PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS

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INTRODUCTION

Some of the earlier workers on root(wilt) disease attributed this malady to certain physiological factors. Bourdilon(1907) speculated that the lack of nutrients and saturated soil water might be the cause of the disease. Rover(1911) characterised this disease as purely physiological in nature, resulting from unfavourable soil conditions. Shepherd(1912) supported Rover and emphasised that, too much or too little soil water disturbed the physiological process of the palms and render them prone to the disease. It was also suggested that certain weak fungi like Diplodia might attack the palms under disturbed physiological conditions and cause the disease symptoms. Varghese(1934), who examined all the known factors associated with root(wilt) disease, reported that this disease is not physiological but, fungi, virus and certain toxic substances produced by microorganisms living around the root zone of the palms might also contribute to produce the symptoms. However, in the absence of definite proof of any biological agent being the cause of the disease, physiological investigations assumed importance and were intensified from 1951. An attempt is made in this paper to review the work done on the physiological and biochemical aspects of root(wilt) disease.

STUDIES ON ROOTS

Michael(1964) studied the extent of root damage caused by root(wilt) disease in two to ten year old healthy and diseased palms and reported that the number of active roots and the diameter of the bole(root forming portion of the stem) were drastically reduced in diseased palms. Percentage of dead roots was found to be higher in diseased palms than in healthy ones. Regenerating capacity of roots in diseased palms was also considerably reduced.

A method for the collection of root sap was devised by Davis(1964). His studies on the nature and composition of the root sap indicated that the root sap of healthy palms was

acidic in nature, odourless, clear and was rich in K_2O and MgO contents; whereas the root sap of diseased palms was neutral to alkaline in nature, foul smelling and was poor in K_2O and MgO contents (Davis, 1964). Ramadasan(1964) noted that the root sap of diseased palms contained 65-72% more total solids than that of healthy palms.

A method was standardised to collect root exudates from coconut palms(Ramadasan, Radha and Michael,1967). However, no authentic information is available on the nature and composition of the root exudates from healthy and diseased palms.

Ramadasan(1967) reported that tomato seedlings grown in root sap collected from diseased palms developed epinasty and bending of leaves, while no such symptoms were noticed on seedlings grown in healthy root sap or in distilled water. This indicated the possible involvement of a toxin in root (wilt) affected palms. However, when the experiment was repeated with potted coconut seedlings, typical root(wilt) symptoms could not be reproduced in them. This study needs further pursuation with particular emphasis on the biochemical nature of the root sap.

PERMEABILITY CHANGES

Permeability of leaf and root tissues was damaged in diseased palms. This was indicated by the release of more electrolytes from the leaf and root tissues of diseased palms. The flow of electrolytes from diseased leaf samples was 29% more during summer and 9% more during rainy season than from healthy samples. This increase from diseased roots was 22% during summer and 8.2% during rainy season(Michael,1977).

WATER RELATIONS

Studies on water relations revealed that absorption of water was very much reduced in diseased palms. When water was fed through the cut end of roots it was found that a single functioning root of healthy palm could absorb, on an average 250 cc, while the same in diseased palm was only 150 cc (Davis, 1964). The water absorbing capacity of the excised root tissue was also found to be low in diseased palms (Chacko Mathew and Ramadasan, 1968).

The uptake and upward transport of water through the trunk in diseased palms was found to be 35% less than that of the healthy palms(Ramadasan, 1964). Significant increase in the transpiration rate was noticed in diseased palms as compared to that in healthy palms. The mean rates of transpiration from healthy palms were 4.4, 4.5 and 3.8(mg/dm^2/minute) respectively for the first fully opened, middle and outer whorls of leaves, while the corresponding rates from diseased palms were 7.1, 7.5 and 5.2. An excess of transpiration normally leads to progressive decrease in the water content of leaf tissues. But in root(wilt) affected palms the leaf water content was significantly higher than in healthy palms. The frequency of cells and stomata per unit area of leaf was also greater in diseased palms(Table 1). However, the stomatal index did not differ significantly between healthy and diseased palms. These studies indicated an imbalance in the water economy of the diseased palms(Chacko Mathew(unpublished).

Table I. Cell and stomatal frequency and stomatal index in the leaves of healthy and root(wilt) diseased coconut palms

Condition	No. of cells(mm^2)		No. of stomata(mm^2)		Stomatal index	
	First fully opened leaf	Middle leaf	First fully opened leaf	Middle leaf	First fully opened leaf	Middle leaf
Healthy (mean of 20 palms)	1272	1280	182	171	13.5	13.0
Diseased (mean of 20 palms)	1413 **	1398 **	228 **	219 **	15.1 NS	14.8 NS

**tvalue significant at p = 0.01

NS - Not significant

MINERAL NUTRITION

A statistically laid out sand culture experiment was conducted to study the effect of N, P and K nutrients on the growth of coconut seedlings(Chacko Mathew and Ramadasan,1960). Results revealed that the role of nitrogen in the growth of coconut seedlings is much more significant than that of phosphorus or potassium. The role of phosphorus seemed to be

only indirect in that this element probably enhances the uptake of potassium. The seedlings showed maximum response to the treatment of N, P, K combinations as compared to other treatments. The deficiency symptoms of N, P and K nutrients on the seedlings were not comparable to root(wilt) disease syndrome.

A detailed study on the effect of micronutrients on root (wilt) disease was conducted for a period of 10 years. A total of 384 palms in a disease prevalent sandy tract consisting of trees in all stages of disease were supplied with micronutrients and magnesium singly as well as in all possible combinations. The nutrients were given as soil application for the first five years and for the rest of the period as root injection. It was observed after the 10 year period that the yield of these experimental palms had generally increased irrespective of their diseased condition. Magnesium had significant positive effect on the yield (Davis and Pillai, 1966). However, not only have these treatments failed to improve the diseased condition of the palms, but also more than 50% of the healthy palms under the experiment contacted disease during the course of the experiment.

Yellowing of outer whorls of leaves is of common occurrence in healthy as well as root(wilt) infected palms. One type of such yellowing is characterised by the development of the same from the tips and margins of leaves. Pandalai(1958) discussed the association of a high K/Mg ratio with such type of foliar yellowing and had suggested K induced Mg deficiency as a probable reason for the same. Attempts on the control of such type of yellowing revealed that 2% $MgSO_4$ given as foliar spray at quarterly intervals completely cured yellowing in 16% of palms while reduction in yellowing was noted in another 60%. K/Mg ratio in the leaves during the pre-treatment period ranged from 3.1 to 5.1 while at the end of the treatment period it ranged from 2.1 to 3.1(Chacko Mathew and Ramadasan, 1967). In a recent study, Varkey, Kamalakshyamma, Ramadasan and Nambiar, 1979) observed that foliar sprays with 2% Mg SO_4 was effective in checking foliar yellowing both in the healthy and diseased area. They have further noted that basal application of $MgSO_4$(500 g/palm/year) and foliar spray with 1% $FeSO_4$ also checked foliar yellowing to a considerable extent.

RESPIRATION AND PHOTOSYNTHESIS

Michael(1978) determined the rate of respiration in healthy and root(wilt) affected palms, employing Warburg's manometric techniques. It was significantly higher in diseased palms than in healthy. The increase in respiration rates of the leaves of diseased palms was 18-21% in the advanced stages and 2-5% in the early stages of the disease. The roots of palms in advanced and early stages of the disease recorded 14.3% and 11.4% more respiration over that of apparently healthy palms. The respiration rates differed markedly between healthy and apparently healthy palms also.

The effect of root(wilt) disease on photosynthesis reflected marked variation between healthy and diseased palms in the photosynthetic CO_2 fixation. The leaves of the diseased palms fixed 11-70% less CO_2 than those of healthy ones. The first fully opened and middle leaves of healthy palms had almost equal photosynthetic rates, but in diseased palms the first fully opened leaf recorded significantly higher rate of CO_2 fixation.(Dwivedi, Chacko Mathew, Michael, Ray and Sumathykutty Amma, 1978).

Table II. Respiratory rate in leaf and root tissues of healthy and root(wilt) affected palms(expressed as O_2 consumed per $mm^2/mg^{-1}/hr^{-1}$)

Condition of palms	Summer season	South-West monsoon season
Leaves		
Healthy	.30	.20
Apparently healthy	.69	.55
Diseased	.70	.62
Roots		
Healthy	1.7	1.6
Apparently healthy	3.5	2.9
Diseased	4.9	3.6

OTHER METABOLIC DISORDERS

Studies on changes in carbohydrate content of root(wilt) affected palms revealed that total, reducing and non-reducing sugars were significantly higher in the leaves of infected palms. But a depletion of these sugars occurred in the roots

of diseased palms. This situation indicated a possible derangement in the translocation and distribution of sugars in diseased palms. In spite of the higher sugar content in the leaves total carbohydrates and starch content were lower in both leaves and roots of diseased palms(Chacko Mathew,1977).

Gross derangement in the path of nitrogen resulting in considerable increase in the non-protein nitrogen content, concomittant with a sharp decrease in the water soluble nitrogen and protein nitrogen fractions was observed in the diseased leaf tissue. The total and organic phosphorus were found to be higher in diseased palms than that in healthy. A reduction in the C/N ratio in the roots and leaves of diseased palms was also noticed(Varkey et al. 1969).

BIOCHEMICAL FACTORS

Pillai and Shanta(1965) reported accumulation of certain free amino acids in wilt affected leaves and they have suggested that probably the accumulation of amino acids predisposes the wilt infected palms to 'leaf rot'. The quantity of amino acids in the leaves were found to increase with the incidence and intensity of disease. Arginine increased from minute traces in healthy to considerable quantity with increase in the intensity of disease. Predominance of glutamic acid and arginine was noted in the root saps of healthy palms, while arginine was completely absent in the root saps of the diseased (Annual Report, CCRS , 1968).

Tannin content in the middle and outer leaves of diseased palms was found to be more than that of healthy ones. However, in the tender leaves the increase was not statistically significant(see Lal, 1968). DxT hybrids contained higher amount of phenols and tanins in their leaves, as compared to W.C.T. palms(Chacko Mathew and Joseph, 1974). Total phenols in the outer and middle leaves of diseased palms were higher than that of healthy ones, but in the other leaves reverse trend was noted(Dwivedi, C.Mathew, Ray and B.S.K.Amma, unpublished)

The activity of carbonic anhydrase was studied with a view to finding out the disorder of zinc nutrition in root (wilt) affected palms. CA activity was found to be low in the leaves of diseased palms as compared to that in healthy palms(Dwivedi, Ray, Sumathykutty Amma, Chacko Mathew, Padmaja and C. Ninan,1977). This indicated reduced concentration of biologically active zinc in diseased palms.

Table III. Carbonic anhydrase activity ($MgCO_2$/100 mg/ protein/20 min.) in healthy and root(wilt) diseased coconut palms

Condition of palms	Spindle leaf	First fully opened leaf	Middle leaf	Outer leaf
Healthy	32.5	17.5	12.0	8.1
Apparently healthy	20.6	5.8	5.6	4.1
Diseased	18.3	3.6	2.9	3.5

In another study CA activity was assessed in unfertilised female flowers, one month old and two month old buttons from WCT variety of coconut. The titratable acidity was also determined in these samples. A positive correlation was indicated between CA activity and yielding potential of palms, ie., the high yielding palms had greater CA activity. The activity of CA progresstively increased with the maturity of buttons. A reciprocal relationship was obtained between CA activity and titratable activity (Padmaja, Sumathykutty Amma, Chacko Mathew, Ray and Dwivedi, 1980)

The role of cellulase enzyme in root decay associated with root(wilt) disease was investigated. Non-decayed and decayed roots collected from both healthy and diseased palms were subjected to enzyme assay. No activity was observed in non-decayed roots of healthy and diseased palms, but decayed roots showed activity in both the cases. The mean enzyme activity recorded in healthy palms was 4.856 (ug of glucose liberated per 100 ug protein per hour) while the same was 12.817 in diseased palms (Padmaja and Sumathykutty Amma, 1979)

EFFECT OF DISEASE ON FLOWERING

Though the appearance of the disease syndrome can occur at any stage of the growth of the palm, the most vulnerable stage to disease attack was found to be flowering/nut bearing age(5-10 years). The onset of disease during the nut production and stabilising stage(10-15 years) of the palms leads to fast deterioration in growth and yield. However, when the palms of middle and old age(25-50 years) were attacked their decline was at a slow pace(Lal, 1964). Ramadasan, Shanta and Lal, 1971) observed that in young palms bearing was often delayed indefinitely and yields were much less than those palms which became diseased after the onset of bearing.

Studies undertaken on pollen morphology (Varkey and Davis,1960) have shown that pollen grains from diseased trees were of irregular and smaller size and showed poor germination. The percentage of pollen grains germinating from healthy palms was found to be 75% while the same figure in disease in its early, middle and advanced stages was 52, 49 and 39% respectively. Pollen tube growth was also very much reduced in diseased palms.

RADIOTRACER STUDIES

Since the disease caused retardation of growth and reduced yield in palms, it was presumed that there might be certain imbalance in absorption and translocation of nutrients and also in the distribution of photosynthates and other metabolites. It was also realised that the incorporation of inorganic nutrients into organic complexes might be hindered in diseased palms. To understand these phenomena in greater depths and with more accuracy than with the conventional methods, studies using tracer techniques, were initiated from 1976.

Radioactive $KH_2{}^{32}PO_4$ was fed to coconut leaves and palms under laboratory and field conditions. In healthy palms the absorption of phosphorus by roots and its accumulation in spindle and first fully opened leaves was found to be significantly higher till 9th hr. as compared to that of diseased palms, but at later stages the reverse pattern was observed. The time required for ^{32}P to reach the spindle(top-most leaf) situated at 9.5 M height in both healthy and diseased palms was found to be 3 hr only. The analysis of total and different fractions of phosphorus and entry of ^{32}P in the different forms of organic phosphorus indicated that although total P was more in diseased palms, the organic phosphorus especially the neuclic acid P was significantly less as compared to healthy ones. This revealed less utilization of absorbed P in the synthesis of P-constituted organic substances in diseased plants(Dwivedi, Ray and Ninan, 1979)

The efficiency of different methods of applying ^{32}P through soil(circular trenches, holes, strip and basin) and plant parts(growing root-tip, cut root end, stem and leaf axil) was also studied (Ray, Dwivedi and Ninan, 1979). Out of four soil placement methods application through circular trenches showed maximum recovery of ^{32}P throughout

the experimental period upto 30 days. The ^{32}P activity was detected in the palm after 7,8,8 and 11 days respectively in hole, circular trench, strip and basin application methods. ^{32}P absorption was equally efficient in the palm receiving irrigation daily and once in a week only. Plant injection methods were found more economical and efficient than soil application. Among the plant injection methods, the application of ^{32}P through cut end of roots was most efficient. Radio activity in the palm was detected after 8, 12 and 18 hr of application through stem, leaf axil and growing root tip respectively.

In order to evaluate the efficiency of different sources of phosphatic fertilizers (Ray, Ninan and Dwivedi, 1977) applied four phosphatic fertilizers, namely single superphosphate, rock phosphate, nitrophosphate and ammonium dihydrogen orthophosphate tagged with ^{32}P in sandy loam soil under field conditions. Rock phosphate was found to be superior to other phosphatic sources for this type of soil.

BIOCHEMICAL TEST TO DETECT THE DISEASE

Attempts have been in progress to develop a suitable test for the diagnosis of the disease. Thomas Joseph and Shanta(1963), Holmes, Lal and Shanta(1965), Padma, Summanwar and Chandra(1973), Solomon, Sasikala and Shanta (1976) have reported their attempts on this aspect. But, all of the tests developed by these workers are time consuming and reported to involve sophisticated equipments. Dwivedi et al(1977) worked on this line and developed a rapid biochemical test to detect the disease. The solvent/extractant used in this test, was 0.3 M EDTA, which is known to extract biologically active organic substances/pigments constituted in certain secondary and micronutrients by the process of chelation. The spindle and root extract of diseased palms showed yellowish light brown and dark brown colour respectively. On the contrary in healthy palms the spindle and root extract exhibited yellowish brown and brown colour respectively. When transmittance ratio of root/spindle was worked out, it was found to be 1.86, 4.19 and 5.01 respectively for healthy, early diseased and disease advanced palms respectively. Though this test was found to be simple and quick, it did not hold good in all the cases tested. Only in about

75% cases it was found true. Attempts are, therefore, under way to modify this test and make it more sound.

ATTEMPTS ON CHEMICAL CONTROL

Several attempts were made to control the disease with chemicals(Nair and Radha;1959, John, Channy and Varghese, 1959; Rajappan, Varghese and Sankaranarayanan,1959; Davis and Pillai; 1966; Sahasranaman, Radha and Pandalai;1967 and Lal; Radha and Shanta,1970). Although temporary improvement in the condition of palms and increase in yield were noticed in some of the chemical treatments, none of them was found effective in curing or controlling disease. A recent experiment conducted by Dwivedi, Sumathykutty Amma, Chacko Mathew and Ray;1980 has shown that ascorbic acid, certain phenols and micronutrients have good effect in checking disease symptoms. These chemicals, especially ascorbic acid not only did reduce disease symptoms drastically but also improved the yield of the diseased palms significantly. A more elaborate experiment, started recently in the farmers' fields, is expected to give further information on the curative effects of these chemicals under farmers' field conditions.

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