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PHYSIOLOGY AND BIOCHEMISTRY

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Diseased plants generally exhibit impaired metabolism. A number of physiological and biochemical derangements occur consequent on disease development. In root (wilt) affected palms also derangements were noticed in the root functioning, water relations, mineral nutrition, respiration and photosynthesis, phenol metabolism and similar other processes.

Michael (1964) studied the extent of root damage caused by root (wilt) disease in two to ten-year-old palms and reported that the number of functioning roots and the diameter of the bole (root forming portion of the stem) were drastically reduced in diseased palms. In diseased palms, the percentage of dead roots was higher and the regenerating capacity of roots was also considerably reduced.

An attempt to regenerate roots in root (wilt) disease affected palms was made by Amma and Patil (1982) using hormones, phenols and amino acids. Application of these chemicals in the debarked region of the stem of coconut palms just above the bole induced production of new roots. The palms which received indole butyric acid (IBA) 500 ppm+phenols 400 ppm produced maximum number of roots (Table 19) followed by naphthalene acetic acid (NAA) + glutamic acid. Palms in which regeneration of roots was noticed, the disease indices also tended to decrease with an improvement in the foliar condition of the palm (Table 19).

A method for 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 apparently healthy palms was acidic in nature, odourless, clear and rich in K_2O and MgO contents. The root sap of diseased palms was neutral to alkaline in nature, foul smelling and poor in K_2O and MgO contents. Ramadasan (1964) noted that the root sap of diseased palms contained 65.72% more solid contents than that of healthy palms. Tomato seedlings placed in root sap collected from diseased palms developed epinasty and bending of leaves, while no such symptoms were noticed on seedlings placed in the root sap of healthy palms. Davis (1964) reported the absorption of 250 to 500 ml water per day by a single root of healthy palm, as against less than 150 ml per day by that of diseased palms. The uptake and transport of water through the trunk in diseased palms was reported to be 35% less than that of healthy palms (Ramadasan, 1964). Studies on the profile of soil moisture depletion by the roots of healthy and diseased palms in irrigated plots also revealed poor depletion by wilt affected palms (Rajagopal *et al.*, 1986b). This was further supported by studies with labelled phosphorus (Dwivedi *et al.*, 1979).

Table 19. Effect of growth hormones and phenols on root regeneration and foliar condition of (wilt) affected coconut palms after one year of treatment (Amma and Patil, 1982)

Sl. No.	Treatment	No. of palms treated	No. of palms producing roots	Total No. of roots	Disease index	
					Pre-treatment	Post-treatme
1.	IBA 500 ppm	3	Nil	-	19	23
2.	IBA 1000 ppm	3	1	3	32	28
3.	IBA 500 ppm + Thiamine 250 ppm	3	1	4	38	35
4.	IBA 500 ppm + Phenols 400 ppm	3	3	51	35	26
5.	NAA 500 ppm + Glutamic acid 500 ppm	3	2	33	38	30
6.	Control	3	2	2	21	22

Root (wilt) diseased palms were seen to have higher stomatal frequency than that of the healthy ones (Mathew, 1981). Rajagopal *et al.*, (1986a) found abnormal stomatal opening in the infected palms with impaired regulation leading to excessive water loss, irrespective of the time of the day or season or growing condition (Fig. 14). Thus, the stomatal resistance at 14.00 h was only 5.5 sec. cm^{-1} in the leaves of diseased palms, as against 14.9 sec. cm^{-1} in those of healthy palms. With the advancement of disease, there was greater disturbance in stomatal regulation resulting ultimately in excessive water loss. They found palms in the early stage of disease with an index of 20 and a transpiration rate in the range of 4.20 to 4.35 mg. cm^{-2} sec^{-1} while those in the advanced stage with the index above 50 transpired at the rate of 9.15 to 10.50 mg. cm^{-2} sec^{-1}.

Root (wilt) affected palms had consistently lower leaf water potential than the healthy palms at any given time (Rajagopal *et al.*, 1987). The nature of symptoms on the leaflets of different whorls of leaves reflected the changes in leaf water potential (Table 20).

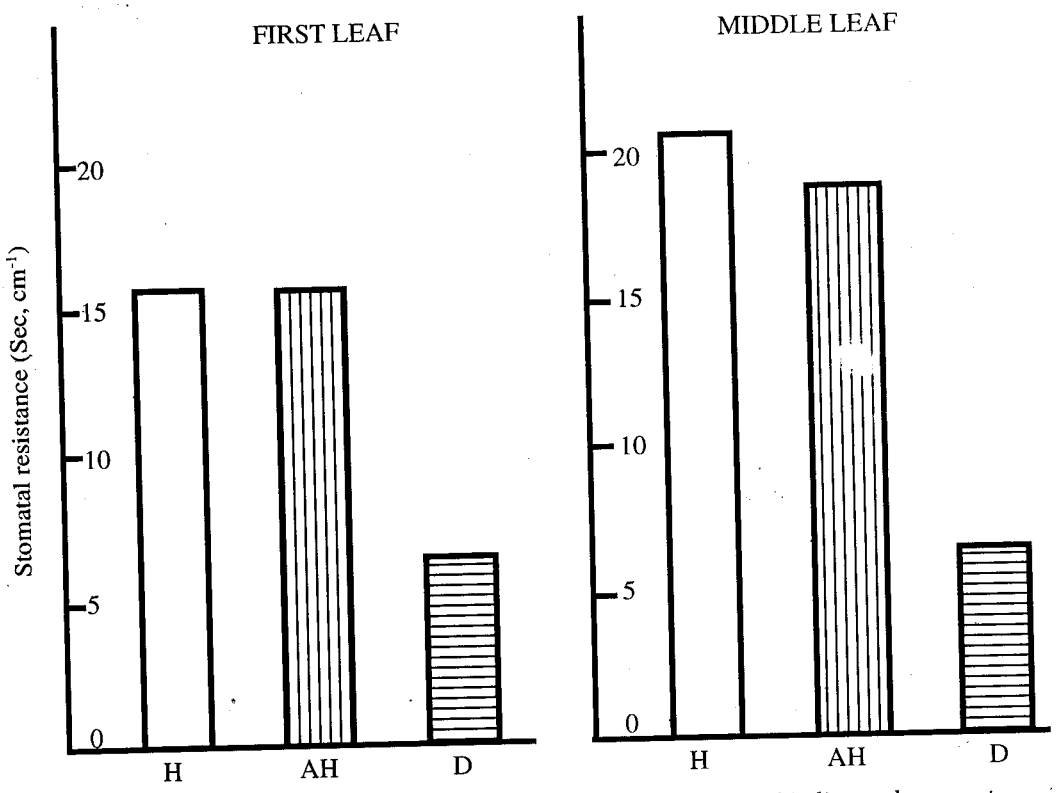
Permeability of leaf and root tissues was found damaged in diseased palms. This was revealed by the release of more electrolytes from root and leaf tissues of diseased palms than those of healthy palms (Anon., 1977).

Studies on the effect of N, P and K nutrients on the growth and incidence of root (wilt) disease of coconut seedlings revealed that nitrogen had a significant effect on the growth. The symptoms noticed on seedlings due to deficiency of N P K nutrients were not, however, comparable to the root

Table 20. Changes in leaf water potential with leaf age and leaflet condition in root (wilt) affected palms (Rajagopal *et al.*, 1987)

Position	Apparently healthy palm	(MPa)	Diseased palm	(MPa)
Spindle	Yellow to light green thick, stiff	-0.37 ±0.02	White to dull cream, thin papery, brown spots	- 0.2 ±0.06
First whorl	Green, normal, erect	-0.68 ±0.06	Light green, slight flattening and bending at the tip	-1.24 ±0.08
Middle	Dark green normal, erect	-0.79 ±0.07	Flaccidity, yellowing necrosis	-1.28 ±0.08
Outer whorl	Dull green, senescent but normal	-0.89 ±0.07	Ribbing, necrosis abnormal, senescence	-1.26 ±0.09

(This experiment was carried out in the 'wet' season (September, 1983). Values are means of six palms ± SE of the mean). * MPa = Mega-Pascal



H Healthy coconut, AH Apparently healthy coconut, D Root (wilt) diseased coconut

Fig. 14 Stomatal resistance in coconut leaves (Rajagopal *et al.*, 1986)

(wilt) disease syndrome (Mathew and Ramadasan, 1971).

Pandalai (1958) discussed the association of a high K/Mg ratio with foliar yellowing and suggested K induced Mg deficiency as a probable reason for the same. Studies on the control of yellowing revealed that 2 per cent $MgSO_4$ given as foliar spray at quarterly intervals, completely cured yellowing in 16 per cent palms, while reduction was noticed in another 60 per cent of palms (Mathew and Ramadasan, 1971). The effect of $MgSO_4$ in reducing foliar yellowing was also confirmed by Varkey *et al.* (1979). They further observed that basal application of $MgSO_4$ (500 g/palm/year) and foliar spray with 1% $FeSO_4$ helped in checking foliar yellowing in coconut.

Michael (1978) reported a significantly higher rate of respiration in root (wilt) diseased palms compared to healthy palms (Table 21).

Table 21. Respiratory rate in leaf and root tissues of healthy and root (wilt) affected palms (expressed 0_2 consumed per $mm^2mg^{-1}hr^{-1}$) (Michael, 1978)

Condition of palms	Summer season	South West monsoon season
Leaves		
Healthy	0.30	0.20
Apparently healthy	0.69	0.55
Diseased	0.70	0.62
Roots		
Healthy	1.70	1.60
Apparently healthy	3.50	2.90
Diseased	4.90	3.60

A marked variation in the photosynthetic CO_2 fixation was noticed between apparently healthy and root (wilt) affected palms, the former recording significantly high rate of CO_2 fixation than the latter. The increase in the CO_2 fixation rate was 11% in the first fully opened leaf and 70% in the middle whorl leaf of apparently healthy palms. Percentage increase of the chlorophyll content in the first fully opened and middle leaves of healthy palms was 16 and 70 respectively, over that in diseased palms (Dwivedi *et al.*, 1978).

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

Padmaja *et al.*, (1981) reported an increase of 34.2%, 15.9%, 19.9% and 10.1% in the alkali extractable protein fractions in the spindle leaf, first fully opened leaf, middle leaf and outer leaf, respectively of healthy palms compared to diseased palms. The increase in the water extractable protein and ethanol extractable protein was 59.0%, 50.2%, 49.5% and 47.8% for the former in the four whorls of leaves, respectively, and 21.6%, 42.8%, 4.1% and 12.4% for the latter in the respective leaves. The low protein values obtained in the diseased palms may be the net effect of decelerated protein synthesis and accelerated protein breakdown.

Gross derangement in the path of nitrogen resulting in considerable increase in the non-protein nitrogen content, with a concomitant sharp decrease in the water soluble nitrogen and protein nitrogen fractions was observed in the diseased tissue. A reduction in the C/N ratio was also noticed in the roots and leaves of root (wilt) diseased palms (Varkey *et al.*, 1969).

Pillai and Shanta (1965) reported accumulation of certain free amino acids in root (wilt) affected leaves and they opined that accumulation of amino acids might predispose the diseased palms to 'leaf-rot' infection. The quantity of amino acids in the leaves was found to increase with the incidence and intensity of the disease. Arginine increased from minute traces in healthy to considerable quantity with increase in the intensity of the disease.

The activity of carbonic anhydrase (CA) enzyme was studied with a view to finding out the disorder of zinc. Carbonic anhydrase activity was found to be low in the leaves of diseased palms as compared to that in healthy palms (Anon., 1979). This indicated reduced concentration of biologically active zinc in palms.

The higher enzyme activity observed in diseased palms indicates that cellulase might be one of the factors responsible for the vast decay noticed in diseased palms (Padmaja and Amma, 1979).

Pectin lyase activity was found to be nearly six times higher in the roots of diseased palms as compared to the healthy ones (Amma and Patil, 1985).

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 for nine hours after application, as compared to diseased palms; but at later stages, reverse pattern was observed. The time required for ^{32}p to reach the spindle situated at 9.5 M height of both healthy and diseased palms was found to be three hours only.

The analysis of total phosphorus and different fractions of the same indicated that although total P was more in diseased palms, the organic P, especially the nucleic acid phosphorus, was significantly less in diseased palms than that in healthy palms. This revealed less utilization of absorbed phosphorus in the synthesis of P constituted organic substances in diseased palms (Dwivedi *et al.*,1979).

Joseph and Jayasankar (1973) recorded that the highest concentration of polyphenols was recorded in the samples of roots collected from healthy tract as compared to that in apparently healthy (healthy palms in diseased tract) or diseased palms. With the increase in disease intensity the concentration of total phenol was found to decrease to half.

Studies on phenol oxidising enzymes such as polyphenol oxidase and peroxidase indicated a corresponding increase in their activities with the incidence and increase in intensity of the disease. A positive correleation has been noticed between the activities of these enzymes and disease index (Joseph *et al.*,1976). The

orthodihydroxy phenol content in the roots of coconut palms has also decreased with increase in intensity of the disease. A marked increase in the level of phenylalanine ammonia lyase (PAL) in the apparently healthy, disease early and disease advanced palms has been observed with a negative correlation between enzyme activity and disease indices (Joseph and Jayasankar, 1979). The activities of polyphenol oxidase and peroxidase have a negative correlation with orthodihydroxy phenol while the activity of phenylalanine ammonia lyase has a positive correlation. The isozyme pattern of polyphenol oxidase has indicated distinct difference between the healthy, apparently healthy and diseased palms, resolving into one, three and five bands, respectively (Joseph, 1983).

The rate of flow of phloem sap from the inflorescences of coconut depends on the nature of palms. Apparently healthy palms had a rate of flow of 5 ml/hr until 25 days, but shot up at 32 days with a rate of flow of 22 to 25 ml/hr. This trend continued for 70 days followed by a rapid decline. With the intensity of disease, the rate of flow decreased (Rajagopal *et al.*, 1989). The rate of sap collection differed between day and night, in that the flow was greater during the day than during the night. The data in Table 22 clearly indicates the differences in pH, osmotic concentration and sugars between the apparently healthy and diseased palms. Chempakam and Rajagopal (1989) reported the biochemical constituents of the phloem sap. Diseased palms had much less content of reducing sugars, proteins and free amino acids, phenols, lipids and sterols than in the apparently healthy palms. Arginine content alone was relatively high in the sap of diseased palms (Table 23). Further analysis with gas liquid chromatography revealed the levels of individual amino acids, organic acids and sugar (Chempakam *et al.*, 1991). Arginine and aspartic acid among amino acids, malonic acid and lactic acid among organic acids and glucose and galactose among sugars registered higher values in the sap collected from the inflorescences of diseased palms than that from the apparently healthy ones (Table 24). These biochemical constituents of the phloem sap might have favoured the growth of MLOs in the diseased palms.

Table 22. Differences in the phloem sap collected during the day and night from the inflorescences of coconut palms (Rajagopal *et al.*, 1989)

	Apparently healthy		Diseased	
	Day	Night	Day	Night
Rate of sap flow ($ml.h^{-1}$)	17.9	11.4	9.9	4.1
pH	6.9	6.1	7.2	6.5
Osmotic concentration (m mol Kg^{-1})	720	765	660	690
Total sugars (mg g^{-1} sap solid)	250	180	180	190
Reducing sugars (mg g^{-1} sap solid)	52.0	45.8	51.1	24.6

Table 23. Biochemical constituents of vascular sap. Values expressed as mg g^{-1} sap solids (Chempakam *et al.*, 1991)

Constituents	Apparently healthy	Diseased
Total sugars	299.50	286.20
Reducing sugars	98.10	65.90
Proteins	4.90	2.50
Free amino acids	45.90	18.30
Arginine*	0.41	0.68
Phenols	0.60	0.20
Lipids	0.83	0.57
Sterols*	0.40	0.22

* Values expressed as mg ml^{-1} sap.

It has, thus, become evident that the root (wilt) disease has altered many of the physiological and biochemical processes of the coconut palm. The flaccidity symptom of the disease could be correlated with the development of internal water stress, obviously caused by the pathogen.

Table 24.Composition of amino acids, organic acids and sugars identified in the phloem sap from the inflorescences of apparently healthy and root (wilt) diseased coconut palms. Values are expressed as mg g^{-1} sap solids. (Chempakam *et al.*, 1991)

Compounds identified	App. healthy	Diseased
A) Amino acids (average of 5 palms) :		
Cystine + Cysteine	0.25	0.20
Arginine	0.19	0.28
Serine	0.13	0.09
Glycine	0.11	0.09
Aspartic acid	0.19	0.25
Glutamic acid	0.12	0.06
Leucine	0.14	0.12
Methionine	0.17	0.13
Phenylalanine	0.27	0.24
Tyrosine	0.06	0.23
B) Organic acids (average of 5 palms) :		
Oxalic acid	1.54	1.03
Malic acid	1.29	1.02
Fumaric + succinic acids	0.41	0.29
Maleic acid	0.90	0.64
Malonic acid	75.07	82.05
Citric acid	11.73	12.93
Lactic acid	13.67	23.70
C) Sugar (average of 4 palms) :		
Sucrose	135.50	128.30
Glucose	25.50	39.40
Galactose	18.10	28.90
Mannose	11.50	9.50
Lactose	16.30	14.70
Raffinose	20.40	17.50

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