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BIOCHEMICAL STUDIES ON THE YELLOW LEAF DISEASE ON ARECANUT PALMS

By

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THERE exists no rigid definition of a perfectly normal and healthy plant. Variations in the environment and nature may produce abnormalities which are termed diseases in extreme cases. Fungi and Bacteria are capable of producing pathological symptoms and these in turn produce very great changes in the ratios of normal proteins in the metabolism of the plant. Immunity is produced in some instances after prolonged attacks and these phenomena may be related to specify proteins. But these have not so far been isolated. (Gaumann, 1950).

The pathological picture of a virus is entirely different. The virus is not capable of multiplying independently but more virus accumulates inside the host plant. This naturally is consequent on the altered protein synthesis in the affected plants. Virus infection chemically is an induced abnormality of the nucleoprotein synthesis in such a way that more virus is synthesised (Bawden and Pirie, 1953).

All the known viruses so far studied come under the group of nucleoproteins, mostly derived from the ribonucleic acid of the plant cell nucleus. Based on these, the following investigations have been carried out with Yellow Leaf of arecanuts, (causing considerable havoc in the Kerala State,) at this Station.

This paper details the experiments conducted during the preliminary studies.

Preparation of infected extracts

The absence of pathogenicity in inoculated fungal isolates on the healthy arecanut palms necessitated the search for the causal organism of the Yellow leaf disease in soil and in the field of virus research.

Soil analysis showed lack of Nitrogen and Phosphorous. Proper cultivation methods and manuring may go a long way to make the palms resistant to the attack of the disease.

Two hundred gms. of the healthy and 200 gms. of diseased leaves were chopped to very fine pieces with a knife and later crushed with a laboratory pestle and mortar. The highly fibrous nature of the palm leaves made the process very difficult and the material finally was kept under-20°C for 48 hours after which it was possible to mince the leaves to some extent. This work was carried out under low temperature to keep the leaves brittle. The diseased leaves pulverised more easily than the normal healthy leaves.

Isolation of nucleic acids and Associated Proteins from plant tissues

The frozen minced healthy and diseased leaves were taken in separating funnel. 500 ml. of ethanol were added to each. The suspension was not boiled as it was not sure whether the particular nucleoprotein in the extract is thermostable. The suspension was filtered in a sintered funnel. Two hundred ml., of acetone was layered above the solid. The acetone was allowed to percolate through the layer of tissue using very slow suction in order to remove the leaf pigments which may interfere in the work of isolation. To ensure complete removal of these the tissues were again washed with acetone, the powder was dried at room temperature. The acetone powder was suspended in 500 ml. of 0.5 M. Nacl. The solution was filtered using thick pleated filters. Even under these conditions the filtration proceeded very slowly. The pH of the solution was found to be 5.1–5.4 The pH was controlled to 3.2 and 7% cold trichloracetic acid was added to the extract. The mixture was shaken very thoroughly. The filtrate was centrifuged, at 1000 r. p m. The precipitate was collected and again centrifuged at 1500 r. p. m. A slightly coloured precipitate settled at the bottom. This precipitate was tested further in the following experiments.

A part of the residue was shaken with ammonium sulphate and the precipitate after centrifuging tested for activity.

The residue left over was hydrolysed with strong alkali (NaOH) for 18.23 hrs. The diseased extract was reddish in colour while the healthy was green. 7% trichloracetic acid was added to the filtrate to make the pH 3.3. Mixture after shaking was centrifuged at 1000 r.p.m. and precipatate tested for activity.

Tests for protein reactions in the diseased and healthy plants

1) Electrophoresis

The separation of the nucleoproteins have been effected by the filter paper method The method used was that of Davidson and Smellie (1952). The extract of normal and diseased leaves were applied as spots with a calibrated pippette. The paper was removed and for tagging the separated nucleoproteins the moist paper was dipped directly into a solution of 1% bromophenol blue in ethyl alcohol saturated with $HgCl_2$ (14.41). After 2 minutes the paper was removed and washed with dilute acetic acid solution (0.1%) Fig. 1. gives the pattern of electrophoresis.

2) Paper Chromatogram

Trichloracetic acid precipitate of healthy and diseased leaves were taken up in distilled water and spots were applied to Whatman No. 1 paper and chromatogram run in the solvent isopropanol (170 ml.) Conc. HCl. (44 ml.) H_2O (250 ml.). Development was continued for 16 hrs. The chromatogram was dried for a day at the ordinary room temperature. Paper was sprayed with bromothymol blue (10 cc in 40 cc ethyl alcohol). Spots developed turned blue in colour.

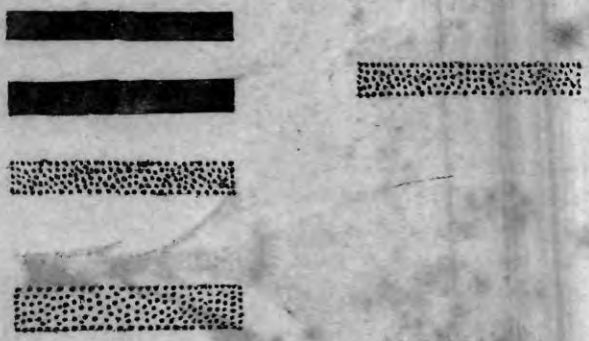
Fig. 2 gives the diagramatic representation of the chromatogram.

Spots were developed in the diseased extract while no spots were recorded in the healthy plants; bromothymol blue spraying gave better results than brom-cresol green.

3) Colour reaction with Folin Phenol Reagent

Apart from the above tests nucleoproteins in the diseased and healthy leaves were tested by the colour reaction. Method adopted was that of Glick in Biochemical analysis.

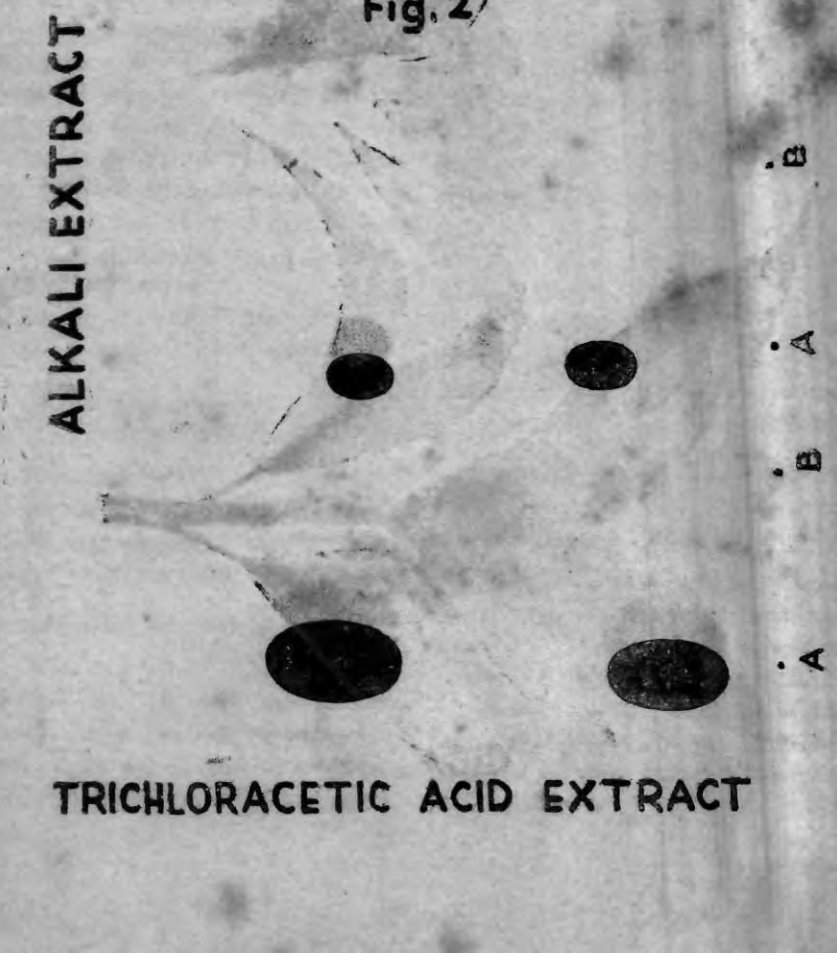
Fig. 1



DISEASED NORMAL

16 hrs, Run ELECTROPHORESIS

Fig. 2



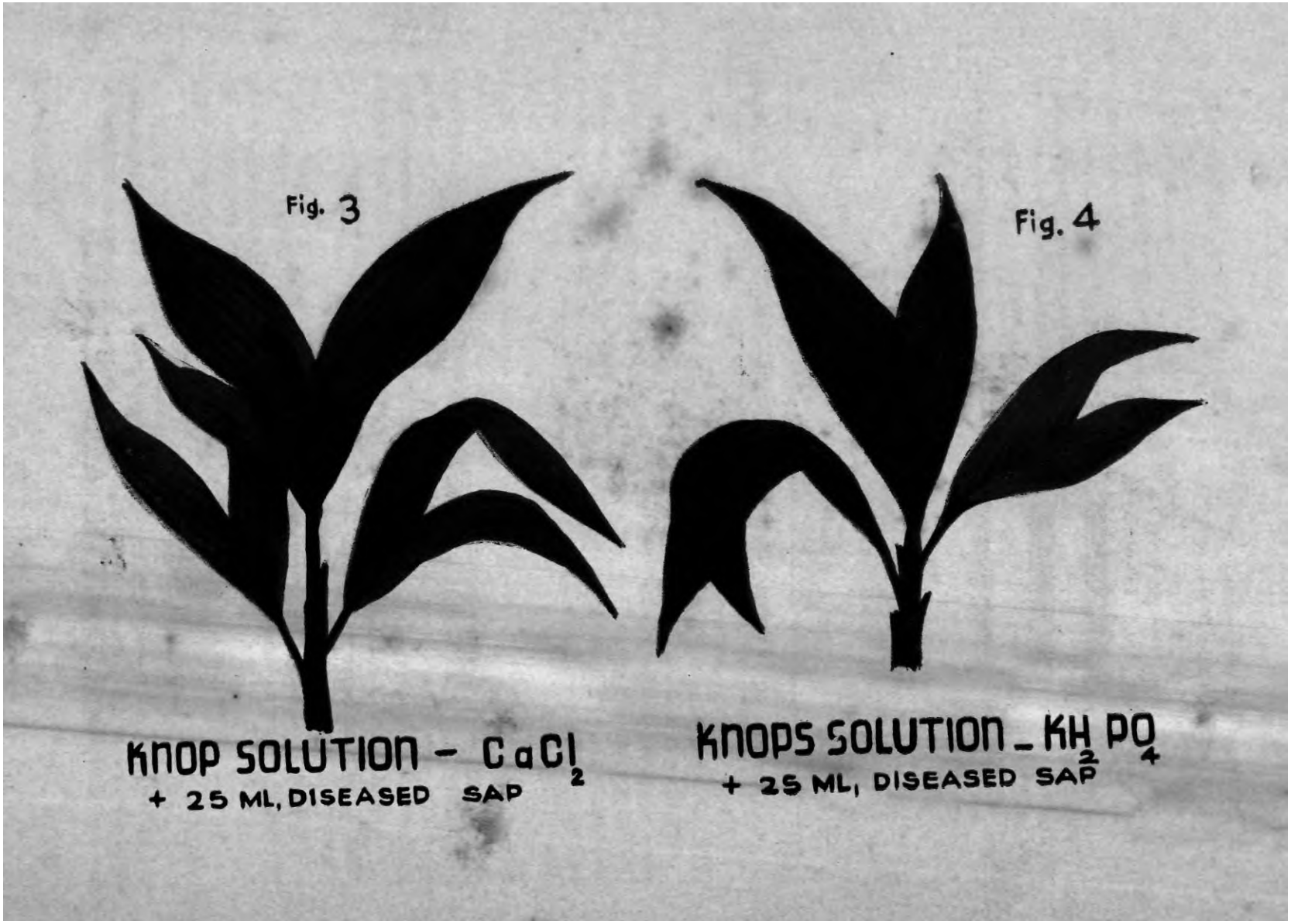


Fig. 3
Fig. 4
KNOP SOLUTION - $CaCl_2$
+ 25 ML, DISEASED SAP
KNOPS SOLUTION - KH_2PO_4
+ 25 ML, DISEASED SAP

Ten gm. of sodium tungstate, 2.5 gms. of ammonium molybdate, 70 ml. of water, 5 ml. of 85 % phosphoric acid and 10 ml. of Conc. HCl were refluxed gently for 10 hours. 15 gms of lithium sulphate was added to the mixture. 5 ml. of H_2O and a few drops of bromine was introduced later. The solution was cooled, diluted to 100 cc and filtered. It was then diluted with two volumes of water as phenol reagent.

Ten ml. of 5 M. NaoH and 3 ml. of dilute phenol reagent were added to 5 ml. trichloracetic acid filtrate. Phenol reagent was added as rapid stream of drops and tube was shaken. After 2–10 minutes the solution was read in a colorimeter against copper sulphate standard solution. A green colour was developed in the diseased extract showing the presence of proteins.

Folin Phenol reagent	Colour density
1. Trichloroacetic acid extract (Diseased)	Blue Green
2. Healthy	Nil
3. Alkali extract (Diseased)	Dull green (Olive)
4. Healthy	Nil

Bromothynol blue reagent

This reagent was prepared as follows:– Three ml. of formalin was added to 0.1 ml. of 60% aqueous KoH and 20 ml. of 0.15% (w/v) bromothynol blue in 95% alcohol. The chromatogram was sprayed with the solutions. Brilliant blue spots developed indicating the nucleoproteins in the diseased leaves. Fig. 1.

4) Spot test

On a spot plate the test solutions were placed with a drop of an alcoholic solution of pottasium salt of tetraboromophenolphthlein ethylester and then acidified with one to two drops of dilute acetic acid. A blank test changed to yellow but the colour remained blue green in the diseased leaves.

Reagent 1) K salt of tetrabromophenolphthalein ethyl ester of 0.1% in alcohol.
2) Acetic acid 0.2 N.

Conclusion

All the biochemical tests carried out in the above experiments indicate a positive test for infectious nucleoprotein present in the diseased leaves as compared with the normal healthy leaves of the arecanut palms.

A sample experiment was carried out to test the infectivity of the diseased and healthy leaves. 1 year old arecanut seedlings were kept in nutrient cultures (Knop's solution.) Two solutions were complete and from each of the others one essential element omitted. Twenty five C.C. of clarified sap was introduced in the solutions. Records were taken for the development of Yellow leaf symptoms. After 2 weeks the symptoms were noticed, more acute in those solutions were phospherous and calcium salts are absent. Fig. 3 & Fig. 4.

The clarification is carried out by centrifuging the extract at 1500 r.p.m., later dialysing the solution against running water and a second centrifuging. The clear brown supernatant is tested for activity.

Serological tests have given positive precipitate reaction isolations, and biochemical experiments are under way.

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