pseudomonas* sp. and an isolate of *Aspergillus niger* showed maximum solubilization of the bound phosphate to the extent of 48.95% and 49.69%, respectively. This was also associated with a fall in the pH of the culture filtrate to 3.5 and 3.0, respectively mainly due to the production of ketogluconic acid by *Pseudomonas* sp. and citric acid by *Aspergillus niger*.

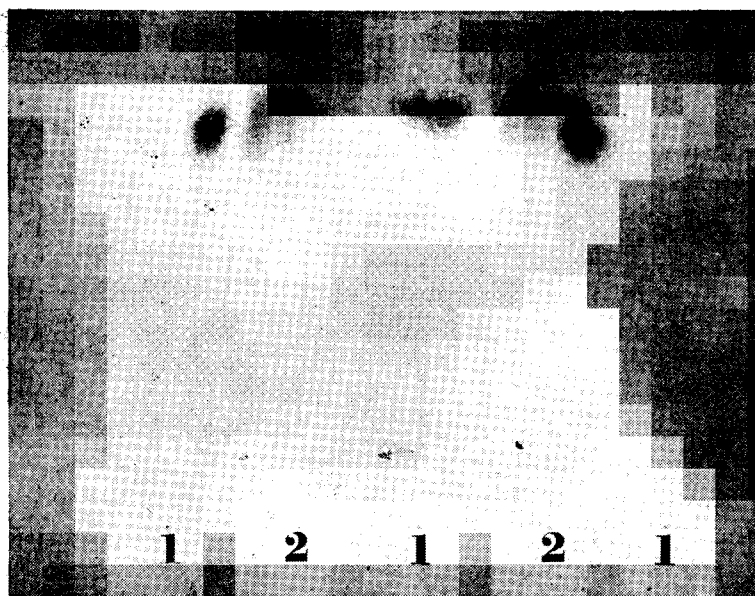


Fig. 3. A paperchromatogram showing 1 Gibberellin-like substances in ethylacetate extract of the culture filtrate of *A. flavus* and 2 standard gibberellic acid.

Production of IAA and gibberellin-like substances (GLS).

Among the bacteria and fungi isolated during the present investigation for production of growth substances, an isolate of *Escherichia* sp. was found closely associated with the root surface of coconut in more than 80% of the root samples examined. The isolate produced IAA equivalent to 0.2710 $\mu\text{g}/\text{mg}$ dry weight of bacterial cells in pure culture.

Among the many rhizosphere fungi tested, two isolates, one of *Aspergillus flavus* and the other of *A. fumigatus* were found to produce gibberellin-like substance (GLS) when the culture filtrates were screened by paper chromatography. The *R_f* value of GLS was 0.87 while that of gibberellic acid was 0.91 (Fig. 3). The production of GLS was also confirmed by bioassay using rice seedlings (Fig. 4). It was noteworthy that the population of *A. flavus* in the rhizosphere was $3 \times 10^4/\text{g}$ and that of *A. fumigatus* was $4 \times 10^4/\text{g}$ of dry rhizosphere soil.

In conclusion, it can be said that mixed cropping of cacao in coco-

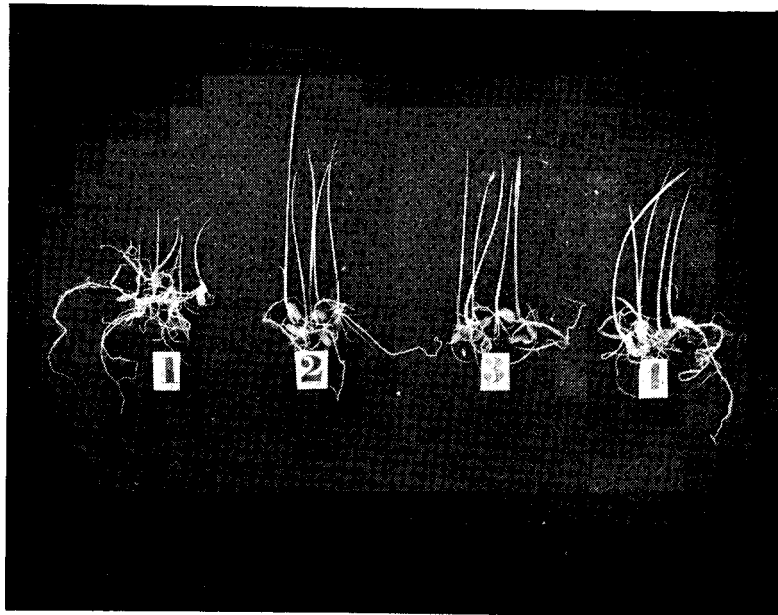


Fig. 4. Responses of rice seedlings to 1 distilled water, 2 standard gibberellic acid, 3 ethylacetate extract of gibberellin-like substance from culture filtrates of *A. flavus* and 4 crude culture filtrate of *A. flavus*. Note elongation of seedlings in 2, 3 and 4.

nut plantations enhances the microbiological activity in the rhizosphere of coconut, possibly due to improvement in the organic matter of soil due to cacao litter. However, increased yields of coconut cannot be solely ascribed to increased rhizosphere activity although a better mobilization of phosphate coupled with fixation of nitrogen and production of growth substances (GLS and IAA) in the rhizosphere may be regarded as possible explanations behind the observed enhanced yield of coconut under the influence of cacao as a mixed crop. Other reasons behind the beneficial effect of cacao cultivation in coconut plantations are being further investigated at the Central Plantation Crops Research Institute, Kasargod, Kerala, India.

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