MICROBIOLOGICAL ASPECTS

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INTRODUCTION

An attempt has been made in this review to place in chronological order the investigations carried out by the discipline of microbiology on this coconut disease of uncertain etiology. Unlike most reviews it will not reflect the inclination of mind of different investigators but is confined to the work carried out by a handful of personnel during a limited period of eight years. No claim is made on a penetrating analysis of the subject or specific conclusions. The major thrust of the discipline at the time of its inception was the Coconut Root (wilt) disease and it will not be out of place to consider the basis on which the projects were formulated. The interaction of a three tiered system - the host, the pathogen and the environment - was the main consideration and it needs time and date for the crystallisation of a fourth dimension of significance in a perennial crop. From the purview of microbiology, in the literal sense, the association of bacteria with the disease has been recognised as a gap in multidisciplinary approach to the problem. Phytobacteriology in its pioneering era has been concerned mainly with symptomatology, infection cycle, dispersal of pathogen and control of the disease. But the development of general bacteriology has resulted in paying attention to basic studies on phytopathogenic bacteria. Lack of adequate information on the possible host-pathogen interaction in coconut has been a limiting factor in restricting studies to the role of phenolics. A range of phenolics and phenol oxidising enzymes has been implicated in host pathogen interactions. It will be undisputed that the interaction of the plant and its environment is best reflected in the rhizosphere: (a term coined by Hiltner, 1904). By definition rhizosphere is "that zone of soil in which the microflora are influenced by plant roots". The results presented under appropriate heads may be viewed against this background.

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ON BACTERIAL ETIOLOGY

Pioneering observation in this context is the vascular streaming movement of bacteria typical of a root pathogen, a feature characteristic of coconut Root (wilt) (Srivastava, Shekhawat and Rao, 1969). Earlier attempts from this laboratory were concerned with isolating the bacteria from the vascular tissues of surface sterilized root bits of disease affected and healthy palms using nutrient agar, soil extract agar and potato sucrose agar media. Repeated isolations failed to establish the consistent association of any major group of bacteria in the Root (wilt) affected palms. This situation was overcome by altering the conditions of sampling and isolations. Under the newly designed conditions, stelar portions of freshly collected and surface sterilized roots from the growing tips were plated in an enriched medium containing 15% (w/v) coconut root extract, solidified with agar (Mathew George, Potty and Jayasankar, 1976). An off white bacterium forming transluscent, smooth, flat and glistening colonies with entire margin was isolated which was conspicuous by its absence in the coconut roots collected from the Root (wilt) disease-free areas. The bacterium was rod shaped, Gram negative and motile. Cultural characteristics were the fermentative utilisation of glucose, sucrose, sorbitol, mannitol, arabinose and salicin, positive response to VP and a negative reaction to MR and indole tests. The bacterium produced hydrogen sulphide, liquefied gelatin, but did not hydrolyse starch or polygalacturonic acid. Arginine decarboxylase, catalase and nitrate reductase were present while lysine decarboxylase, phenylalanine deaminiase and urease were absent. The bacterium has been classified as Enterobacter cloacae (Mathew George, Potty and Jayasankar, 1976). The identification has been confirmed by the Commonwealth Mycological Institute as E. cloacae (Jordan) Hornaeche and Edwards.

The coconut Enterobacter isolates produced alcohol precipitable polysaccharide-like materials in growing culture filtrates when cultivated as still culture in nutrient broth. An aqueous extract of the polysaccharide-like materials reversibly wilted tomato seedlings in vitro (Mathew George, Potty and Jayasankar, 1976). The alcohol precipitable materials

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harvested from the culture filtrates of coconut Enterobacter grown in yeast extract dialysate medium was passed through Dowex columns. The effluent was freeze dried to give the crude toxin. Aqueous extract of this crude toxin material also wilted tomato cuttings in vitro. The time required for wilting was inversely proportional to the toxin concentration. An aqueous suspension of the material also produced ribbing, waving and bending of tender coconut leaflets. Further investigations showed the presence of a soluble and insoluble fractions in the coconut Enterobacter toxic substances. The soluble fraction contained materials absorbing like a nucleoprotein in the ultraviolet region. The fraction after dissociation got separated into nine separate bands under polyacrylamide slab gel electrophoresis (Unpublished). The bacterium does not belong to conventional plant pathogenic genera; however, species of Enterobacter have been implicated more recently in plant diseases (Rohrbach and Pfiffer, 1976; Hopkins and Elmstrom, 1977).

Investigations on this aspect concerning the association of Enterobacter in coconut root (wilt) were extended to isolated pockets of infection of the disease and to the border areas demarcating the spread of the disease (Mathew George and Jayasankar - in press). In all, fourteen plots were selected of which four plots were located in each of the two mildly infected areas around Shertallay and Karunagappally while the remaining six plots were distributed in the northern and southern borders. Isolation of bacteria from the stelar portions of root samples collected from these areas was made in two seasons (pre- and post-monsoon). Consolidated data on the distribution of Enterobacter revealed its comparative abundance in palms in the early stage of the disease. The abundance of saprophytes in the root samples collected during the post-monsoon period indicated the advisability of restricting bacterial isolations to the pre-monsoon period.

With a view to formulating control trials the coconut Enterobacter isolates were screened against a number of chemicals in vitro. The organism was sensitive to oxytetracycline, tetracycline and streptomycin. Oxytetracycline at a concentration of 0.8/ug per ml completely suppressed the growth of the bacterium. Large scale control trials have been started in the field with streptomycin, tetracycline and oxytetracycline.

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Positive indications on the ability of oxytetracycline (Terramycin tree formulation of M/s. Pfizer, Bombay) in checking the deterioration of the root (wilt) diseased condition of palms in cultivators' gardens have been brought out (Unpublished).

HOST PATHOGEN INTERACTIONS

The total phenol content was estimated in samples of roots collected from healthy (palms in the healthy tract), apparently healthy (healthy palms in the root (wilt) affected region and root (wilt) affected (both early and advanced) palms (Joseph and Jayasankar, 1973). The highest concentration of polyphenols was recorded in samples of roots collected from healthy palms. There was a fall in the concentration of total phenols with the incidence and increase in intensity of the disease (Joseph and Jayasankar, 1973). In the root (wilt) affected tracts the levels of polyphenol oxidase and peroxidase were found to increase in the roots with an increased intensity of the disease (Joseph, Potty and Jayasankar, 1976). The correlation between concentration of polyphenol oxidase and root (wilt) disease index based on foliar symptoms in the range of 11-50 was highly significant and positive. In this range, the activity of peroxidase also increased with increase in the root (wilt) disease index (Joseph, Potty and Jayasankar, 1976).

Ethanolic extracts of roots collected from the apparently healthy and root (wilt) affected coconut palms in the disease affected areas were characterized using thin layer chromatography (cellulose) with 2% formic acid as the solvent system. Three fluorescent compounds with Rf values, 0.29, 0.44 and 0.57 were separated. Though qualitative difference was not indicated, quantitatively the intensity of the spots was less in the samples collected from the diseased palms. The choice of the solvent system was arrived at after exhaustive study using 14 different solvent systems that are in vogue in the characterization of phenolic compounds.

An ethanol extractable phenolic compound which was present in the leaves of West Coast Tall (WCT) palms cultivated in the root (wilt) disease-free region was absent in the WCT palms cultivated in the diseased region (Joseph, Potty and Jayasankar, Communicated). The compound was fluorescent under ultraviolet and showed a Rf value of 0.36 in thin layer

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chromatography (cellulose) with 2% formic acid. The purified compound had a melting point of 86°c and showed an absorption maximum at 285 nm in ethanol. The compound was also present in the leaves of hybrid like the T x D, D x T and T x G but showed depletion only in the WCT palms in the root (wilt) affected region (Joseph, Potty and Jayasankar - Communicated).

Further studies indicated certain interesting trends with respect to the biosynthesis of phenolic compounds. Phenylalanine ammonia lyase (PAL) which provides the phenylpropanoid skeleton for the synthesis of phenolic compounds was assayed based on the ability of enzyme preparations to convert L-phenylalanine to trans cinnamic acid. Initial studies were restricted to quantify the enzyme in the roots of palms cultivated in the disease-free areas and the diseased palms in the disease-affected areas. The specific activity of the enzyme in the healthy samples was significantly low (0.9 µg/mg protein/4 h) compared to the diseased samples (18.0 µg/mg protein/4 h) (Joseph and Jayasankar, 1979). Further, the PAL activity was highest in the apparently healthy palms in the disease affected areas compared to the other two categories of palms. With the incidence and increase in intensity of the level of the enzymes showed a negative correlation with disease intensity (Unpublished). Likewise, the orthodihydroxyphenol levels also showed a negative correlation with disease intensity.

Partially purified preparations of polyphenol oxidase were resolved using polyacralamide gel electrophoretic system when the isozyme patterns showed distinct differences between the three categories of palms with one band in the healthy palms from the disease-free areas, three bands in the apparently healthy palms from the disease-affected areas and five bands in the diseased palms in the disease-affected areas (Unpublished).

ON RHIZOSPHERE MICROFLORA

The soil and rhizosphere microflora of the coconut palms were investigated in detail with particular reference to coconut root (wilt) disease (Potty, 1977). Besides collecting the rhizosphere and non-rhizosphere samples of healthy

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palms (palms cultivated in the disease-free tracts) and diseased palms (palms cultivated in the root (wilt) affected tracts) sampling was extended for enumeration to coconut palms cultivated at 10 KM away from either sides of the border areas demarcating the spread of root (wilt) disease in different soil types. Investigations revealed significantly higher population of actinomycetes and bacteria in coconut soils of root (wilt) affected region compared to the healthy. The occurrence of these organisms was higher in the top soil at a depth of 0-25 cm. Irrespective of the diseased condition the coconut rhizosphere harboured higher numbers of actinomycetes, bacteria and fungi. The number of the different microflora was more in the rhizosphere of diseased palms compared to healthy. However, the difference in the quantitative distribution of the various microorganisms between the young and old roots was not consistent. Similarly the pattern of occurrence of the different microflora in the coconut rhizosphere varied in different soil types irrespective of the diseased condition of the palms. Within the root (wilt)-affected region a higher population of the different microflora was noticed with high levels of water table. This trend was more pronounced in the case of bacteria. Gram positive bacteria were the dominant in the rhizosphere of healthy and root (wilt) affected palms. Similarly, the dominant fungi in the coconut rhizosphere consisted of Aspergillus, Penicillium and Fusarium.

More conclusive observations were recorded on the investigations carried out on the microflora of the coconut palms under mixed cropping in the root (wilt) affected region. Preliminary investigations on this aspect of study revealed higher values of total bacteria and ratios of nitrogen-fixing organisms to denitrifiers in coconut soils cultivated with the fodder crop Stylosanthes gracilis alone and in combination with hybrid napier (Sahasranaman, Pillai, Jayasankar, Potty, Varkey, Kamalakshi Amma and Radha, 1976). On similar investigations it was observed that mixed cropping of healthy and root (wilt) affected coconut palms with hybrid napier enhanced the total bacteria and nitrogen-fixing organisms in the coconut rhizosphere, irrespective of the condition of the palms (Sahasranaman, Pillai, Jayasankar, Potty, Thomas Varkey, Kamalakshy Amma and Radha, 1976). There was also a similar

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increase in the number of phosphate solubilising bacteria in the coconut rhizosphere with the root (wilt) affected palms harbouring a higher number (Potty, Mathew George and Jayasankar, 1977). An exhaustive investigation on the influence of crop mixing Centrosema pubescense and Stylosanthes gracilis besides hybrid napier, on the rhizosphere microflora of the coconut palms also revealed similar trends (Potty, 1977). Crop mixing resulted in an increase in the load of fungi, bacteria, actinomycetes and indole producing organisms in the coconut rhizosphere.

Mixed cropping also favoured the proliferation of nitrogen-fixing organisms but no consistent difference was noticed between the healthy and diseased palms. Beijerinckia and Azotobacter which constituted the bulk of the nitrogen-fixing organisms in the coconut rhizosphere fixed nitrogen in vitro in the range of 10.6 to 19.0 and 14.6 to 16.8 mg per 100 ml. respectively. Relatively higher numbers of phosphate solubilising actinomycetes and bacteria were noticed in the rhizosphere of root (wilt) affected palms irrespective of treatment combinations. The phosphate solubilising actinomycetes, bacteria and fungi mineralised phosphorus in the range of 20-40 µg/ml, 42-89 µg/ml and 34-81 µg/ml respectively. Physicochemical factors of the coconut rhizosphere like pH, organic carbon, nitrogen and phosphorus showed correlations with the microflora.

Yet another indication made during the course of this investigation was on the basis of a physiological classification of the actinomycetes isolated from the coconut rhizosphere (Potty, 1977). The traits studied were phosphate solubilisation, nitrate reduction, cellulose digestion, gelatin hydrolysis and starch hydrolysis. Among the palms under mixed cropping a difference in the proliferation of physiologically distinct actinomycetes in the coconut rhizosphere was noticed either in response to a fodder grass or legumes. Among the fodder legumes no difference was noticed on the pattern of physiologically distinct actinomycetes. Results were indicative of the possibility of altering the microflora of the coconut rhizosphere as a result of inter and mixed cropping. Extensive studies on the influence of inter and mixed cropping in coconut gardens with emphasis on yield and disease condition are in progress in cultivators' gardens.

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