# Studies on the microflora of coconut soils - Observations on the microbiological activity of coconut soils with reference to the root ( wilt ) disease.

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## INTRODUCTION

The importance of soil micro-organisms in determining the fertility status of the soil has long been recognised. For a given soil sample the quantity of carbon dioxide evolved from it and its capacity to produce and accumulate nitrates will serve as indices of its microbiological activity. According to Waksman (1952) 85% of the total carbon dioxide evolved from a soil is due to the activity of the micro-organisms. Nitrate accumulation, bacterial numbers and $Co_2$ content of soil were found to be related phenomena by Russel and Appleyard (1952). Thus the rate of production of carbon dioxide itself under certain conditions of moisture and temperature may be looked upon as an indication of the rate of the microbial activity of the soil. However, the nature and availability of the decomposing material in the soil remains as a conditioning factor on the microbiological status. Further the interactions of higher plants and soil micro-organisms should also be considered in view of the observations made by Timmonin (1940), Katznelson (1946) and Agnihothrudu (1954) on the rhizosphere microflora in relation to the nature of the host plant. Timmonin (1940) has shown that the susceptible and resistant nature of plants to any specific disease may influence the rhizosphere microflora, higher numbers of micro-organisms having been observed in the rhizosphere of the susceptible than of corresponding resistant plants. Katznelson (1946) observed that mangel roots exerted a striking selective action on the micro-organisms depending on the nutrient status of the soil and the vigour of growth of the plant. Plant growing in manured soil harboured an abundant rhizosphere microflora during the period of most vigorous growth while those in the unmanured soil manifested full selective power when approaching maturity. Agnihothrudu (1954) working on pigeon-pea wilt showed that the rhizosphere of the resistant variety was not favourable to the causal organism, *Fusarium udum* due to a predominance of antagonistic actinomyctes.

In this paper the microbial activity of soils collected from the base of healthy as well as palms affected by root (wilt) disease as measured by the quantity of carbon dioxide evolved from and the number of bacterial colonies yielded by them was studied.

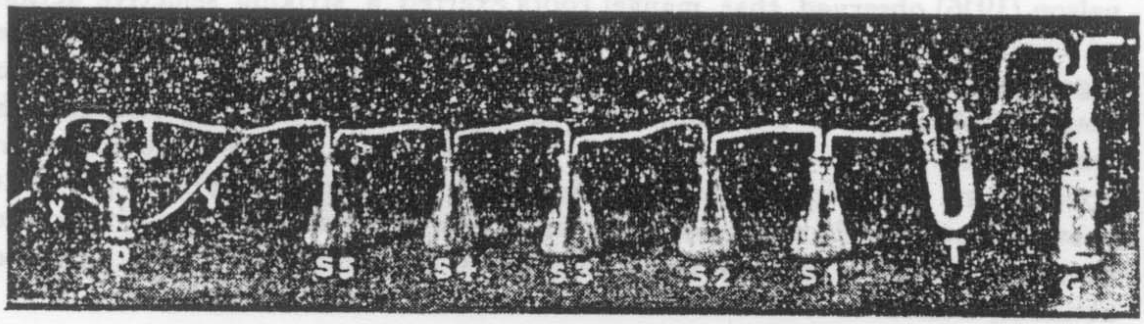
Appleman (1927), Lundigardh (1927), Waksman and Starkey (1924) and Marsh (1928) had followed different methods for the determination of carbon dioxide liberated from the soil. In the present study the principle adopted by Waksman and Starkey (1924) was followed. The main feature of their method is collection of representative soil samples from the field and testing them for their capacity to liberate carbon dioxide under arbitrarily prescribed conditions.

*Sampling and preparation of soil*

Soil samples were collected from the proximity of the root surface from a uniform depth of 2 ft by opening trenches about 4 ft away from the bole of palms. The collections were made from the different blocks of the Central Coconut Research Station Farm, Kayangulam at different periods. All the samples of a particular period were collected on the same day itself so as to avoid variation due to climatic changes. The palms selected for sampling in the different blocks were approximately of the same age and were being given the same treatments. The samples were brought to the laboratory in sterile screw-capped bottles. They were partly air dried to a known moisture percentage. Later the samples were crumbled, sifted and weighed in lots of 100 g. into 250 cc. Erlenmeyer flasks. The moisture content of the soil was brought to 15% by adding the required quantity of sterile tap water and the flasks were connected to the collecting system.

*Experimental*

A general plan of the apparatus used for the determination of carbon dioxide is given in the Fig. below




Five Erlenmeyer flasks ($S_1$-$S_5$) with soil samples (prepared as mentioned above) were connected in series. Each flask had two tubulatures — one to bring in the air, and the other which reaches almost to the surface of the soil, to draw out the $Co_2$ laden stratum of air from the soil as soon as it was formed. $Co_2$ free air was passsed through the soil in the flasks by working a filter pump at the opposite end. The removal of $Co_2$ from the air was done by passing it first through strong KOH solution (N. strength) contained

in a gas wash bottle (G) and then through a U-tube (T) containing soda lime. The continuous air current thus removed the $Co_2$ produced by the activity of the micro-organisms in the soil. This air, having passed through the soil in the flasks was again passed through 0.5 N. KOH solution kept in a potash bulb (P) where the $Co_2$ was completely absorbed. All the connections of the apparatus were made by pressure tubings and each joint was wired tightly. The atmospheric air in the system was drawn out through XY before the experiment was started each time. The collecting system was worked for 24 hours continuously at a uniform speed and the amount of carbon dioxide produced during the period was determined by titrating the alkali against 0.5 N. $H_2So_4$ using phenolphthalein and methyl orange as indicators. The amount of $Co_2$ shown by the titration was taken as the amount of $Co_2$ produced by the microbial activity of the total quantity of the soil in the flasks ($S_1$-$S_5$). From this the amount of $Co_2$ produced from 1 kg of soil was calculated and was expressed in mg. per kg. soil.

The estimation of bacteria in the soil samples was done by the dilution plate method of Waksman and Fred (1922) using the bacterial medium advocated by them. The bacterial number was expressed in lakhs per gram moisture free soil.

Altogether 3 sets of soil samples were studied. Each set consisted of 18 samples collected at a time from three different blocks viz. Block I, III & VI of the Central Coconut Research Station Farm. Six samples, three from the base of healthy palms and three from the base of diseased were collected from each block during each set of sampling, thus each set consisting of 18 samples. Collections were made from the base of the same trees during subsequent periods of sampling also. The details of soil sampling are given in Table 1.

TABLE I

*Details of soil samples taken from the Central Coconut Research Stat on Farm, Kayangulam.*

| Block | Healthy Tree No. | Diseased Tree No. | Date and Number of samples taken 24-4-1962 | 12-6-1962 | 3-7-1962 |
|---|---|---|---|---|---|
| Block I | 216 | 207 | 6 | 6 | 6 |
| | 335 | 212 | | | |
| | 340 | 344 | | | |
| Block III | 89 | 17 | 6 | 6 | 6 |
| | 210 | 34 | | | |
| | 234 | 47 | | | |
| Block VI | 457 | 402 | 6 | 6 | 6 |
| | 432 | 472 | | | |
| | 479 | 440 | | | |

TABLE 2

*Set I. Samples collected on 24-4 1962. Quantity of $Co_2$ in mg per Kg soil and bacterial counts in lakhs per g. moisture free soil.*

| Locality | Tree No. and condition of palm | Bacterial counts in lakhs per g soil | $Co_2$ in mg per Kg soil | Average Bact No. | Average $Co_2$ |
|---|---|---|---|---|---|
| Block I C. C. R. S. | 207 Diseased | 4.2 | 26.4 | | |
| | 212 Do | 4.1 | 17.6 | | |
| | 344 Do | 1.4 | 22.0 | 3.2 | 22.0 |
| | 216 Healthy | 19.0 | 48.0 | | |
| | 340 Do | 30.1 | 56.0 | | |
| | 335 Do | 31.0 | 56.0 | 26.6 | 53.3 |
| Block III | 17 Diseased | 6.05 | 26.0 | | |
| | 34 Do | 7.8 | 15.4 | | |
| | 47 Do | 8.0 | 21.0 | 7.3 | 20.8 |
| | 89 Healthy | 20.1 | 52.0 | | |
| | 210 Do | 20.3 | 60.0 | | |
| | 234 Do | 21.0 | 60.0 | 20.4 | 57.3 |
| Block VI | 407 Diseased | 10.0 | 17.6 | | |
| | 472 Do | 11.0 | 17.6 | | |
| | 440 Do | 8.3 | 11.4 | 9.8 | 15.5 |
| | 432 Healthy | 22.2 | 48.0 | | |
| | 457 Do | 21.3 | 42.0 | | |
| | 479 Do | 30.0 | 53.0 | 24.5 | 47.6 |

TABLE 3

*Set II. Samples collected on 12-6-62. Quantity of $Co_2$ in mg per kg soil and bacterial counts in lakhs per g moisture - free soil.*

| Locality | Tree No. and condition of palms. | Bacterial counts in lakhs per g soil. | $Co_2$ in mg per kg. soil. | Average Bacterial number. | Average $Co_2$ |
|---|---|---|---|---|---|
| Block I | 207 Diseased | 36.5 | 168.0 | | |
| | 212 do | 7.5 | 146.0 | | |
| | 344 do | 8.0 | 132.0 | 17.3 | 148.6 |
| | 216 Healthy | 89.0 | 252.0 | | |
| | 340 do | 87.5 | 270.0 | | |
| | 335 do | 100.0 | 284.0 | 92.1 | 268.6 |
| Block III | 17 Diseased | 38.0 | 132.0 | | |
| | 34 do | 11.2 | 146.0 | | |
| | 47 do | 15.0 | 132.0 | 21.4 | 136.6 |
| | 89 Healthy | 91.7 | 242.0 | | |
| | 210 do | 102.0 | 264.0 | | |
| | 234 do | 103.8 | 220.0 | 99.1 | 242.0 |
| Block VI | 407 Diseased | 10.0 | 158.0 | | |
| | 472 do | 14.0 | 162.0 | | |
| | 440 do | 10.0 | 144.0 | 11.3 | 146.0 |
| | 432 Healthy | 64.0 | 224.0 | | |
| | 457 do | 53.0 | 236.0 | | |
| | 479 do | 63.0 | 247.0 | 60.0 | 235.6 |

TABLE 4

*Set III. Samples collected on 3-7-62. Quantity of $Co_2$ in mg per kg soil and bacterial counts in lakhs per g moisture - free soil.*

| Locality | Tree No. and condition of palm | Bacterial counts in lakhs per g. soil | $Co_2$ in mg. per kg. soil | Average Bacterial number | $Co_2$ average |
|---|---|---|---|---|---|
| Block I | 207 Diseased | 32.0 | 134.0 | | |
| | 212 do | 23.0 | 120.0 | | |
| | 344 do | 25.0 | 110.0 | 33.3 | 121.3 |
| | 216 Healthy | 80.0 | 239.0 | | |
| | 340 do | 74.0 | 260.0 | | |
| | 335 do | 93.0 | 223.0 | 82.3 | 240.6 |
| Block III | 17 Diseased | 31.0 | 115.0 | | |
| | 34 do | 28.0 | 99.0 | | |
| | 47 do | 20.0 | 120.0 | 26.3 | 111.3 |
| | 89 Healthy | 67.0 | 200.0 | | |
| | 210 do | 75.0 | 194 0 | | |
| | 234 do | 59.0 | 199.0 | 67.0 | 197.6 |
| Block VI | 407 Diseased | 19.0 | 98.0 | | |
| | 472 do | 9.0 | 70.0 | | |
| | 440 do | 7.0 | 88.0 | 11.6 | 85.3 |
| | 432 Healthy | 48.0 | 173.0 | | |
| | 457 do | 51.0 | 184.0 | | |
| | 479 do | 50.0 | 195.0 | 49.6 | 184.0 |

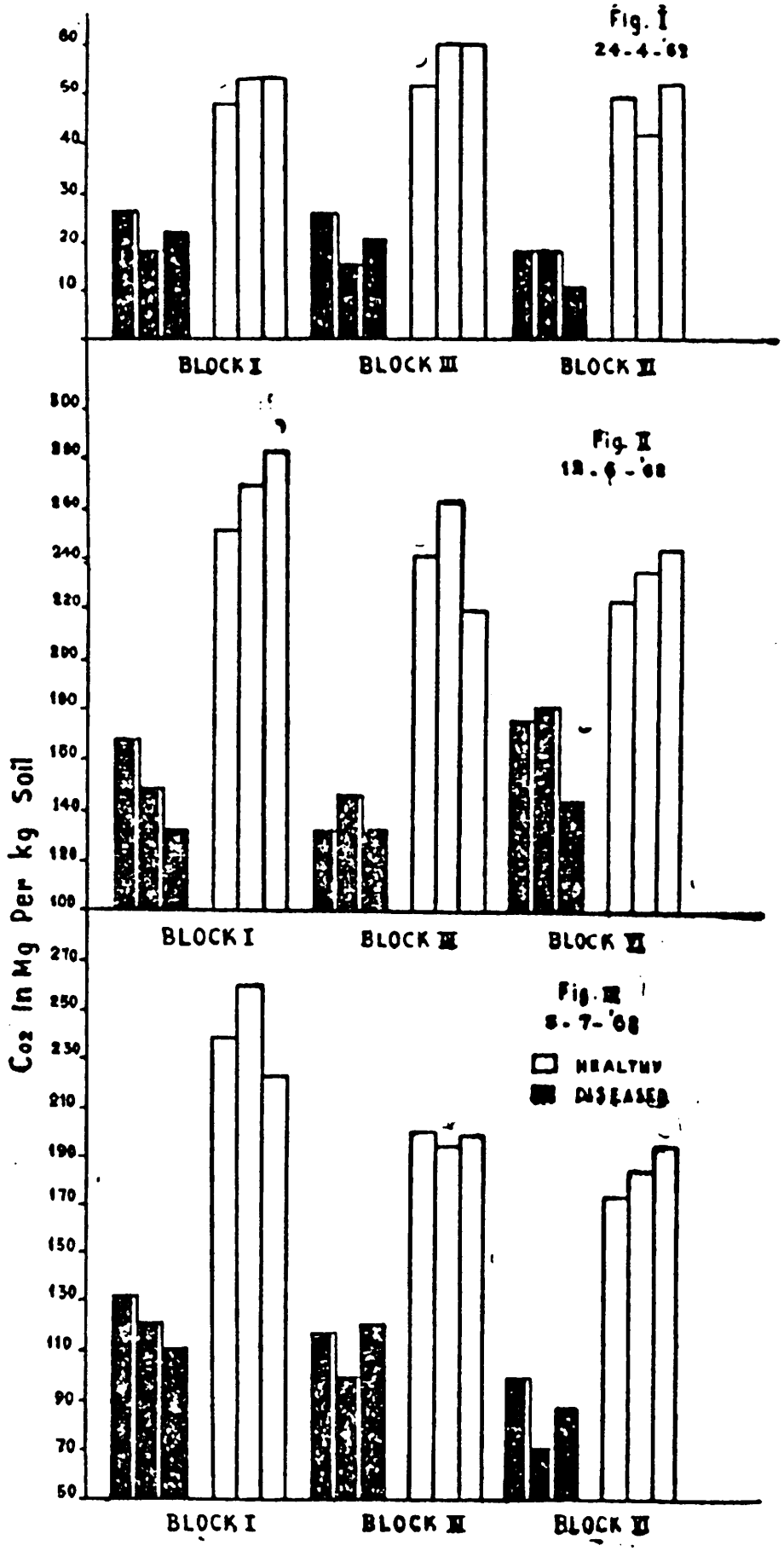


Fig. I
24-4-'68
60
50
40
30
20
10
BLOCK I
BLOCK III
BLOCK II
Fig. II
18-6-'68
300
280
260
240
220
200
180
160
140
120
100
BLOCK I
BLOCK III
BLOCK II
Fig. III
8-7-'68
HEALTHY
DISEASED
270
250
230
210
190
170
150
130
110
90
70
50
BLOCK I
BLOCK III
BLOCK II
Co2 in Mg Per kg Soil

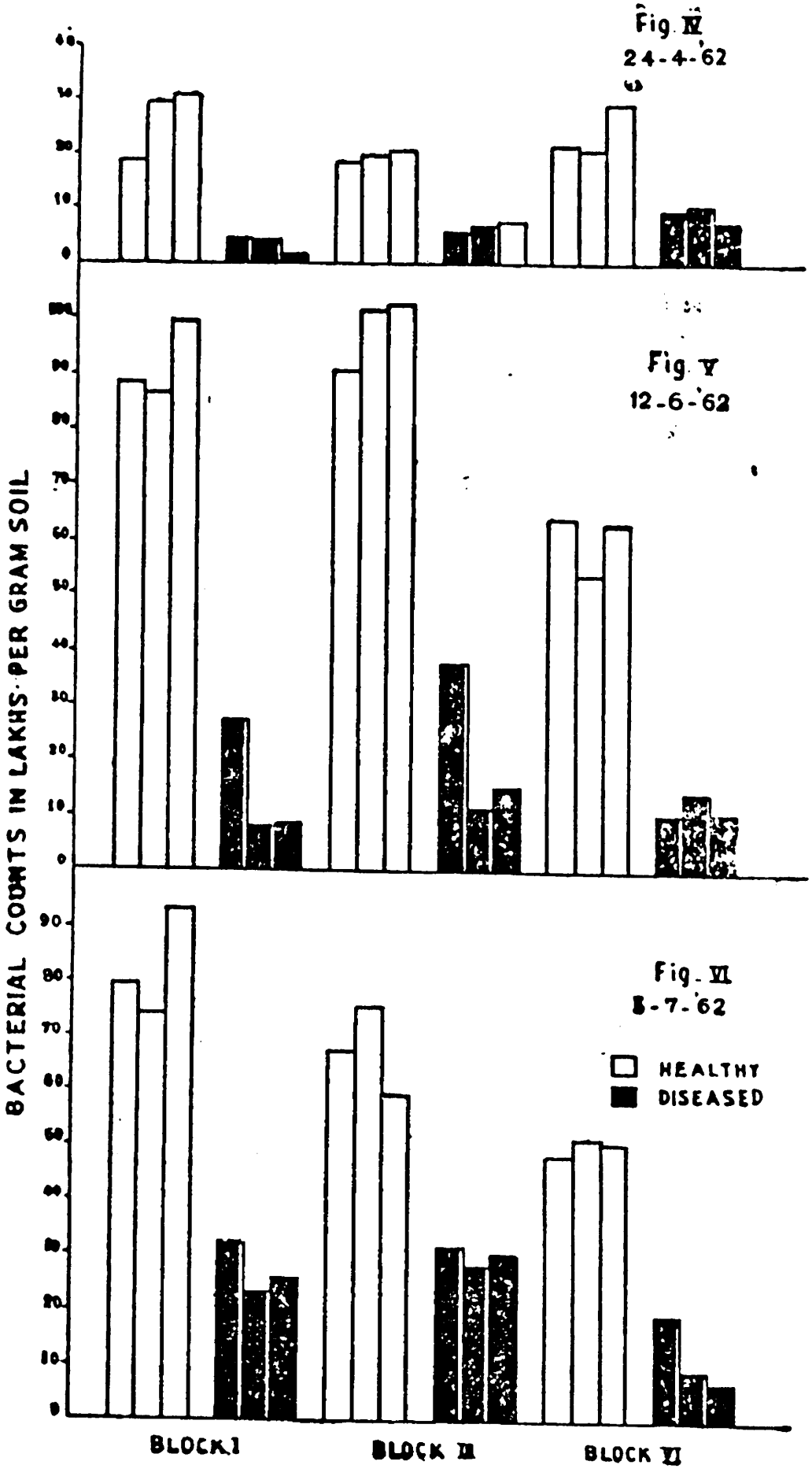


Fig. IV
24-4-'62
Fig. V
12-6-'62
Fig. VI
5-7-'62
HEALTHY
DISEASED
BACTERIAL COUNTS IN LAKHS PER GRAM SOIL
BLOCK I
BLOCK II
BLOCK III

## RESULTS

The results of each set of samples are given below:-

*A. Samples collected on 24-4-1962 (Table 2 and Figs I & IV).*

1. The samples collected from the base of healthy palms yielded greater amount of carbon dioxide as compared to those collected from the base of the diseased in all blocks.

2. Among the healthy samples those from block III yielded the maximum amount of $Co_2$ while those of block VI yielded the minimum.

3. Of the diseased samples those of blocks VI again yielded the lowest quantity of $Co_2$. The diseased samples of blocks I & III gave almost similar results.

4. The bacterial counts show that all the healthy samples yielded greater number of bacteria as compared to the diseased. The maximum number was recorded from the healthy samples of block I, the minimum being recorded from block III. In contrast to this, the diseased samples from block I yielded the minimum number of bacteria, the maximum being recorded in samples of block VI.

*B Samples collected on 12-6-1962 (Table 3 and Figs. II & V).*

1. As in the previous set here also the healthy samples yielded greater amount of $Co_2$ and greater number of bacteria as compared to the diseased.

The $Co_2$ evolution and bacterial number in both healthy as well as diseased samples were highly enhanced as compared to the samples of the previous set.

2. Among the healthy samples those of block I yielded the maximum quantity of carbon dioxide and those of block VI the minimum.

3. Among the diseased samples those of block III yielded the lowest quantity of $Co_2$ whereas samples of blocks I & VI had given almost similar results.

4. Bacterial counts show that a higher number was recorded in all the healthy samples as compared to the diseased. In both healthy and diseased samples the lowest number was recorded in block VI whereas the samples from the other two blocks gave almost similar results.

*C. Samples collected on 3-7-1962 (Table 4 and Figs. III & VI).*

1. The healthy samples had yielded higher amount of $Co_2$ and greater number of bacteria as compared to the diseased.

2. Among the healthy samples those of block I had yielded the maximum quantity of $Co_2$ and bacteria as compared to those of blocks III & VI.

3. There was a gradual decrease in $Co_2$ evolution and bacterial number in both healthy as well as diseased samples from blocks I to VI.

## DISCUSSION

The increase in the microbiological activity of healthy soil samples as measured by the quantity of $Co_2$ liberated may be attributed to the healthy condition of palms and the consequent increase in the rhizosphere microflora. Radha and Menon (1954) recorded abundant rhizosphere microflora in the healthy coconut palms as compared to the diseased. Joshi (1958) during his investigations on the band disease of coconut, observed significantly higher microbiological activity in the soils from the base of healthy palms than the soils from the base of the band affected ones. Thus the condition of palms seems to be a determining factor so far as the microbiological activity of coconut soils is concerned. Root (wilt) disease being a systemic one, the metabolic activities of the palms get disturbed and this in turn might affect the microbial activity of the soil in the sphere proximal to the root surface. This finding has been amply justified by the increase in the bacterial number in the healthy samples as compared to the diseased in each block. Radha and Rawther (1959) further observed that environmental factors like rainfall and soil moisture influence the soil microflora. Moderate rainfall was found to be favourable to the microflora whereas heavy rains adversely affect it. In the present instance the rainfall received during a period prior to the collection of soil samples perhaps influenced the microbiological activity of the soil. The samples collected in June when moderate rains were received recorded the highest microbial activity. Similarly the low values for $Co_2$ liberated and bacterial number from samples collected in April might partially be the result of the dry soil condition which prevailed during that month. The samples of July had recorded a slight reduction in microbial activity and bacterial number as compared to those of June possibly due to the heavy rains during the month. More recent observations recorded by us (unpublished) on the seasonal variations in bacterial population indicate that the trend in microbiological activity noticed here during the different periods is positively correlated with the bacterial population.

The variations in the microbial activity among the samples from the different blocks may be explained as due to the difference in the fertility status of the soil. Further studies on the influence of soil conditions, seasonal factors and fertility status of soil on the activity of soil microflora are in progress.

## SUMMARY

1. Observations on the microbiological activity of the soil collected from the base of healthy and diseased coconut palms were recorded, the criteria being the amount of $Co_2$ evolved from a known quantity of soil per unit time and number of bacterial counts yielded by unit weight of soil.

2. The data collected from 54 soil samples indicate considerable variation between the healthy and the diseased. The healthy samples consistantly recorded higher microbiological activity and bacterial number than the diseased.

3. Indication of the influence of environmental conditions like rainfall and soil fertility on microbial activity are also forthcoming.

## ACKNOWLEDGEMENT

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* *These papers were not seen in original.*