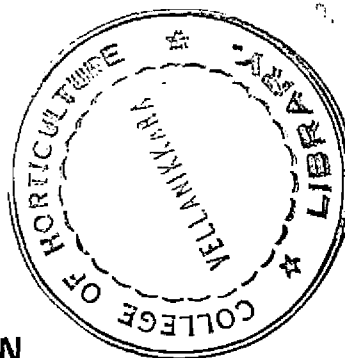


**PHYSIOLOGICAL AND BIOCHEMICAL BASIS  
OF DEGENERATION IN ROOT (WILT)  
AFFECTED COCONUT PALM**

BY  
**SHEELA CHERIAN**



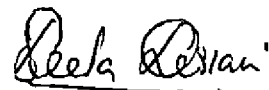
THESIS  
SUBMITTED IN PARTIAL FULFILMENT OF  
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DEPARTMENT OF AGRICULTURAL BOTANY  
PLANT PHYSIOLOGY SECTION  
COLLEGE OF AGRICULTURE  
VELLAYANI, TRIVANDRUM

1983

## DECLARATION

I hereby declare that this thesis entitled,  
"PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF DEGENERATION  
IN ROOT (WILT) AFFECTED COCONUT PALM" is a bonafide  
record of research work done by me during the course  
of research and that the thesis has not previously  
formed the basis for the award to me of any degree,  
diploma, associateship, fellowship or other similar  
title, of any University or Society.



(SHEELA CHERIAN)

Vellayani,  
18 May 1984.  
~~18 December 1983.~~

C E R T I F I C A T E

Certified that this thesis entitled,  
 "PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF DEGENERATION  
 IN ROOT ( WILT) AFFECTED COCONUT PALM" is a record of  
 research work done independently by Smt. SHEELA CHERIAN  
 under my guidance and supervision and that it has not  
 previously formed the basis for the award of any degree,  
 fellowship or associateship to her.



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## INTRODUCTION

## INTRODUCTION

The coconut palm (*Cocos nucifera* L.) family *Palmaceae* is an important and extensively grown crop of Kerala. By virtue of its unique position in the day to day life of the people, it has earned the name 'Kalpavriksha' the tree of heaven and Burkhill (1966) rightly described it as one of nature's greatest gifts to mankind. The importance of the palm lies in the fact that it supplies food, drink and shelter and it also provides raw materials for a number of industries.

Coconut occupies about 32 per cent of the area under cultivation in Kerala. Thirty six per cent of the State's agricultural income and approximately 60 per cent of the revenue is derived from coconut and related industries. The coconut industry is intimately connected with the economic and domestic life of the inhabitants of the coconut growing countries. This extreme dependence has been described by Pieris (1954) that human life would become intolerable if there were no coconut palms.

Burkhill (1935) pointed out that the tree demanded certain conditions for its growth viz. aeration of the root provided by the movement of air through the sandy soil provided by rising and falling tides, moisture at the crown afforded by the sea breeze and nearness to water or sea for its effective dispersal.

According to Mayuranathan (1938) its high degree of tolerance to salinity and saline moisture and adaptability for dispersal make it a sea side plant.

Blatter (1926) indicated that wherever the coconut ventured beyond the limits of the tropics it lost its elegance, productivity, sickened off and died after 8 or 10 years. In support of this he pointed out that in the Sandwich Islands at the edge of the Torrid zone, the coconut had a mean look. He therefore, stated that the plant can be grown successfully only in the tropics at low and medium elevations and as evidenced by its distribution confined essentially to the coastal areas of the tropics.

Despite its hardy nature, coconut palm is prone to several diseases all over the world and for many the etiology is still obscure. Root (wilt) disease is one,

which is causing great havoc to the plantations in Kerala.

The root (wilt) disease of coconut palm was found in some form and reported from areas now in Kerala since many decades. Bulter (1908) referred to the malady as the root disease probably because root rotting was recorded as the prominent symptom of the diseased palms. A later terminology referred to the disease as 'wilt disease' as the visible symptom was flaccidity of the foliage. But Mathen, Gopinathan Pillai and Radha (1976) after examining the various suggestions made a compromise and referred the disease as coconut root (wilt) disease until such time that an alternate name can justifiably replace it.

#### Symptomatology, spread and extent of damage

Many attempts have been made to depict the symptom picture of the root (wilt) disease. (Menon and Nair (1951), Menon (1963); Lal (1969); Radha and Lal (1972); Radha (1974) and George Tharakan (unpublished). The characteristic symptoms of the disease are general wilting of leaves from the middle whorl outwards followed by yellowing and marginal necrosis of leaflets.

The abnormal bending or ribbing of leaflets termed as flaccidity is a typical feature of the disease. In young palms this is most often the only symptom whereas in adult palms foliar yellowing and necrosis are also associated.

Symptoms may appear singly or in combination with general chlorosis of the leaflets. Growth rate is retarded and the number and length of leaves and leaflets are reduced. Leaf production stops and a few withered leaves linger on the palm. Premature nut fall and gradual decline of yield are other common characteristics of the disease. The number of nuts and the quality of endosperm produced are reduced, the endosperm progressively disintegrates and finally nut production dwindles out. Rotting of root tips is common and decaying starts from tip backwards. The vascular tissues of the root of the diseased palms show disintegration and later root production is completely curtailed. A high percentage of the pollen produced is sterile. The affected palms live with the disease for 15 to 20 years or more depending on management practices. The tapering, thin trunks with a small tuft like crown abound in the affected areas.

In most cases root (wilt) diseased palms are affected by leaf rot also.

The century old root(wilt) disease of coconut is spreading slowly and steadily in all directions. The disease originally reported in 1882 at Erattupetta is now established almost in a stretch of eight districts viz. Triyandrum, Alleppey, Quilon, Pathanamthitta, Kottayam, Idikki, Ernakulam and Trichur. The incidence of the disease in more than 30 per cent of the 750,000 ha under coconut in Kerala is causing an estimated annual loss of about 340 million nuts.

Gopinathan Pillai et al. (1973) indicated that the disease was prevalent in all the major soil types in Kerala state and the spread of the disease was observed to be fast in light textured, sandy loam and alluvial soils as well as in heavy textured clayey soils than in the laterites. The incidence was relatively higher in waterlogged, low lying areas adjacent to rivers and canals. In 'Kari' soils too the disease incidence was heavy. Varghese (1934); Menon and Nair (1951); Pillai and Pushpadas (1966) suggested the possible involvement of surface and sub-surface soil

moisture in the rapid spread of the disease. Subsequent investigations by Gopinathan Pillai (1976) support the view that areas close to river banks and lying near canals are invariably infected heavily by the disease. Some interesting observations were the occurrence of healthy pockets surrounded by diseased tracts and presence of apparently healthy palms in the midst of palms in the advanced stages of disease.

The disease is causing a severe drain on the economy of the State. It is estimated to cause an annual loss of Rs.500/- million. The area under coconut cultivation has increased two to three fold from 1956-57 to 1970-71 at the rate of 3.5 per cent per year. The average yield of a palm is reduced from 35 to 25 nuts per tree per year due to root (wilt) disease.

The present study aims at providing a possible explanation for the pattern of deterioration of the root (wilt) affected palms, as the symptoms clearly point to widespread aberrations in the physiological processes and functions. These changes are also the result of many interactions in the different centres

of growth. Accumulation of free amino acids especially aspartic acid, serine, glycine etc. in tender leaves as the disease advances to the middle stages when all the typical symptoms are fully manifest on the foliage indicate that protein synthesis is affected (Pillai and Shanta, 1965). There is evidence that roots are sites of synthesis and interconversion of cytokinins and gibberellins respectively. (Jones and Phillips, 1966); (Crozier and Reid, 1971). Root system plays an important role in the overall hormonal physiology of the plant. Since the roots especially root tips are severely affected in this syndrome, the synthesis supply and interconversion of cytokinins and gibberellins to the top are in disarray and hence a reduction in all phases of leaf growth and functioning. Further the reduced root growth drastically affect the absorption of water and nutrients and these factors also affect metabolism and growth. When leaf growth is limited photosynthesis is very much curtailed and supply of photosynthates and other essential metabolites including growth regulating substance to the plant as a whole and specifically to the roots is severely reduced. This brings about a further reduction in the overall growth processes

and particularly root growth and development creating a vicious cycle of cause and effect. The symptoms further aggravate and the lanky trunk with a degenerated crown is the final result. These various physiological stresses could also result in an accumulation of abscissic acid.

An earlier study in this laboratory (unpublished) has shown that considerable changes occur in the content of cytokinins, proteins, non-protein, amino acids and chlorophyll between healthy and wilt disease infected palms. So in the present study estimation of gibberellins and abscissic acid from roots, leaves and developing nuts of healthy, apparently healthy and root (wilt) affected coconut palms at various intensities is included. The data were statistically analysed and discussed. The results of this investigation may lead to the formulation of effective methods for the control of root (wilt) disease or its predisposing factors.

## **REVIEW OF LITERATURE**

## REVIEW OF LITERATURE

The following review highlights some of the relevant work done on physiological, biochemical and anatomical aspects of the root (wilt) disease.

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. He concluded that the disease was hereditary and spread from tree to tree. Its rapid and free spread must be due to some predisposing factors weakening the trees attacked, either a want of proper mineral constituents in the soil or too stiff and damp a soil from which there is no proper drainage and through which the roots cannot properly penetrate. 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 the disease is not physiological but fungi, virus and certain toxic substances produced by microorganisms living around the root zone of the palms contribute to produce the symptoms. However, in the absence of a definite proof for any biological agent being the cause of the disease, physiological investigations assumed importance and were intensified from 1951.

#### 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. Ramadasan (1964) reported that the root sap of diseased palms contained 65 to 72 per cent more total solids than that of healthy palms.

Though a method was standardised for collection of root exudate from coconut palms (Ramadasan, Radha and Michael, 1967) no authentic information is available on the nature and composition of the root exudate 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.

#### 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 per cent more during summer and 9 per cent more during rainy season than from healthy samples. This increase from diseased roots was 22 per cent during summer and 8.2 per cent 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 transport of water through the trunk in diseased palms was found to be 35 per cent 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<sup>2</sup>/min) 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. 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, 1981).

### Mineral Nutrition

Yellowing of outer whorl 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. Pandarai (1959) 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 per cent  $\text{MgSO}_4$  given as foliar spray at quarterly intervals completely cured yellowing in 16 per cent of palms while reduction in yellowing was noted in another 60 per cent. K/Mg ratio in the leaves during the pretreatment period ranged from 3.1 - 5.1 while at the end of the treatment period it ranged from 2.1 - 3.1 (Chacko and Ramadasan 1967). In a recent study Varkey, Kamalakshyamma, Ramanandan and Nambiar (1979) observed that foliar sprays with 2 per cent  $\text{MgSO}_4$  was effective in checking foliar yellowing both in the healthy and diseased area. They have further noted that basal application of  $\text{MgSO}_4$  (500 gm/palm/year) and foliar

spray with 1 per cent  $\text{FeSO}_4$  also checked foliar yellowing to a considerable extent.

#### Respiration and Photosynthesis

Michael (1978) determined the rate of respiration in healthy and root (wilt) infected palms. It was significantly higher in diseased palms than in healthy. The increase in respiratory rates of the leaves of diseased palms was 18-21 per cent in the advanced stages and 2-5 per cent in the early stages of the disease. The roots of palms in the advanced and early stages of the disease recorded 14.3 per cent and 11.4 per cent more respiration over that of apparently healthy palms. The respiratory rates differed markedly between healthy and apparently healthy palms also.

Shanta, Thommen and Menon (1959) noticed that the chloroplastids are highly affected by the disease, the disintegration and degeneration of which result in the partial or complete disappearance of chlorophyll. Chacko Mathew and Ramadasan (1968) also observed a reduction in the chlorophyll content in the diseased

palms when compared to the healthy ones. The effect of root (wilt) was evident from the marked variation between healthy and diseased palms in photosynthetic  $\text{CO}_2$  fixation. The leaves of the diseased palms fixed 11.70 per cent less  $\text{CO}_2$  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  $\text{CO}_2$  fixation (Dwivedi, Chacko Mathew, Michael, Ray and Sumathykutty Amma, 1978).

#### Anatomical studies

There is much controversy regarding the extent of damage in roots. Varghese (1934) reported severe rotting of root cortex which led to temporary starvation of the palm. The roots actually rot only when in contact with subsoil water and only some roots become functionless. Menon and Nair (1949) reported that during the early stage of the disease with visible external symptoms, rotting was confined to the tip of a small number of roots. Nagaraj and Menon (1955) reported that there was no difference in root damage between apparently healthy and early diseased palms.

Abnormal shedding of female flowers and immature nuts was noticed in the diseased plants (Menon and Pandalai, 1958). Shanta, Thommen and Menon (1959) reported degeneration of chlorophyll in diseased palms as the reason for chlorosis.

Varkey and Davis (1960) reported that a higher percentage of pollen was sterile. The percentage of germination of the pollen dwindles as the disease aggravates. Radha, Sahasranaman and Menon (1962) reported that the yield of nuts was reduced from 43-80 per cent, in root (wilt) affected palms depending on intensity of the disease. Joseph and Shanta (1964) reported a reduction in wall thickness of sclerenchymatous tissue in the leaf and increase in the number of upper epidermal cells, both of which may be contributory to the development of flaccidity. Thomas Joseph and Shanta (1964) investigated the anatomy of tender unopened leaves of healthy and root (wilt) affected palms. According to them the stomata on the abaxial side of leaflets are slightly narrower in diseased than in healthy palms which correspond with the general stunting of the epidermal cells. This resulted in higher percentage distribution of stomata per unit area, in the leaflets of

diseased palms.

Michael (1964) reported that the diseased palms were found to give considerably lower values for all the characters studied like number, thickness, colour etc. of the main roots and number of rootlets and breathing roots. Percentage of dead roots is greater in the diseased palms. Quantitative characters of the shoot length and circumference of the bole were also low in diseased palms. Indira and Ramadasan (1968) observed internal browning of vascular elements extending to the cortex and sometimes accompanied by disintegration of vascular elements also in 60 per cent of the roots of diseased palms while mild internal browning of tissues was observed in 33 per cent of the roots of apparently healthy palms growing in diseased soil. Development of tyloses was also noted in the vessels of majority of roots of diseased palms examined. None of the above derangement were noticed in the root tissue of healthy palms examined. Govindankutty and Vellaichamy (1976) observed the development of tyloses in xylem vessels and degenerate phloem in roots from diseased palms as characteristic of the disease. Over 60 per cent of the externally healthy roots from diseased palms had

internal browning (Anonymous, 1976). Further investigations showed that roots from palms of healthy and diseased categories failed to develop the discolouration in the presence of antioxidants (Dwivedi, Potty, Amma, Govindankutty, Solomon and Jayasankar; 1978). They observed that vascular browning does not exist in situ in externally healthy roots of diseased palms to any significant level. Govindankutty (1979) reported the presence of fungal hyphae and tyloses in xylem vessels, necrosis of phloem tissues and burrowing nematode lesions in the cortex of disease affected palms. Root tissues of palms not manifesting foliar symptoms were also found to be damaged in some cases. The disease affected palms had narrower and thinner leaflets. The phloem was degenerated in certain vascular bundles and leaflets.

#### Other metabolic disorders

Gross derangement in the path of N resulting in considerable increase in non-protein N content, concomittant with a sharp decrease in the water soluble N and protein N fractions was observed in the diseased leaf tissue. The total and organic P were found to be

higher in diseased palms than in healthy. A reduction in the C/N ratio of the roots and leaves of diseased palms was also noticed ( Varkey et al. 1969).

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). Michael, Thomas and Rajan (1978) reported an increased quantity of sucrose ( 2.2 per cent) compared to the apparently healthy palms ( 1.91 per cent). Starch content was high in healthy palms (16.02 per cent) compared to diseased (13.13 per cent).

#### 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 acid predisposes the wilt infected palms to 'leaf rot'. The quantity of amino acid 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 the 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).

Joseph and Jayasankar (1973) estimated total phenol in roots collected from healthy, apparently healthy and root (wilt) affected (both early and advanced) palms. The highest concentration of polyphenols was recorded in samples of roots collected from healthy palms. With the incidence and increase in intensity of disease, there was a fall in the concentration of total phenols. Tanin 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 significant (Chacko Mathew and Joseph, 1974). Joseph, Potty and Jayasankar (1976) reported that in root (wilt) affected tracts the level of polyphenol-

oxidase and peroxidase increased in the roots with an increase in the intensity of the disease. The correlation between concentration of polyphenol oxidase and root (wilt) index formulated on the basis of foliar symptom was positive.

The activity of carbonic anhydrase was low in the leaves of diseased palms as compared to that in healthy palms ( Dwivedi, Ray, Sumathykutty Amma et al. 1978). This indicated reduced concentration of biologically active zinc in diseased palms. In another experiment a positive correlation was indicated between carbonic anhydrase activity and yielding potential of palms. The activity of carbonic anhydrase progressively increased with the maturity of buttons.

Though coconut palm is the most important and extensively grown crop of Kerala and root (wilt) is the most destructive disease associated with it, except some elaborate work on cytokinins from this laboratory by George Tharakan (unpublished) no work is reported in the literature on the involvement of growth regulators in this disease.

The gibberellins are detected in a wide range of plants and are present in bacteria, fungi, algae, mosses, ferns and higher plants. Already about 58 of them have been detected and identified. No single plant contains all of them. Many contain more than one.  $GA_1$ ,  $GA_3$  and  $GA_4$  are the most widespread. Gibberellins can be detected in all plant parts, in germinating seeds, cereal stems, roots, leaves and fruits. Jones and Phillips (1966) reported that the leaf primordia rather than the stem apex is the main site of synthesis of gibberellins. Leaf primordia continues to produce gibberellins throughout its period of cell division. Carr et al. (1964) pointed out that the root system is a major site of gibberellin synthesis. Synthesis occurs in embryos, roots, seeds and also tubers. Crozier and Reid (1970, 71) studied the effect of root apex removal on the gibberellin content of leaves, stems and cotyledons of light grown phaseolous seedlings. They found that  $GA_1$  was the main gibberellins present in control plant and that root apex removal resulted in the disappearance of  $GA_1$  but observed accumulation of  $GA_{19}$  in the remaining leaves, apical buds and sub-apical root parts. The  $GA_1$  present in the cotyledons was unaffected by the root apex removal. They concluded

that  $GA_{19}$  is apparently synthesised in the shoot and subsequently transported to the roots to be converted to  $GA_1$ .

Jacobs and Pruetz (1973) demonstrated the basipetal polar movement of gibberellins in zea roots which is opposite to the polarity of auxin transport. A similar movement of gibberellins was shown in Phaseolus coccineus root segment by Hartung and Phillips (1974). However, they found that the polarity of the transport was restricted to the stele and absent in the cortical tissues. Gibberellin like substances occur in the sieve tube of several plants which indicate phloem transport of gibberellins. Xylem transport of gibberellin like substances has been deduced from the presence of gibberellin like substances in the xylem exudate from a number of plants. Zweig et al. (1961) using  $^{14}C$ - gibberellins provided evidence for phloem as well as xylem transport of gibberellins in beans and for phloem transport in corn. Gibberellin like substances are shown to be present in the bleeding sap of tomatoes. It is demonstrated that these substances can promote stem elongation in intact tomato plants. Waterlogging of the root system

inhibits its growth and severely reduces the export of gibberellins from the root to the shoot xylem sap. It is suggested that decline in growth rate following waterlogging may in part be due to the reduction in the supply of gibberellins to the shoot (Reid and Crozier 1969).

Phillips (1964a) reported that the root system serve as a centre for the oxidative inactivation of shoot synthesised auxin, thus regulating shoot auxin level. He observed that an adequately aerated root system supplies some growth promoting and hyponasty inducing principle to the shoot. The effects of this principle were found to be similar to gibberellins. So an early effect of flooding on plants would be to increase shoot auxin level and also to stop the synthesis of non-auxin shoot growth hormone in the roots. The induction of wilting of the shoot by flooding was found to be correlated with low root temperature.

Exogenous application of  $GA_3$  showed a rapid increase in respiration, photosynthesis and transpiration after one or two hours of application. A maximum rate was reached five or six hours after spraying and was followed by a rapid decline. Except for transpiration,

which returned to its initial rate, the respiration and photosynthesis of the treated plants remained higher than those of the checks (Coulombe and Paguin (1959) Lambeth and Das (1965) observed an increase in the top, root ratio and reduced root growth by an exogenous application of  $GA_3$  at 200 ppm. Apical dominance was greatly enhanced with an increase in the top : root ratio.  $GA_3$  also increased cation exchange capacity per unit root weight of 40 day old beans. Mc Comb (1966) reported that  $GA_3$  brought about an increase in the rate of dry weight incorporation into expanding internodes of intact dwarf pea seedlings. The effect of gibberellins upon internode expansion is mediated through the change in dry weight. Part of the increase in dry weight brought about by gibberellins was attributed to an increase in the weight of cell wall material. It is possible that the increased rate of wall synthesis which follows gibberellin treatment allows an increase in the rate of cell expansion.

Application of  $GA_3$  at various stages of bloom resulted in much higher than normal per cent fruit set and large increases in yield in Cranberries. Application of  $GA_3$  also caused abnormal elongation of the vines

and inhibited terminal bud development (Robert, Devlin and Demoranville, 1967).  $GA_3$  treatment of one-month old seedlings of some tree species increased the fresh and dry weights of both the shoots and roots. The increase in dry weight of the shoots were proportional to increase in fresh weight. With some species however increase in shoot weight were indirectly associated with decrease in root weight (Shanmughavelu, 1967). Fletcher, Oegema and Horton (1969) reported that the level of endogenous gibberellins in leaf tissue of Taraxacum officinalis was high during leaf growth and expansion but declined progressively during leaf senescence. Chin and Beevers (1970) noticed a progressive decline in gibberellin like components and an increase in ABA like components. Pretreatment of detached leaves with  $GA_3$  or kinetin prevented changes in the levels of endogenous growth regulators and delayed senescence.

In plants of winter pea, total contents of free gibberellin like substances at the vegetative growth stage were 1.50 mg equivalent  $GA/100$  gm fresh weight in the leaves, 1.60 mg in the stem and 1.11 mg in the roots and bound gibberellin like substances 1.2, 0.82

and 0.75 mg respectively. At flowering gibberellin like substance contents decreased in the leaves and roots and slightly increased in the stems, whereas their contents were high in the tendrils, 7.83 mg and very high in the flowers 15.39 mg. At the seed formation stage GA like substance decreased except in the leaves where they reached 1.37 mg of free gibberellin like substance and 2.38 mg of bound gibberellin like substance. Different fractions of gibberellin like substances detected often showed some specificity to plant parts. RF 0.9 fraction was predominantly connected with bound GA like substance and 0.3 - 0.7 with free GA like substance (Banishevskaya et al. 1976).

Taylor and Railton (1977) reported that wilting of etiolated, dwarf pea seedlings resulted in significant reduction in the conversion of bioactive gibberellin  $A_{20}$  into its less biologically active derivative, gibberellin  $A_{29}$ . Increasing the degree of wilting resulted in further reduction in the amount of conversion. Aharoni and Richmond (1978) noticed a sharp decline in gibberellins accompanied by a moderate rise in ABA before the onset of chlorophyll degradation in detached lettuce leaves. As senescence advanced no gibberellins could

be detected and a considerable rise in ABA was observed. They concluded that the senescence in detached lettuce leaves is connected with a depletion of free gibberellins and cytokinins, which is thereafter followed by a great surge in ABA.

Williams and Mallorca (1982) investigated the content of endogenous gibberellin like substance in roots and root nodules of soyabean by a combined TLC-dwarf pea epicotyl bioassay technique. Gibberellins were more concentrated in root nodules than in the roots totalling 1.34 and 0.16 nm  $GA_3$  equivalent/gm dry weight respectively. Comparison of the gibberellin components of plants and bacterial culture medium extracts suggested that rhizobial gibberellin production may contribute to nodule gibberellin content.

The root apex is the major site for the synthesis of gibberellins and cytokinins and when this region of the root is subjected to anaerobic conditions their synthesis and translocation will be reduced. Many of the symptoms of flooding injury seen in shoots are growth effects which are characteristic of hormone imbalance. Phillips (1964b) speculated that reduced growth rate of flooded plants could be due to a substance

inhibiting gibberellin synthesis in the roots. Reid et al. (1969) examined the gibberellin content of the xylem sap of tomato plants before and after waterlogging of the root system. After one day there was a 40 fold reduction in the level of acidic GA like activity.

By the third day there was no detectable activity. Reid and Crozier (1971) followed the changes in the gibberellin content of roots and shoots during waterlogging. In the shoots the gibberellin level declined six fold on the first day and a further four fold on the second day. However, on the third day the gibberellin content of the roots increased but this increase coincided with the appearance of adventitious roots, so the extra gibberellin activity may have originated from there.

Endogenous gibberellin activity declined rapidly in stressed lettuce leaves and rose again during a subsequent recovery period (Aharoni et al. 1977). The fall in GA like activity preceded the rise in ABA content of the same tissue. Gibberellins are known to be synthesised in roots as well as leaves (Torrey 1976) but effect of water stress on sites of synthesis and transport of gibberellins have not been studied.

It is strange that so little attention has been directed during the past 45 years to the study of gibberellins in relation to plant diseases. Most of the effort appear to have been in the direction of exogenous application of gibberellins in order to relieve symptoms of individual diseases and this approach has not provided any answers to basic problems concerning the role of gibberellins in plant diseases (Leuis Sequeira, 1973).

One of the first studies on gibberellins from infected plant tissues was that of Radely (1961) who showed that ethanol extracts of root nodules from pea and bean contain substantially greater amounts of gibberellin like substances than those from normal root extracts. Dullaart and Duba (1970) have confirmed and expanded these observations on the basis of lettuce hypocotyl and barley endosperm bioassay and they reported that the nodule tissue of Lupinus luteus contains as high as 3340 mg GA<sub>3</sub> equivalent/kg fresh weight whereas gibberellins were very low or undetectable in the normal roots. The variability in the activity of extracts from nodule tissues was very high however.

Plants of the creeping thistle systemically infected with the rust fungus Puccinia punctiformis undergo very striking morphological changes such as longer internodes, incurled leaf margins, reduced leaf area and lack of inflorescence. Bailiss and Wilson (1967) determined that systemic infection caused a four fold increase in stem length as compared with healthy plants. Based on results of lettuce hypocotyl assay they observed higher levels of gibberellin like substance in infected roots which closely paralleled the measured growth increase. In the early stage of infection most of the activity was due to gibberellins that separated at the Rf of GA<sub>1</sub> or GA<sub>2</sub> but as shoots became more mature, a second zone of activity appeared at the Rf of GA<sub>3</sub>. They concluded that the constantly higher level of gibberellins in diseased shoots was always correlated with the period when they grow faster than the healthy shoots.

Evans and Wilson (1971) made a comparable study to that of Bailiss and Wilson involving an obligate parasite with a stunting syndrome. The infection which is systemic and perennial induces an overall dwarfing of the plant. Lettuce hypocotyl bioassays of extracts of healthy and

infected plants three, five and seven weeks after emergence of shoots showed at all times a reduction in the level of a single major gibberellin like substance corresponding to  $GA_3$  which closely paralleled the reduced growth of the infected plant.

Russel and Kimmins (1971) noticed a decline in a gibberellin like substance corresponding to  $GA_3$  in barley yellow dwarf virus infected plants. Application of  $GA_3$  to infected plants reversed the dwarfing by increasing cell elongation rather than cell division. This effect however, is by no means universal as Yerkes (1960) Stein (1962) and Chant et al. (1963) have shown that  $GA_3$  virus infected plants are always smaller than healthy controls.

Budagyan et al. (1964) working on tobacco found no difference in gibberellin level in tobacco mosaic virus infected plants where stunting was not a main feature of the disease. Bailiss (1968) found only slight difference in gibberellin like substance levels in tomato plants infected with aspermy virus and incomplete reversal by exogenous application of  $GA_3$ . In another study Bailiss (1974) presents clear evidence for a reduction in gibberellins probably  $GA_1$  and  $GA_3$  in

cucumber mosaic virus infected cucumber plants which was correlated with a reduction in root, shoot and leaf growth and net assimilation rate.

Abscissic acid is the most important growth inhibitor detected in a wide range of plants. Higher plants were found to contain abscissic acid in their leaves, fruits, seeds, buds, roots and stems. It appears that chloroplastids are the sites of its synthesis (Loveys 1977). Although abscissic acid is a potential inhibitor of growth, it has also a variety of effects on plant metabolism. Abscissic acid promotes senescence and abscission, initiates and prolongs dormancy and inhibits growth (Addicot, 1970). Inhibition of the synthesis of  $\alpha$ -amylase and of protease by ABA has been demonstrated by rigorous methods; inhibition of the development of activity of a number of other enzymes of germination and growth has also been shown. Promotion of the synthesis of cellulose by ABA has been demonstrated as well as the development of phenyl alanine, ammonia lyase, invertase and ribonuclease. It is probable that at least the development of other enzymes of senescence and abscission is promoted by ABA. The synthesis and composition of several RNA fractions

are especially sensitive to ABA and would appear to account for the altered enzyme patterns induced by ABA.

One of the most striking effects of ABA is its ability to cause the closure of stomata and this function is associated with a rapid 40 fold increase in the ABA content of the leaves, which occur when they wilt or suffer from waterlogging or chilling. Goto (1978) showed that the exogenous  $GA_3$  treatment caused some inhibitory activities to decrease, in the dwarf and tall cultivars of beans and it was thought to involve ABA.

Karmoker and Van Stevenick (1979) reported that ABA increased the accumulation of chlorine and sodium in roots of intact pea seedlings. After an initial promotion, ABA inhibited long distance transport of these ions from the root to the shoot. It consistently inhibited both uptake and transport of potassium in intact bean seedlings.

Guan et al. (1981) reported that in detached rice seedling leaves, ABA accelerated the increase in chlorophyll and protein contents, increased activities of ribonuclease and acid phosphatase and markedly

increased the activity of cellulase. They concluded that the increase in hydrolytic enzyme activities increased the rate of senescence. Biddington and Dearman (1982) reported that addition of ABA to the nutrient solution increased the root to shoot ratio of hydroponically grown cauliflower plants by reducing the dry weight of the shoot and increasing that of the root. At concentrations higher than  $10^{-7}$ M, ABA increased root branching and root hair formation. Root extension was inhibited in plants kept continuously in solutions containing high ABA concentration but following removal from the ABA solutions, root elongation was increased in comparison with plants given no ABA treatment.

When plants are subjected to various forms of stress such as drought, salinity or flooding their endogenous ABA levels undergo rapid and marked changes. Water stress occur in plants when their cells and tissues are less than fully turgid. Such a situation develop when transpiration from the plant surface exceeds the rate of water absorption from the roots and the plants wilt. When plants wilt they accumulate ABA rapidly in their roots. Wright (1969) reported an increase in 'inhibitor  $\beta$ '

content of detached wheat leaves following a period of wilting. The amount of inhibitor accumulating in the wheat leaves was dependant on the length of the period in the wilted condition, the temperature of the wilted leaves and the degree of leaf water deficit. The greater the degree of wilting (ie. upto about 9 per cent loss in fresh weight) the greater the eventual 'inhibitor  $\beta$ ' content.

Tal and Imber (1970) noticed an abnormal stomatal behaviour by hormonal imbalance in flacca, a wilted mutant of tomato. The mutant wilts fast under water deficit because of inability to close its stomata. A much lower amount of substance with ABA like activity was noticed in the mutant compared with the normal plant. The concentration of ABA like substances increased with age in both genotypes. Leaf water deficit induced in plants by withholding water, waterlogging etc. stimulated many fold increases in ABA. The greater the degree of wilting and the longer the duration, the greater the ABA accumulation (Wright and Hiron 1970). Plants wilted by withholding water soon recovered full turgor upon rewatering but the high ABA levels did not return to normal for several days.

Tobacco plants when held for 24 hrs under either high or low relative humidities with or without salts, the amount of ABA in the leaves of salinized plants rose markedly in low, but not in high relative humidity (Mizrahi et al. 1971).

Most (1971) extracted ABA from immature leaf and stem tissue of sugarcane. Mature leaves of well watered plants contained trace quantities of this hormone but plants subjected to drought accumulated relatively large amounts in their mature leaves. Extracts from wilting leaves contained a substance similar to or identical with (+) phaseic acid.

When water was withheld from spinach plants until wilting symptoms were clearly visible the ABA content of the leaves increased more than 10 fold over that of turgid plants (Zeevaart, 1971). In Brussels sprout seedlings (Brassica oleraceae) induced to wilt by withholding water for 66 hrs, the ABA content of the shoots increased 52 fold (Wright, 1972) Similar rise in ABA content on wilting of whole plants have been found in the leaves or leafy shoots of dwarf and tall peas (Simpson and Saunders 1972) dwarf beans (Wright and

Hiron 1972), tomato (Wright 1972) avacado (Milborrow and Robinson 1973), Grapevine, maize etc. It is proposed that ABA may begin to accumulate in plants when leaf water potential decreases - 2 bars beyond the normal day time leaf water potential. Leaf water deficit induced by warm air treatment caused an increase in ABA level in dwarf pea seedlings. A similar increase in level of ABA in leaves occurred in tomato, dwarf bean and wheat plants when they are flooded. In rice, a sp. adapted to a flooded environment, seedlings showed no increase in ABA level as a result of flooding (Hiron and Wright 1973). Hoad (1973) reported an increase in ABA in stem apices attached to ricinus plants during a moisture stress but only slightly in stem apices which were detached and then allowed to lose water. A very marked rise in ABA also occurred in phloem sap of moisture stressed ricinus plants. The results suggested that in apices of moisture stressed plants the increased ABA does not arise in situ but is translocated to the apex after synthesis in the mature leaves. Milborrow and Robinson (1973) reported that the increase in ABA concentration on wilting of avacado leaves and stems occurs by synthesis of ABA rather than by release from a stored

precursor. Submerged ceratophyllous plants and submerged plants of callitriche show a two fold increase in ABA content on wilting whereas the aerial rosette of the latter plant show a six fold increase. Hence they concluded that the occurrence of the 'wilting' induced mechanism is affected by previous growth conditions as well as by the morphology of the tissue.

When pea seedlings lose about 5 per cent of their water content the ABA level of the shoots increased about 20 times and bound ABA content decreased within 24-48 hrs to the level in the control plants (Dorffling et al. 1974). When water was withheld from tomato plants the most rapid rate of increase in ABA occurred in the 24 hr period prior to the appearance of wilting symptoms. Once wilting had set in, the rate declined but this might be due to leaf damage as a result of the increasing stress or to an optimal leaf water deficit for the accumulation of ABA occurring before or near the point of visual wilting. Milborrow (1974) proposed three switching mechanisms operating the stress ABA syndrome. (a) a rapid synthesis of ABA triggered on by wilting (b) a stop message when sufficient ABA has been formed and (c) the commencement of the destruction or cessation of rapid synthesis when

turgour is regained. Zabadal (1974) reported a relationship between ABA concentration and leaf water status. Water potentials were measured in leaves of two species of Ambrosia throughout a period of dehydration of intact plants. In both species ABA began to increase in a critical water potential range (-10 to -12 atm). These data suggest a threshold water potential that stimulates ABA synthesis.

Chen and Itai (1975) investigated the role of ABA in the regulation of senescence in detached tobacco leaves. Leaves senesced in darkness showed a sharp rise in ABA level in the early stage of aging followed by a rapid decline later. The same trend was noticed when leaves were aged in light but the rise in ABA occurred four days later than in darkness. Senescence was slower in light than in darkness while salt stress accelerated the process.

Harrison and Walton (1975) estimated the endogenous ABA level in bean leaves and found that they rose from 0.04  $\mu\text{g/g}$  to approximately 0.5  $\mu\text{g/g}$  fresh weight within four hours after the beginning of a 10 per cent wilt and remained at that level during a subsequent 20 hrs of wilt. In wilted leaves an elevated level of ABA is maintained

## MATERIALS AND METHODS

Coconut palms for the experiment were selected from different areas. Healthy, apparently healthy and root (wilt) affected palms were used as plant material in the present study.

The healthy palms (disease free) were selected from the Instructional Farm of the College of Agriculture, Vellayani which is in the disease free area. Apparently healthy and root (wilt) affected palms were selected from the Regional Agronomic Research Station, Kumarakom. The disease free palms selected from RARS are characterised as apparently healthy. The root (wilt) affected palms were selected on the basis of disease intensity (George and Radha 1973) and at five different intensities of the disease were selected for the study. The different intensity groups are (1) 50 and above (2) 40 ie. between 35 and 45 (3) 30 ie. between 25 and 35 (4) 20 ie. between 15 and 25 (5) 10 ie. between 5 and 15. Below 5 it is almost apparently healthy.

because the rate of synthesis and metabolism are both elevated and approximately equal. Hoad (1975) reported an increase in the level of ABA present in xylem exudate of osmotically stressed sunflower plants. When plants were subjected to increasing soil water stress, increase in stomatal resistance occurred first on the lower leaves and the stomata on the upper surface was the most sensitive to decreasing leaf water potential. Stomatal closure proceeded from the oldest leaves to the youngest as the stress became more severe. Increase in both free and bound forms of ABA were observed in water stressed plants but the largest accumulation occurred in the youngest leaves (Jordan et al. 1975).

Water potentials, stomatal diffusion resistance and ABA levels were measured in the leaves of maize and sorghum plants subjected to drought recovery cycle under controlled environmental conditions. The levels of ABA began to rise and the stomata closed over a narrow range of water potential ( -8 to -10 bars) in both sp. ABA levels continued to rise after the stomata closed. The maximum amount of ABA extracted from maize leaves was about twice that from sorghum but this represented a similar proportional increase over the control level (Beardsell and Cohen, 1975).

Alvin et al. (1976) noticed that the growth of willow tree during spring was preceeded by decreasing levels of ABA and an increase in cytokinin activity in the xylem sap. The onset of dormancy was associated with low levels of cytokinin and high contents of ABA. Boussiha and Richmond (1976) reported that the ABA content and the extent of stomatal opening in the leaves of prestressed tobacco plants were found to be inversely related.

ABA levels in roots of bean plants exposed to a -4 bar stress in the root medium increased about 10 fold within an hour and 16 fold by the end of the second hour. A similar increase in ABA levels in roots of pea and sunflower was also reported (Daniel et al. 1976). Leaves from rapidly wilted intact plants of tomato showed a rapid increase in ABA level, whereas the concentration of ABA was not higher in the leaves of plants adapted to water stress (Rasmussen, 1976).

Walton et al. (1976) reported that like leaves, roots are also able to synthesize ABA atleast in small amounts during water stress. During progressive stages of drought and subsequent recovery through rewatering, foliage of two-year old seedlings of Douglas fir showed a rapid rise in

stomatal resistance and ABA content corresponding to a plant water potential of -10 to -12 bars (Blake and Ferrell 1977). Michael and Hanny (1977) noticed the same effect in water stressed cotton leaves.

Bengton et al. (1978). reported that during stress period both ABA and proline accumulated in the leaves of dwarf grown wheat seedlings. The content of ABA increased upto six times the normal level during water stress lasting for 20 hours. The increase of proline was about three fold for similar treatment after rewatering the leaves, the levels of both ABA and proline rapidly declined and reached 10 hours later, the levels found in unstressed seedlings.

Water stress caused an increase in ABA level in leaf, seed and pod tissues and phloem exudate of white lupin and the concentration of ABA in the sieve tube sap was very high (Hoad 1978). The ABA content of water stressed xanthium leaves increased for four to five hours of wilting and then levelled off. Phaseic acid increased throughout the wilting

period indicating increased turnover of ABA during stress. Conjugated ABA accumulated at a low rate during stress which suggest that free ABA was converted to the conjugated form rather than the reverse. Following rehydration of wilted leaves, the ABA level stopped increasing immediately and after one hour started to decline rapidly over a three hour period to the prestress level with a concomittant rise in phaseic acid level. In contrast to the rapid disappearance of ABA after relief of stress, the high phaseic acid content of rehydrated leaves declined only slowly. The level of conjugated ABA did not change following rehydration indicating that conjugation of ABA was irreversible (Jan and Zeevaart 1980).

Zeevaart (1980) noticed that the levels of ABA in stressed leaves increased for 4-5 hours and then levelled off due to a balance between synthesis and degradation. Similar effects were noticed in bean (Eze et al. 1981), wheat (Quarrie 1981), Sorghum (Samet et al. 1981), Cotton (Ackerson 1982) and Maize (Ilahi and Dorfling 1982).

In mesophytic plants Brafold and Hsio (1983) noticed that the endogenous levels of ABA rise markedly

in response to moderate water stress, apparently due to denovo synthesis. The increase is most dramatic in the leaves but has also been observed in roots, stems xylem and phloem sap.

Hiron and Wright (1973) investigated the effect of flooding on the ABA level of dwarf bean seedlings. The ABA levels of waterlogged plants increased very rapidly over the first 12 hours and then the rate of increase gradually decreased throughout the treatment period. After four days of flooding the ABA level in the waterlogged plants had increased 10 fold and the plants showed no visual symptoms of wilting. It is found that ethylene content increased rapidly in the roots of plants suddenly waterlogged and ethylene stimulated ABA synthesis in certain plant tissues (Mayak and Halevy 1972).

Crawford (1983) reported that ABA increases within hours of flooding in a number of crop plants and this lead to stomatal closure.

Accounts of various diseases, fungus (Pegg and Selman 1959), Virus (Vanstevenick 1959) and bacterial (Steadman and Sequeira 1969) have implicated an increased

inhibitor content in infected tissues.

Steadman et al. (1970) identified (+) Cis ABA in tobacco plants and studied its involvement in pathogenesis. The concentration of the growth inhibitor was unchanged in tobacco plants at various stages of stunting resulting from growth under low light intensity or from tobacco mosaic virus infection. In contrast, concentration of the inhibitor in Pseudomonas solanacearum infected tobacco tissues were higher than in healthy tissues. In diseased stem tissue concentration of the inhibitor was approximately double to that in healthy stems, 12-14 days after infection, the stems, young leaves and apical regions of diseased plants contained more inhibitor than mature leaves. Since P. solanacearum did not produce growth inhibitors in culture, increased levels of inhibitor in diseased plants apparently were due to a host response to the pathogen. Increase in content of the inhibitor was involved in the stunting of tobacco induced by P. solanacearum during early stages of pathogenesis.

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At the Regional Agronomic Research Station, Kumarakom, palms are growing on narrow bunds 2-3 m wide with water channels in between. The water table is very high 1-1½ m and thus these palms and their boles are constantly subjected to waterlogging conditions and many of them were root (wilt) affected and this represents the condition of coconut palms generally in the Kuttanad area where the disease is widespread. The affected palms show typical symptoms of root (wilt) namely flaccidity, yellowing and necrosis (Plates 2 and 3). Apparently healthy palms show no visible symptoms of the root (wilt) disease. The palms had a normal crown with an average of 25-30 leaves and 20-25 nuts yield per year (Plate 1). The selected palms were located at widely separate sites.

Healthy palms were also selected from the bunds at Instructional Farm, Vellayani so that growing conditions are almost similar. All the palms selected were in the age group of 25-30 years.

#### Sampling

For experimental purpose, samples of roots, leaves and developing nuts were taken from each of the three palms in each category.



PLATE 1



PLATE 2



PLATE 3

### Leaves

$N/2$  or  $(N+1/2)$  leaf in the crown (where  $n$  is the total number of leaves; Mathew and Ramadasan, 1974) was sampled and used for estimation of gibberellins and abscissic acid. Leaflets were collected from either side of the rachis. Both ends of the leaflets were cut to obtain almost uniform width of leaflet.

### Roots:

The surface root tips of about 15 cm in length, that is, tender roots produced soon after monsoon were collected by removing the soil carefully.

### Nuts

Tender nuts of 4-5 months age were used for the estimations. The liquid endosperm along with the thin layer of solid endosperm were used in the experiment.

The following estimations were carried out.

1. Endogenous gibberellins of leaves, roots and developing nuts.
2. Endogenous abscissic acid of leaves, roots and developing nuts.

### Experimental

#### Gibberellins

Extraction of gibberellins from root, leaf and nut was carried out according to the procedure of Radely (1958).

### Extraction

About 200 gm of freshly harvested materials (root and leaf) were homogenised in a wareing blender using 500 ml of 80 per cent methanol. The supernatent was decanted into a separate beaker. To the residue 500 ml of excess 80 per cent methanol was added and left overnight at room temperature (Regarding the nut water, about 500 ml was taken and an equal volume of 80 per cent methanol was added and kept over-night). The extract was filtered and methanolic phases were combined. Methanol was evaporated invacuo at 35°C. The aqueous phase was adjusted to pH 2.5 with 10 per cent (w/v)HCl. This acidified phase was extracted with distilled ethyl acetate and the supernatent was taken out. This was repeated thrice and all the ethyl acetate fractions were pooled up. The ethyl acetate fraction was extracted thrice with phosphate buffer pH 6.2. The combined buffer fraction was acidified to pH 2.5 with 5 per cent (w/v) H<sub>2</sub>SO<sub>4</sub>. The acidified extract was re-extracted with fresh ethyl acetate thrice. The ethyl acetate fractions were dried by freeszing out water and filtered through a drilled funnel. It is then distilled off invacuo at 35°C. The residue was dissolved in 80 per cent ethanol and an aliquot was lineloaded on Whatman No.1 chromatography paper.

Unidimensional ascending paper chromatography was employed. The chromatograms were developed in the solvent system chloroform : ethanol : water : formic acid (20:4:2:1 by volume). The dried chromatograms were divided into 10 equal Rf zones from their origin of solvent front, each zone representing an Rf region.

#### Elution

The GA like substance from different Rf regions (0.0 to 1.0) were eluted in methanol ( in 1 x 6" test tubes) overnight. The filter paper was removed from the tube and the elute was evaporated to dryness for further use in the bioassay.

The activity of GA like substance was evaluated on the basis of cucumber hypocotyl elongation bioassay as described by Reeve and Crozier (1975).

#### Bioassay

The seeds of cucumber (Cucumis sativus L) Var. Poinsette were first soaked in running tap water for 8 hours prior to germinating on a damp filter paper in 6" petridishes at 25°C in darkness. After 72 hours uniform seedlings (without seedcoats) were used for the bioassay.

The residue of GA like substance (after complete evaporation of methanol) was dissolved in three ml of glass distilled water and cotton pads were kept at the bottom of tubes. Three uniform cucumber seedlings were placed in the tubes (3/tube) and these tubes were kept under light (3000 lux) for 72 hours. The tubes were covered with polythene sheet to curtail the evaporation of the medium and the tubes were exposed to free air once in a day for proper aeration. After 72 hours the hypocotyl length was measured to the nearest mm. Control maintained using distilled water (-elute).

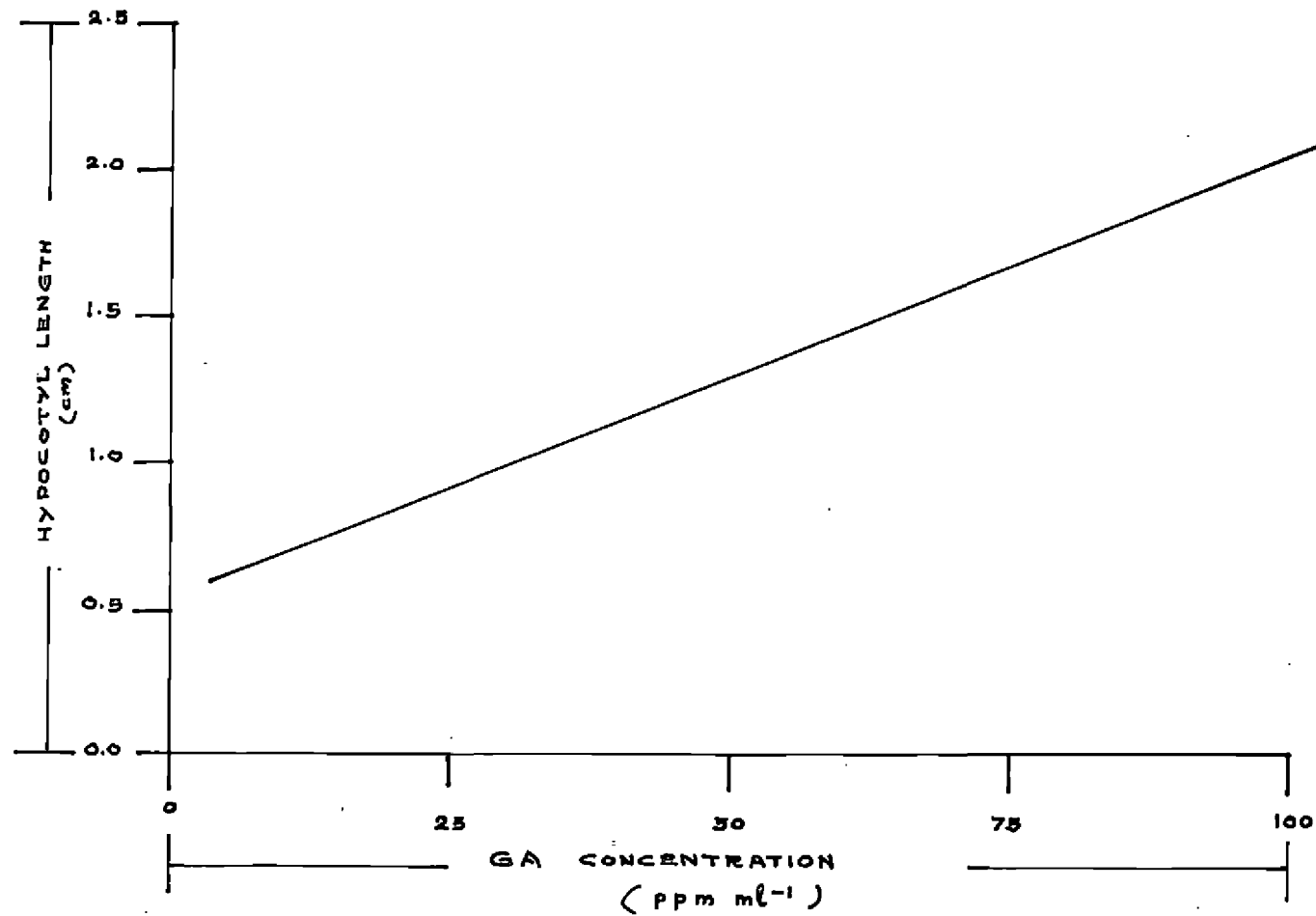
Gibberellin levels were estimated by using a calibration curve prepared from  $GA_3$  and expressed as nanomoles equivalents (Fig.1).

## II. Abscissic acid

ABA like inhibitors were extracted from the root, leaf and nut according to the method of Lenton et al. (1971).

### Extraction

About 200 gm of the freshly harvested (leaf and root) materials were homogenised in a wareing blender using 80 per cent aqueous methanol. The volume of



CALIBRATION CURVE FOR GIBBERELLIC ACID  
(CUCUMBER HYPOCOTYL GROWTH BIOASSAY)

FIG: 1

methanol used in the initial extraction was about 500 ml. The supernatant was filtered and the residue was re-extracted with an additional 500 ml of 80 per cent methanol and filtered. (with regard to nut water, about 500 ml was taken and an equal volume of 80 per cent methanol was added and kept overnight.) The methanol extracts after filtration were pooled up and methanol was evaporated under reduced pressure at 35°C in vacuo. The aqueous solution was frozen, thawed and centrifuged to remove supernatant material. The supernatant was adjusted to pH 3.5 with 10 per cent (w/v) HCl and extracted thrice with equal volumes of diethyl ether. All the ether extracts were pooled up and extracted four times, alternately with one quarter its volume of 5 per cent sodium bicarbonate solution and one quarter of its volume of water. All the aqueous extracts were combined and adjusted to pH 3.5 and reextracted with equal volumes of diethyl ether and all the ether extracts were pooled up and dried over anhydrous sodium sulphate before removing the ether on a rotary evaporator. The residue after evaporation was taken in known volume of 80 per cent ethanol and stored in cold chest until further use. A known volume of this extract was loaded

into Whatman No.1 chromatography paper and separated with isopropanol : Ammonium hydroxide : water (10:1:1 by volume) The chromatograms were dried and divided into 10 equal stripes and eluted in methanol. The methanol was evaporated and the residue were assayed for ABA like inhibitor activity using wheat coleoptile bioassay.

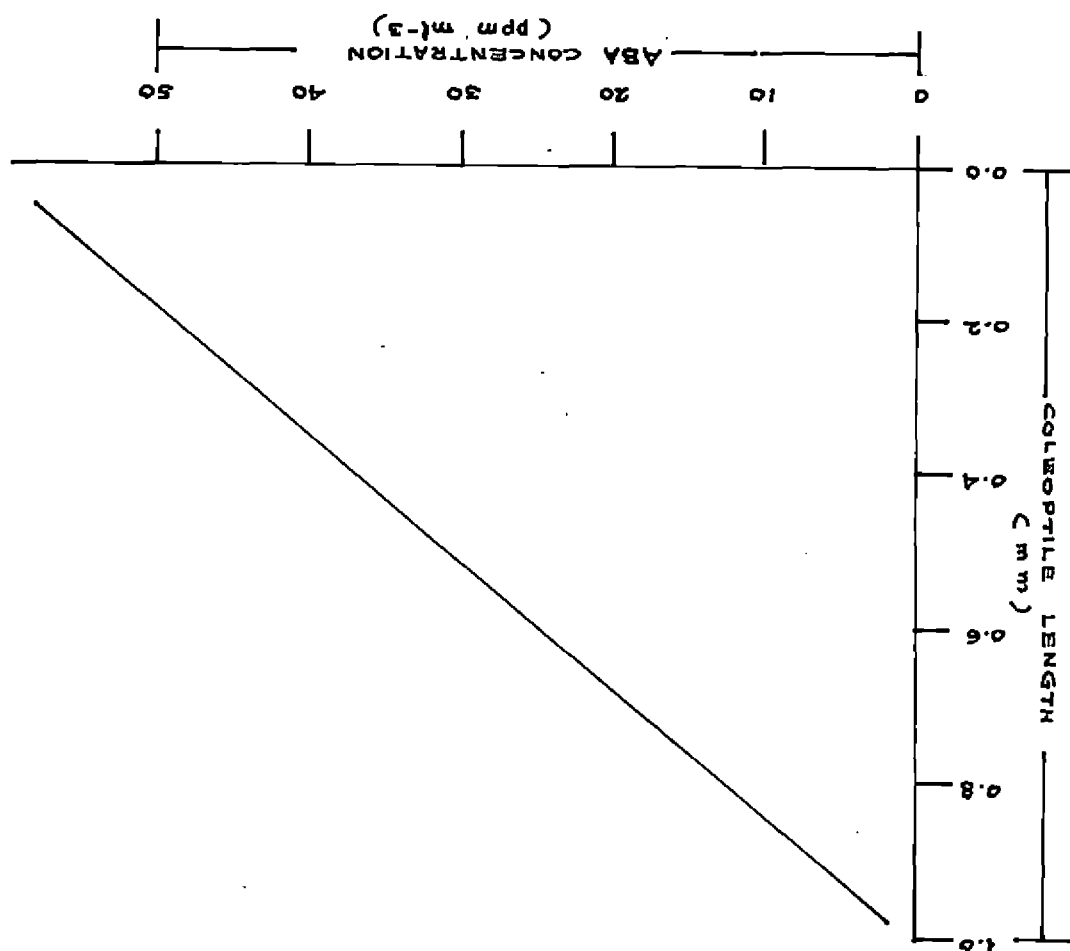
Bioassay:

The straight growth wheat coleoptile bioassay was carried out according to Nitch (1956) and modified by Rudricki (1969). Wheat seeds (Samba variety) were sown on moistened filter paper in 6" petridishes in dark for 60 hours at 25°C. Ten sub apical sections of 4 mm length were incubated in 3 ml. of 5 per cent sucrose for 24 hours in dark either with elutes or sucrose solution alone. ie. for controls. After 24 hours the length of the coleoptile was measured upto nearest mm.

Abscissic acid levels were estimated by using a calibration curve prepared from abscissic acid and expressed as nanomoles equivalents (Fig. 2).

FIG. 2

CALIBRATION CURVE FOR ABA  
(WHEAT COLBOPTILE BIOASSAY)



## RESULTS

## RESULTS

The results obtained in this investigation which include estimation of gibberellins and abscissic acid in the leaf, root and developing nut of healthy, apparently healthy and various intensities of root (wilt) affected coconut palms are presented in this section. The average yield of nuts from these palms were also taken. The mean tables and relevant figures are also presented.

### Gibberellins

The biological activity of GA like substances were determined by the cucumber hypocotyl elongation bioassay. The levels of gibberellins are presented in Table 1 expressed in nanomoles equivalents per kilogram fresh weight of leaf or root or per litre of nut water and their analysis of variance tables are given in appendices I(a), (b) and (c). Fig. 3 indicates the activity of gibberellin like substance in the leaf, root and developing nut (total endosperm) of healthy coconut palm. Healthy palms showed maximum GA like activity in their roots. Their activity were more pronounced

Table 1. Gibberellin content in healthy, apparently healthy and various intensities of root(wilt) affected coconut palm

|                         |    | nmol equivalent kg <sup>-1</sup> fresh weight/l <sup>-1</sup> |        |        |
|-------------------------|----|---------------------------------------------------------------|--------|--------|
|                         |    | Leaf                                                          | Root   | Nut    |
| Healthy                 |    | 214.57                                                        | 225.22 | 154.93 |
| Apparently healthy      |    | 138.70                                                        | 174.58 | 116.97 |
| Root (wilt)             |    |                                                               |        |        |
| affected at             |    |                                                               |        |        |
| disease intensity       |    |                                                               |        |        |
| around                  | 10 | 59.25                                                         | 81.57  | 50.44  |
| "                       | 20 | 52.68                                                         | 59.26  | 31.21  |
| "                       | 30 | 41.67                                                         | 36.08  | 27.20  |
| "                       | 40 | 34.60                                                         | 26.07  | 23.36  |
| "                       | 50 | 28.05                                                         | 30.89  | 18.90  |
| C.D at 5 per cent level |    | 7.56                                                          | 12.91  | 9.01   |

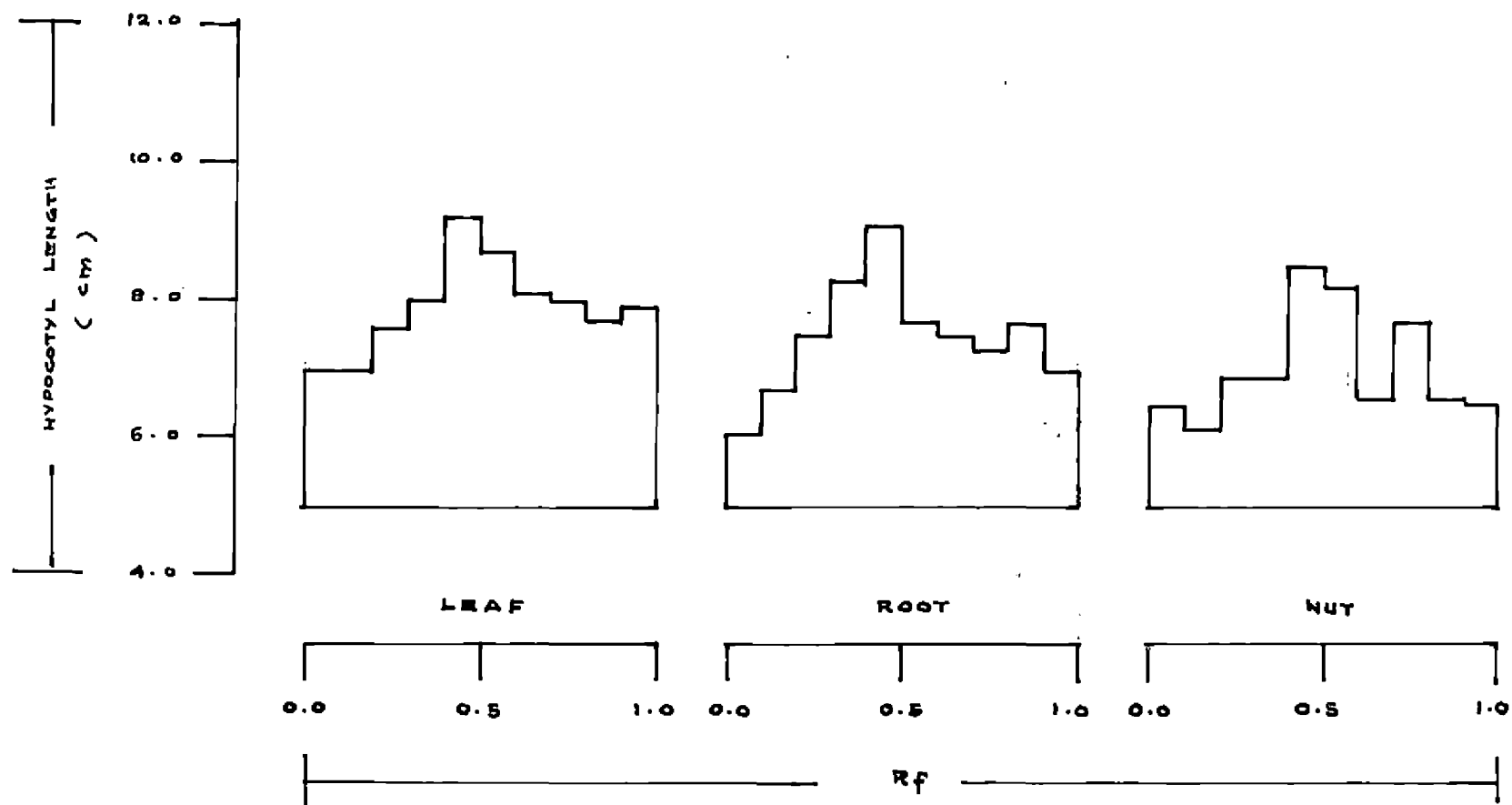


FIG. 3

in the leaves between Rf 0.2-0.5 and 0.5-0.9; 0.3-0.7, 0.8-0.9 in the root and 0.2-0.6, 0.7-0.8 in the nut of healthy palms. No inhibitory zones were observed in the roots, leaves or nuts of healthy palms. All the Rf zones showed promotion and maximum promotive zones were located at Rf 0.4-0.6

Fig. 4 indicates the activity of GA like substance in the leaf, root and nut of apparently healthy coconut palm. The gibberellin like activity was found between Rf 0.1-0.7 in the leaf; 0.1-0.6, 0.8-1.0 in the root and 0.1-0.2, 0.3-0.9 in the nut. An inhibitory zone was observed between Rf 0-0.1 in the roots of apparently healthy palms. Maximum promoting activities were noticed between Rf 0.5 to 0.6 in the leaf; 0.4-0.5 in the root and 0.3- 0.5 in the nuts.

The activity of gibberellin like substance in the leaf, root and nut of various intensities of root(wilt) affected coconut palms are presented in Fig.5. At stage 1 of the disease, the promotive zones were noticed at an Rf of 0.3-0.8 in the leaf; 0.2-0.7, 0.8-1.0 in the root and 0.4-0.6 in the nut. Maximum promotions were noticed at Rf 0.4-0.6 in this stage. A slight inhibitory zone was noticed at Rf 0-0.2 and 0.9-1.0 in the

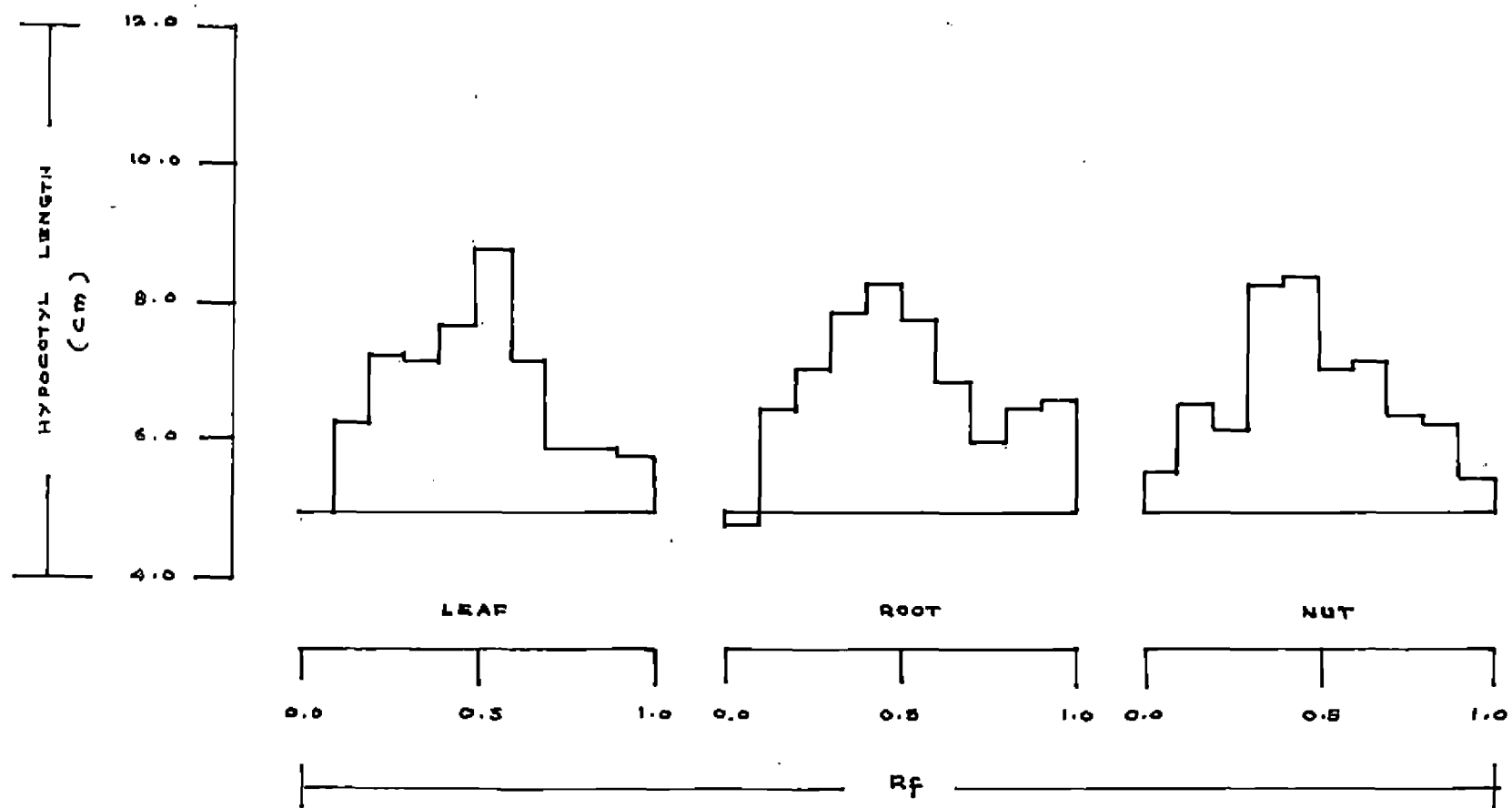
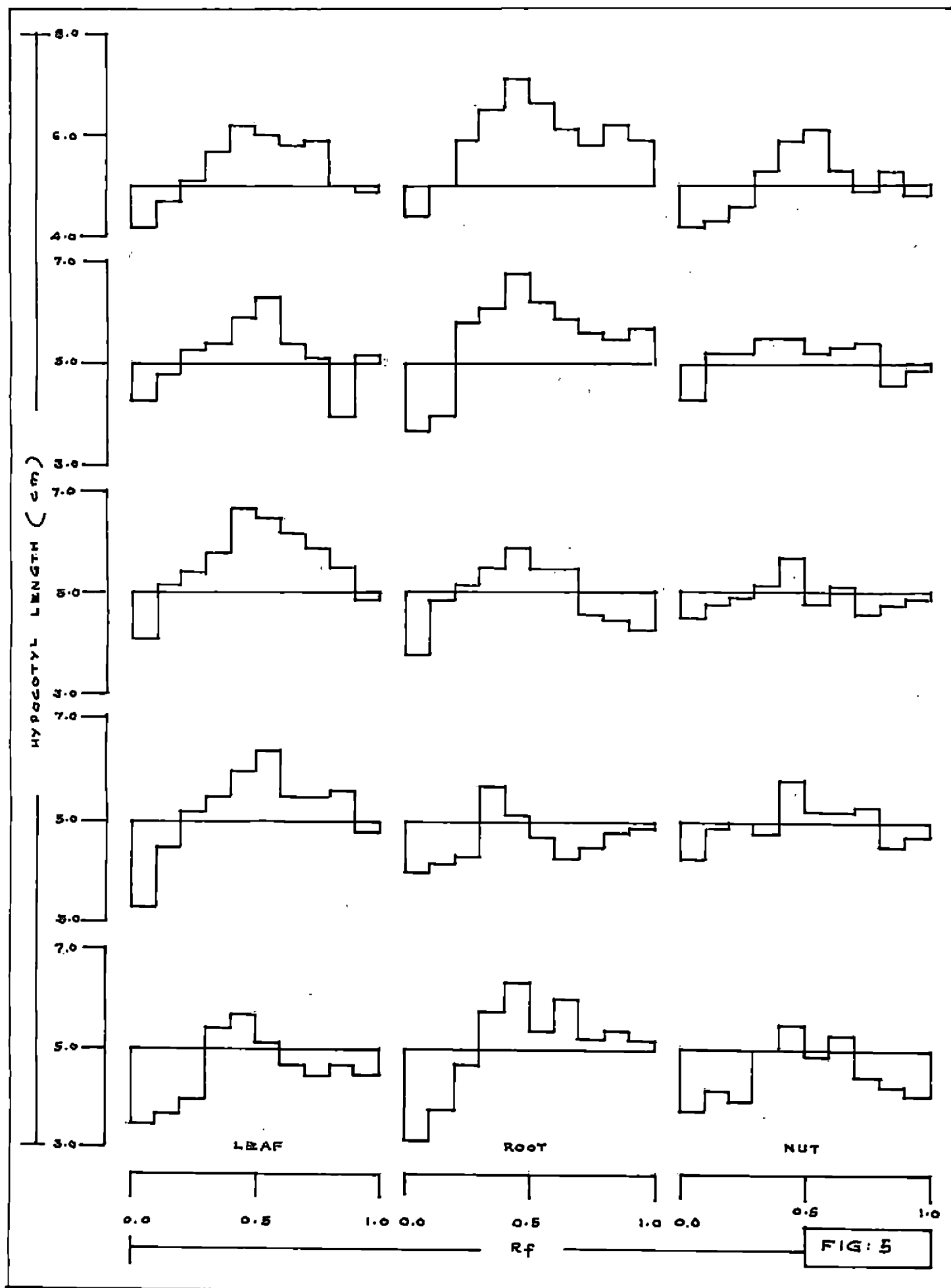


FIG. 4

leaf; 0-0.1 in the root and 0-0.3, 0.7-0.8 and 0.9-1.0 in the nut. At Stage II, leaves showed maximum GA like activity at Rf 0.4-0.6, slight promotion at Rf 0.2-0.4, 0.6-0.8 and 0.9-1.0 and strong inhibition between 0-0.2 and 0.8-0.9. In the root maximum GA like activity was noticed in the Rf 0.2-0.3; 0.6-1.0 and a strong inhibitory zone between 0-0.1. In the nuts only slight promotive zones were there between 0.1-0.8 Rf zones and slight inhibition between 0-0.1 and 0.8-1.0 Rf zones.

At stage III of the disease, a high promotive zone was observed between Rf region 0.3-0.8, slight promotive zones between Rf 0.1-0.2, 0.8-0.9 and inhibitory zones 0-0.1 and 0.9-1.0 in the leaf. Roots showed maximum promotion at the Rf 0.4-0.5 and only slight promotion at 0.2-0.4 and 0.5-0.7. High inhibition was observed between Rf 0-0.2 and 0.7-1.0. In the nut only slight promotive activity between Rf 0.3-0.5 and 0.6-1.0 and a high inhibitory activity between Rf 0-0.3, 0.5-0.7 and 0.8-1.0

At stage IV, only slight promotive zones were there between Rf 0.2-0.9 and slight inhibitory zones between 0-0.2 and 0.9-1.0 in the leaf. Roots showed



more inhibitory zones when compared to leaves, ie. between Rf 0-0.3 and 0.5-0.8. The promotive zones were between 0.3-0.5 and 0.8-1.0 in the roots. Nuts showed only very little promotion between 0.4-0.8 Rf zones and inhibitory zones between Rf 0-0.4 and 0.8-1.0. Maximum promotion was noticed at Rf 0.4-0.5.

At stage V of the disease, promotion was noticed at Rf 0.3-0.6 in the leaf, 0.3-1.0 in the root and 0.3-0.5, 0.6-0.7 in the nut, maximum promotion being at Rf 0.4-0.5. Inhibitory zones were noticed at Rf 0-0.4 and 0.6-1.0 in the leaf, 0-0.4 in the root and 0-0.4, 0.5-0.6 and 0.7-1.0 in the nut.

When compared to the apparently healthy controls, the promotive activity was less in the diseased palms. But the diseased palms showed high inhibitory activity maximum being at 0-0.2 Rf zones. On the whole the activity of GA like substance was low in the diseased palms as evidenced by the cucumber hypocotyl elongation bioassay.

Fig. 6 represents the wide variation in gibberellin content between the leaves of healthy, apparently healthy and root (wilt) affected coconut palms. There is significant difference between them. Similarly,



FIG: 6

Fig.7 indicates the maximum difference in gibberellin content between roots of healthy, apparent healthy and root (wilt) affected palms and Fig.8 indicates the difference between healthy, apparently healthy and root(wilt) affected nuts.

Fig.9 is a histogram where the relative gibberellin content in different groups of palms are shown. The gibberellin content in the leaves of (214.57) healthy palms registered significantly higher levels when compared to apparently healthy (138.7). At the same time apparently healthy palms showed significant difference with the diseased palms. Stage I (59.25) and II (52.68) of the disease do not show significant difference but they differ significantly from the other stages. Stage III and IV (41.67 and 34.6) are on par and stage IV and Stage V (28.05) are also on par but stage III differ significantly from Stage V.

Roots show maximum gibberellin content in healthy palms (225.22), apparently healthy ones contained less (174.58) and there is significant difference between the two. They also differ significantly from diseased ones.

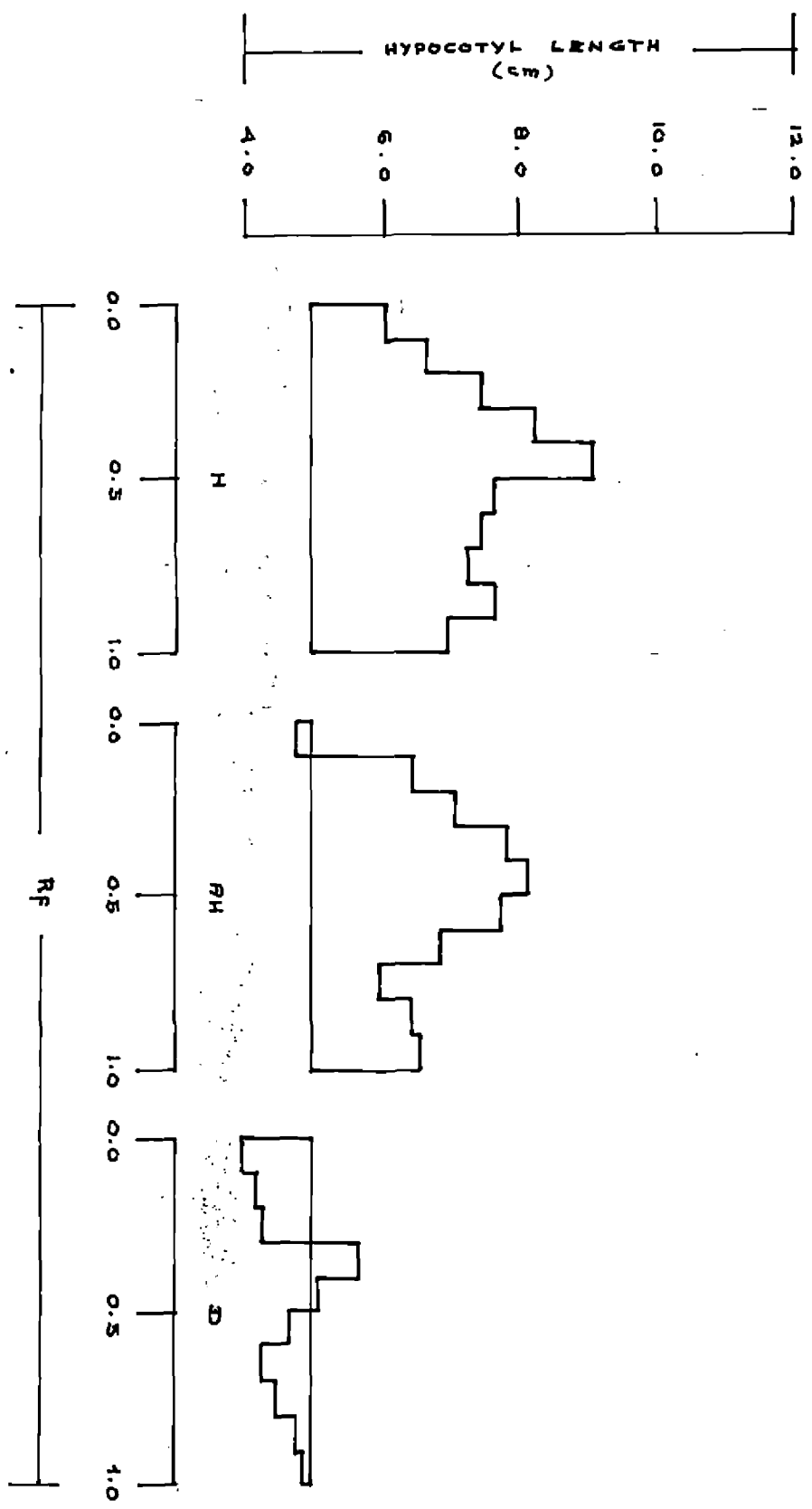
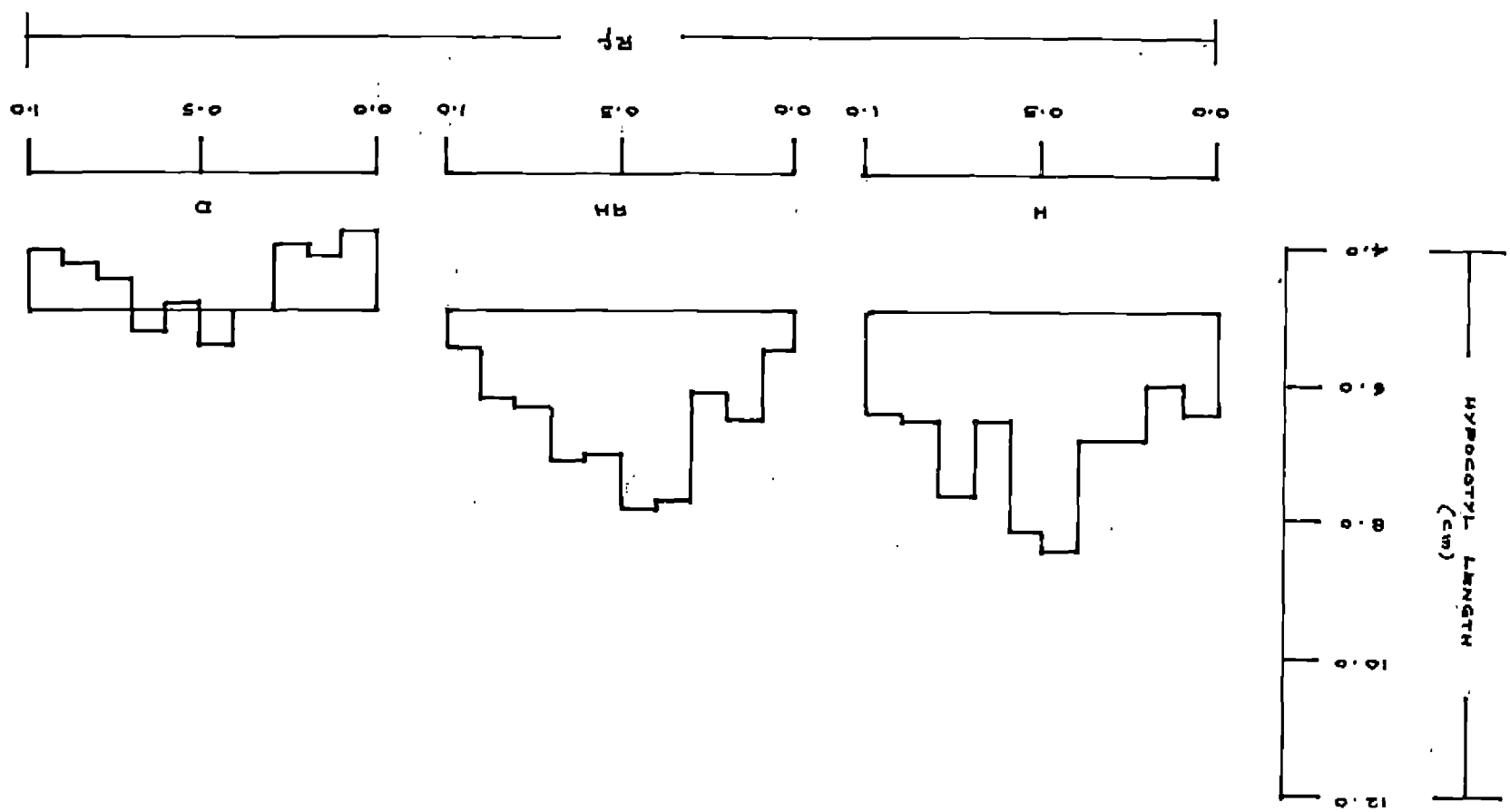
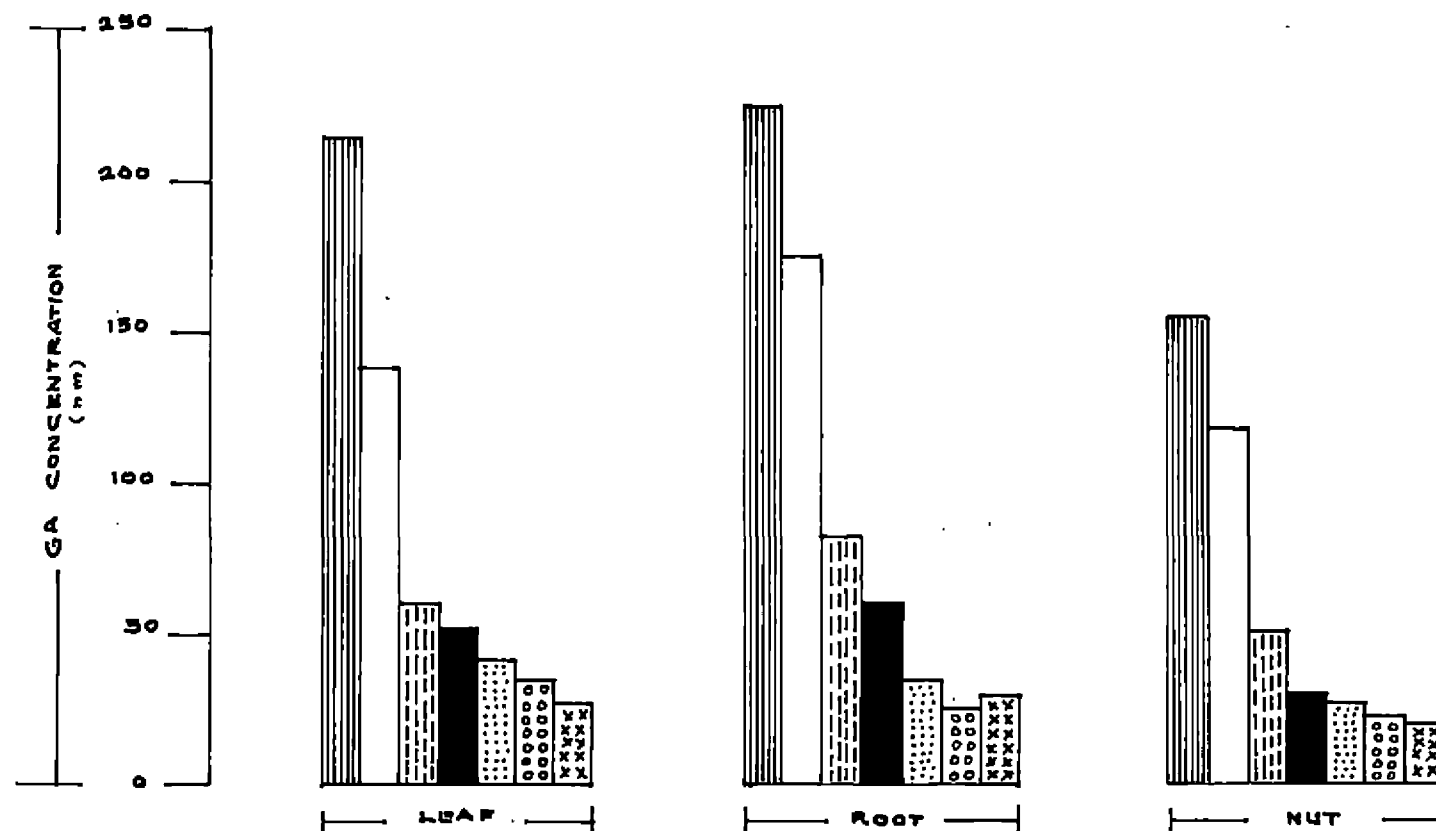


FIG. 17

FIG. 8





- |  |                                     |  |                                     |
|--|-------------------------------------|--|-------------------------------------|
|  | HEALTHY PALMS                       |  | PALMS OF DISEASE INTENSITY ABOUT 30 |
|  | APPARENTLY HEALTHY PALMS            |  | PALMS OF DISEASE INTENSITY ABOUT 40 |
|  | PALMS OF DISEASE INTENSITY ABOUT 10 |  | PALMS OF DISEASE INTENSITY ABOUT 50 |
|  | PALMS OF DISEASE INTENSITY ABOUT 20 |  |                                     |

FIG: 97

Stage I (81.57) and II (59.26) show significant difference from Stage III (36.08) but III, IV (26.07) and (V) (30.89) stages are on par. Stage II differ from the other stages significantly.

In the nut, maximum gibberellin content was noticed in healthy (154.93) and it is significantly higher than in apparently healthy, (116.97). Stage I (50.44) of the disease shows significant difference from apparently healthy and also stage II (31.21), Stage II, III (27.2) and IV (23.36) are on par and Stage III, IV and V (18.9) are also on par but stage III and V differ significantly.

#### Abscissic acid

The biological activity of ABA like inhibitors was assessed by the wheat coleoptile bioassay. The levels of ABA are presented in Table 2 expressed as nanomoles equivalents per kg fresh weight of leaf or root or per litre of nut water, and their analysis of variance tables are given in Appendix II(a), (b) and (c).

Fig. 10 shows the activity of ABA like inhibitors in the leaf, root and developing nut of healthy coconut

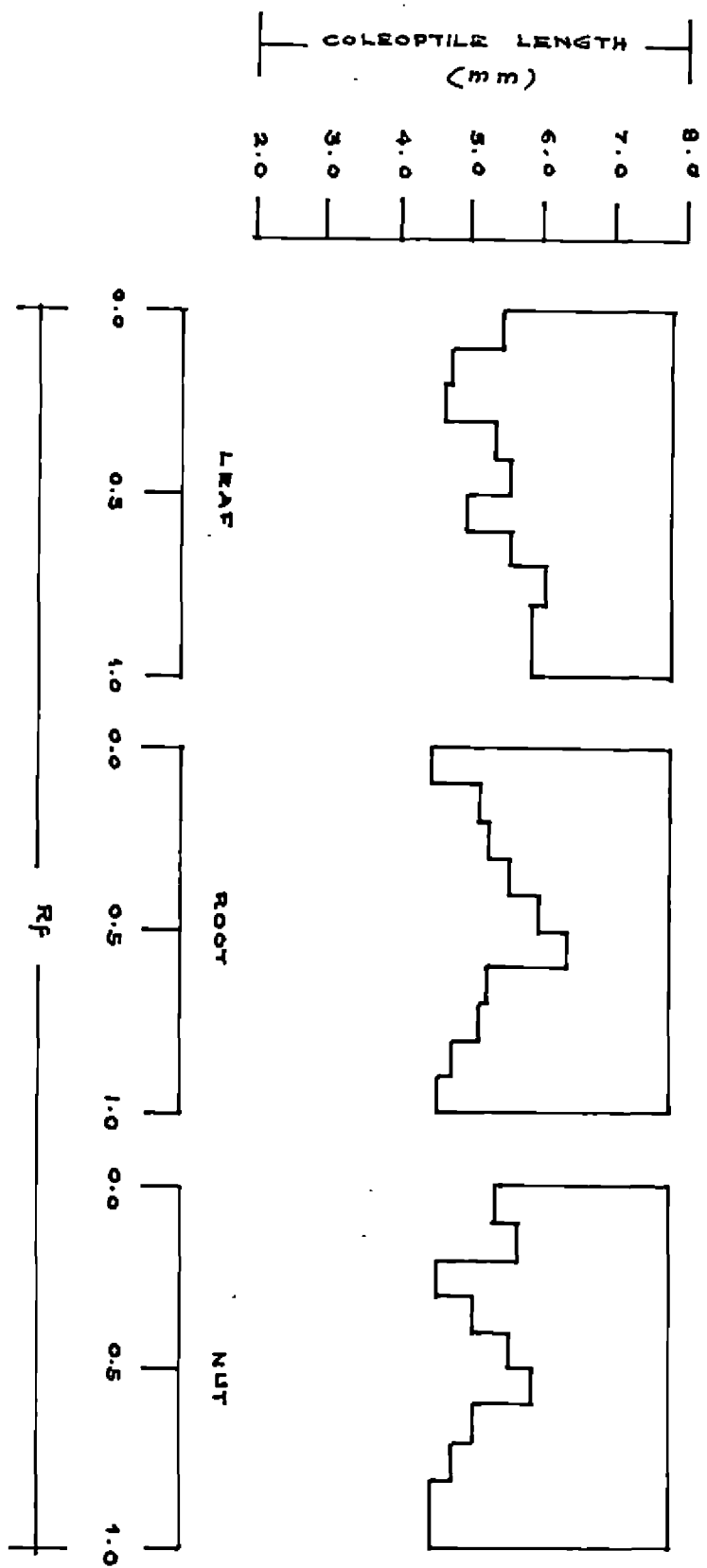
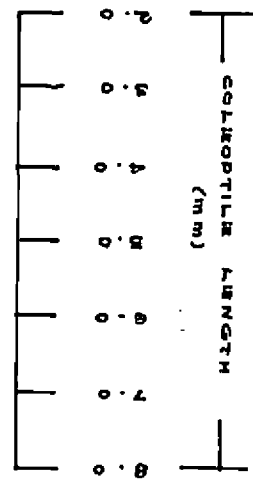
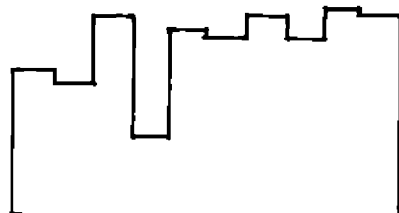
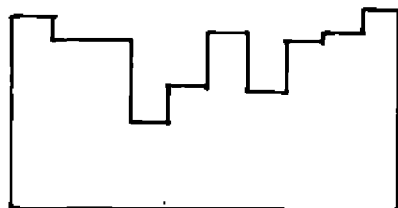
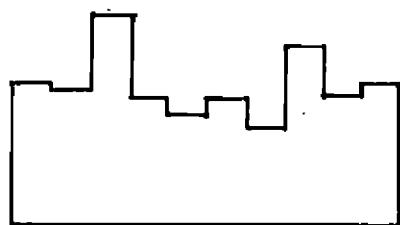
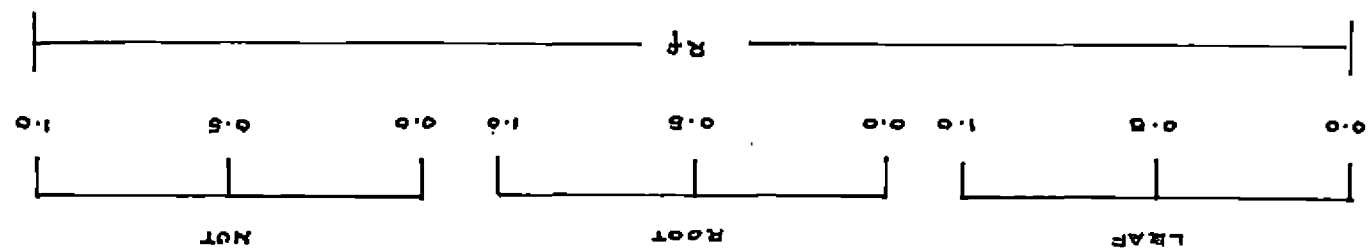


FIG: 10

Table 2. ABA content of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm

|                          |    | nmol equivalents kg <sup>-1</sup> fresh wt/l <sup>-1</sup> |       |       |
|--------------------------|----|------------------------------------------------------------|-------|-------|
|                          |    | Leaf                                                       | Root  | Nut   |
| Healthy                  |    | 69.6                                                       | 58.88 | 54.38 |
| Apparently healthy       |    | 78.36                                                      | 67.74 | 64.67 |
| Root(wilt) affected      |    |                                                            | 0     |       |
| at disease intensity     |    |                                                            |       |       |
| around                   | 10 | 81.82                                                      | 78.43 | 77.03 |
| "                        | 20 | 85.03                                                      | 84.67 | 79.60 |
| "                        | 30 | 88.97                                                      | 87.11 | 86.10 |
| "                        | 40 | 98.98                                                      | 93.74 | 90.62 |
| "                        | 50 | 101.76                                                     | 96.92 | 95.08 |
| C.D. at 5 per cent level |    | 4.13                                                       | 6.55  | 6.06  |

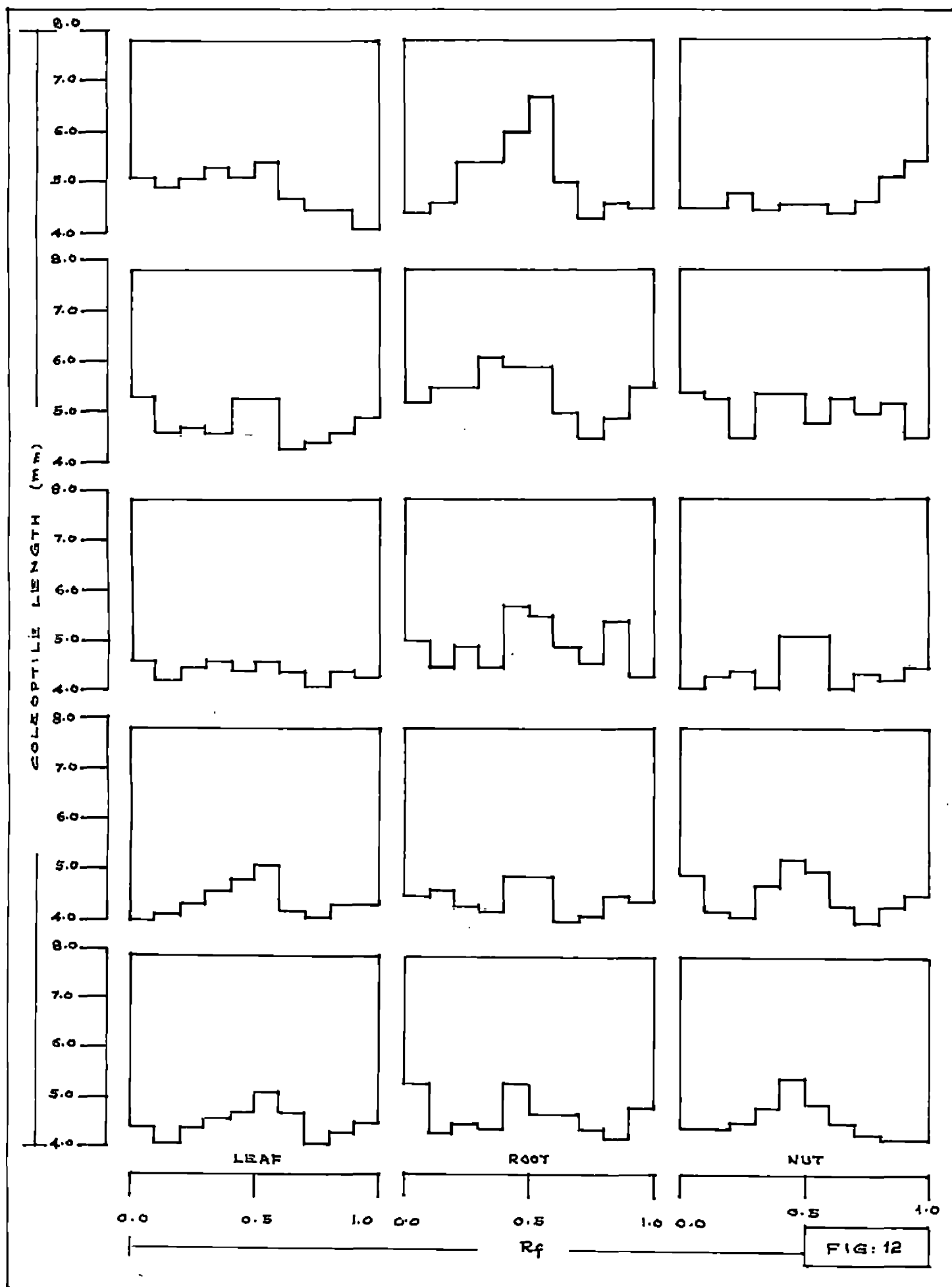
FIG. 11



palm. They show inhibition in all zones and maximum inhibitions are there at the Rf of 0-0.2, 0.3-0.4 and 0.7-0.8 in the leaf, 0-0.1, 0.9-1.0 in the root and 0-.2-0.3 and 0.7-0.8 in the nuts. No promotive zones were observed. The inhibitory activity is relatively low here.

In Fig. 11 the activity of ABA like substance in the leaf, root and nut of apparently healthy palms is presented. Leaves showed maximum inhibition at 0.1-0.3 and 0.5-0.6, roots at 0-0.3, 0.6-1.0 and nuts at 0.2-0.5, 0.6-1.0 Rf zones. Here also no promotive zone was noticed.

Fig.12 presents the activity of ABA like inhibitors in the leaf, root and nut of various intensities of root (wilt) affected coconut palms. At stage I of the disease the inhibitory activity were noticed at the Rf of 0.1-0.5, 0.6-1.0 in the leaf maximum being at 0.7-1.0, 0-0.2, 0.7-1.0 in the root and 0-0.5 and 0.7-0.9 in the nut. At stage II leaves showed maximum ABA like activity at the Rf of 0.1-0.5, 0.6-1.0 in the leaf, 0.5-0.9 in the root and 0.2-0.3, 0.5-0.6 and 0.9-1.0 in the nut. At stage II of the disease the maximum inhibition was located at the Rf 0.1-0.4, 0.5-0.6, 0.7-1.0 in the leaf,



0.1-0.5, 0.6-0.8 and 0.9-1.0 in the root and 0-0.4, 0.6-1.0 in the nut.

At stage IV, leaf showed ABA like inhibitor activity at the Rf 0-0.4, 0.6-1.0 in the leaf, 0-0.4, 0.6-1.0 in the root, and 0.1-0.4, 0.7-0.9 in the root and 0-0.3, 0.5-1.0 in the nut. When compared to the controls the diseased palms showed more inhibitory activity at all Rf regions. Within the diseased palms also maximum inhibition was noticed at Stage IV and V of the disease.

Fig.13 represents the relative, ABA like activity in the leaves of healthy, apparently healthy and root (wilt) affected coconut palms and it shows significant difference. Fig. 14 indicates the maximum difference in ABA content between the roots of healthy, apparently healthy and root (wilt) affected palms and Fig. 15 represents the difference in inhibitory activity between healthy, apparently healthy and root(wilt) affected nuts. All these showed maximum difference.

Fig. 16 is a histogram representing the comparative ABA content in different groups of palms. The ABA content in the leaves of healthy palms (69.6) registered significantly lower levels when compared to apparently healthy (78.36). Apparently healthy did not differ significantly

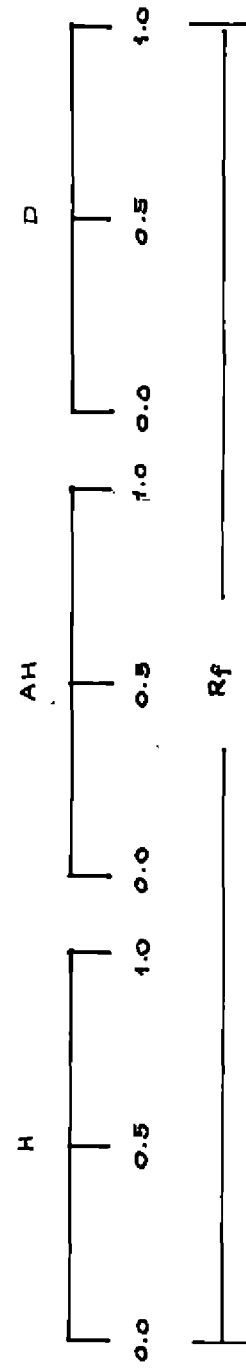
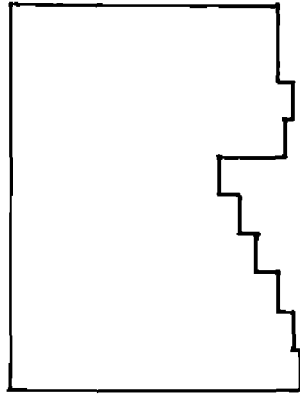
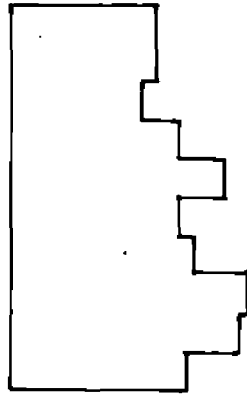
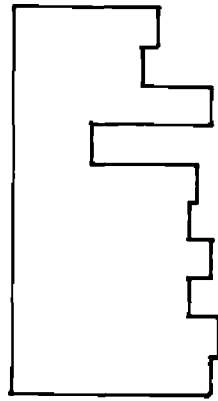
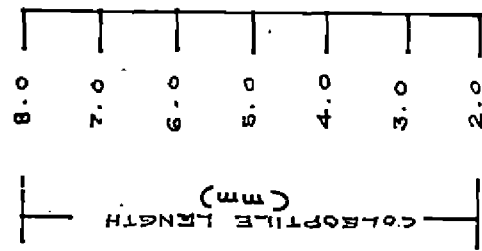


FIG: 13

from the first stage of the disease (81.82) but stage II (85.03) is significantly different from stage I and stage III (88.97). Stage IV (98.98) and V (101.76) are on par but they differ significantly from stage III.

Roots showed maximum ABA content at Stage V of the disease (96.92) followed by stage IV (93.74) which do not differ significantly. Stage III (87.11) and II (84.67) do not differ significantly and stage II and I are also on par. But stage III differ significantly from stage I (78.43). Apparently healthy roots contained much less ABA (67.74) when compared to diseased and it was also significantly higher than that in healthy roots (58.88).

Nuts show least ABA content in healthy (54.38), followed by apparently healthy (64.67) and they differ significantly. Stage I (77.03) and II (79.6) are on par but they differ significantly from apparently healthy. Stage III (86.1) and IV (90.62) are on par, stage IV and V (95.08) are also on par but stage V differ significantly from stage III. Stage III also differ significantly from stage II. Maximum ABA content was observed at stage V of the disease.

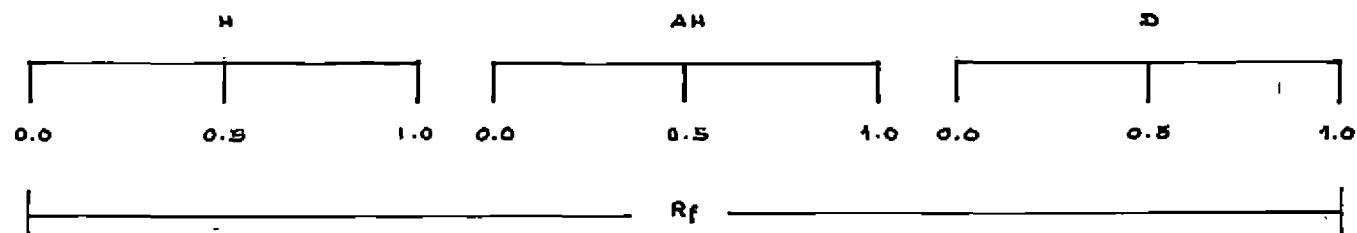
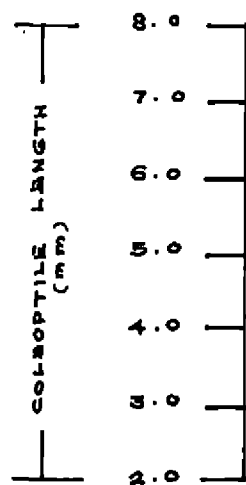


FIG: 14

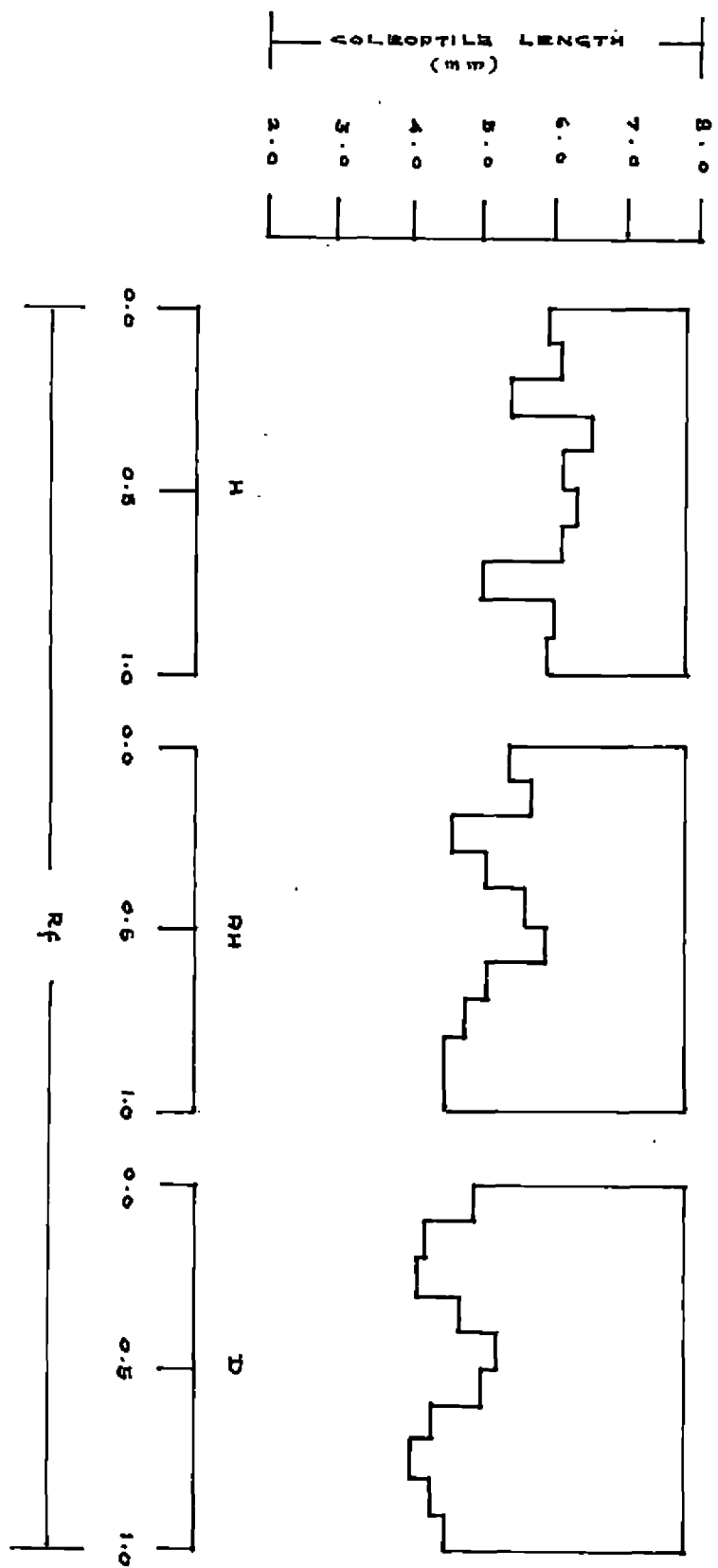


FIG. 15



 HEALTHY PALMS  
 APPARENTLY HEALTHY PALMS  
 PALMS OF DISEASE INTENSITY ABOUT 10  
 PALMS OF DISEASE INTENSITY ABOUT 20

 PALMS OF DISEASE INTENSITY ABOUT 30  
 PALMS OF DISEASE INTENSITY ABOUT 40  
 PALMS OF DISEASE INTENSITY ABOUT 50

FIG. 16

The yield data from the different groups of palms for the last five years was also taken and is presented in Table 3 and its analysis of variance table is given in Appendix III. The data showed significant differences among healthy (55) apparently healthy (23) and diseased. Stage I and II do not show significant difference in yield of nuts ie. (14 and 12). Stage II, III(7) and IV(8) are on par, but stage I is significantly higher than stage IV. Stage III and V are on par but V differ (3) much from stage IV.

The healthy and apparently healthy palms showed almost a steady yield for the last five years with an increasing trend for healthy. But in diseased palms they showed a gradual decline as the disease intensified.

Table 3. Average nut yield from healthy, apparently healthy and various intensities of root (wilt) affected coconut palm for the last five years.

|                                                       |    | No. / palm / year |      |      |      |      |
|-------------------------------------------------------|----|-------------------|------|------|------|------|
|                                                       |    | 1978              | 1979 | 1980 | 1981 | 1982 |
| Healthy                                               |    | 44                | 48   | 57   | 70   | 57   |
| Apparently healthy                                    |    | 23                | 29   | 25   | 20   | 18   |
| Root(wilt) affected<br>at disease intensity<br>around | 10 | 13                | 14   | 20   | 14   | 13   |
| "                                                     | 20 | 13                | 12   | 15   | 11   | 11   |
| "                                                     | 30 | 11                | 3    | 5    | 7    | 10   |
| "                                                     | 40 | 12                | 7    | 7    | 6    | 7    |
| "                                                     | 50 | 6                 | 2    | 3    | 2    | 4    |
| C.D. at 5 per cent<br>level                           |    | 6.43              |      |      |      |      |

## DISCUSSION

## DISCUSSION

The results obtained in the present study are illustrated in figures 3-14 and tables 1-3.

### Gibberellins

The activity of gibberellin like substances of leaf, root and nut (total endosperm) of the three types of palms assessed by cucumber hypocotyl elongation bioassay are presented in figures 3,4 and 5 respectively and gibberellin levels in table 1.

The activity was found to be greater in the case of healthy palm in the leaf, root and nut when compared to the apparently healthy and root(wilt) affected palms. When compared to diseased palms, apparently healthy palms also contained a higher level of GA. Within the diseased palms also the activity was found to decline as the disease intensified.

### Leaf

Fig.6 indicates the activity of gibberellin like substances in leaves of the three types of palms. In the case of healthy palm a greater promotive zone or

activity is seen when compared to apparently healthy and root(wilt) affected palm. The gibberellin content (table 1) in healthy palm also show significant difference from that of root (wilt) affected. The foremost effect of hormone imbalance in leaves as reported by Prisco and O'leary (1972) is enhancement of senescence. During senescence disintegration of chlorophyll is more than that of its synthesis. Gibberellins are involved in retardation of senescence in detached and intact leaves. This is the result of an increase in the rate of chlorophyll synthesis over disintegration. There is also the suggestion that it is associated with a maintenance level of chlorophyll, RNA and proteins (Fletcher and Osborne, 1965) Stoddart (1960) found that GA stimulated the production of RUDP carboxylase in leaves and thereby also an increase in photosynthesis. Reduced gibberellin availability could reduce considerably leaflet and frond size which are late symptoms of the root(wilt) disease syndrome due to the impairment of normal synthetic and metabolic activities.

Anatomical studies by Joseph and Shanta (1964) have revealed reduction in wall thickness of sclerenchymatous tissues. Studies of tender unopened leaves of healthy

and root (wilt) affected palms (Thomas et al. 1964) have shown that there was much abnormality in the distribution of stomata and general stunting of the epidermal cells and reduction in thickness of cuticle finally resulting in the initial symptom termed flaccidity.

The gibberellins are known to be transported through the phloem (Hoad and Bowen, 1968) as well as through the xylem (Phillips and Jones, 1964) (Zweig et al. 1961). So the observations by Indira and Ramadasan (1968); Govindankutty and Vellaichamy (1976) on the development of tyloses in xylem vessels and degenerate phloem in roots indicate the impairment in the transport system of the root(wilt) affected palms, blocking normal distribution to other parts of the plant.

### Root

Fig. 7 indicates the activity of gibberellin like substances in roots of the three types of palms. In the healthy palm a greater promotive zone or activity is seen when compared to apparently healthy and root (wilt) affected coconut palm. A strong inhibitory zone was present in diseased roots. There is evidence

that roots are sites of considerable activity in gibberellin synthesis and or interconversion or both (Jones and Phillips, 1966) (Crozier and Reid, 1971).

In the present study, the gibberellin content in root (wilt) affected palms showed significant reduction from that of the healthy palms, which may be due to the damaged root tips preventing any synthesis or interconversion of the hormones. Varghese (1934) reported severe rotting of the root cortex in root(wilt) affected palms. Actual rotting occurs when they come in contact with subsoil water and some roots become functionless. This condition probably arises in palms which are constantly under waterlogged condition for a few months annually i.e., during monsoons. Phillips (1964a) speculated that the reduced growth rate of flooded plants could be due to a substance inhibiting gibberellin synthesis in the roots. Reid et al. (1969) examined the GA content in the xylem sap of tomato plants before and after waterlogging of the root system. After one day there was a 40 fold reduction in the level of acidic GA like activity. By the third day there was no detectable

activity. There is evidence that the affected palms under waterlogged environment lose considerable quantities of cytokinins by leaching (George Tharakan, unpublished). A similar loss of gibberellins may also occur in the affected palms under waterlogged environment. The new root system under the stress of flooding or drought are not able to provide the necessary gibberellins or water and nutrients to the aerial parts due to their retarded growth, rotting or vascular block and hence the symptoms of chlorosis.

Waterlogging does not necessarily occur only when the soil is inundated, but it often occurs when water fills a critical portion of the soil air spaces, depending upon the species of the plant. Anaerobic conditions result in low ATP yield, accumulation of toxic end products and rapid depletion of organic compounds. Absorption and translocation of water and nutrients in roots are limited by low energy (Makoto Kawase 1981). It is also reported that the synthesis and translocation of gibberellins and cytokinins in the roots are suppressed by root injury resulting from waterlogging. The symptoms of root (wilt) disease also appear to be similar to those of plants subjected to waterlogging and such

injuries result in similar restriction on synthesis and translocation of hormones and hence the abnormal symptoms of the crown (Crozier and Reid, 1971).

#### Nut (Total endosperm)

The activity of GA like substance in Fig.8 provides a clear picture of higher promotive zones in the case of healthy palms when compared to apparently healthy and root(wilt) affected palms and a strong inhibitory zone is indicated in the case of developing nut of root (wilt) affected palms. A significant reduction in gibberellins has been observed in apparently healthy and root (wilt) affected palms over healthy palms (table 1). A drastic reduction in the yield of diseased palms was reported by Radha et al. (1962). Abnormal shedding of female flowers and immature nuts (Menon and Pandalai, 1958) and lack of ability to produce female flowers (Varghese 1934) render the palm unproductive. This abnormal shedding of flowers and immature nuts may be due to hormonal imbalance. It is found that particular endogenous gibberellins in a given type of fruit are involved in the growth of the fruit while other gibberellins are implicated in fruit maturation (Krishnamoorthy, 1975).

Radely (1976) reported that the maximum grain volume in wheat seed is inversely related to temperature and is reached while the dry weight is still small. This stage is associated with a sharp increase in GA. Jackson and Coomble (1966) found an increase in the concentration of GA like substance in the seed, endocarp and mesocarp of apricot during anthesis, attaining a maximum 20 days after anthesis. The time at which GA activity was greatest coincided with the time of greatest growth rate of the seed. Jackson (1968a) reported that in peach fruit, the GA concentration in the seed was closely correlated with the rate of cell expansion. In apple seeds, the gibberellins first appeared 4-5 weeks after full bloom, quickly increased to a peak concentration and then declined rapidly in the final maturation period (Luckwill et al. 1969).

A similar rise in GA like activity during development and a subsequent decline at the final maturation stage was observed in the seeds of almond and plum (Phinney et al. 1960) and pigeon pea seeds (Madhava Rao et al. 1974) which indicate that gibberellins are involved in the development of the seed.

In the present study a reduction in gibberellin content in the total endosperm of the diseased palms is noticed. It may be safely assumed that cytokinins and other organic and inorganic metabolites are also in restricted supply. These could be responsible for poor and improper development of the endosperm due to inadequate metabolite supplies as also a low mobilization capacity. The endosperm under such conditions disrupts and its normal metabolic capabilities are lost.

#### ABA

The activity of ABA like inhibitors of the leaf, root and nut (total endosperm) of the three types of palms assessed by wheat coleoptile bioassay are presented in figures 10,11 and 12 respectively and the ABA levels in table 2.

The inhibitory activity was found to be greater in the leaf root and nut of the diseased palms when compared to apparently healthy and healthy palms.

### Leaf

ABA synthesis has been shown to occur in the chloroplast of the leaves and migrate readily to other parts of plants (Loveys, 1977). All the enzymes necessary to synthesise ABA from mevalonic acid reside in the chloroplast. When plants are subjected to various forms of stress such as drought, salinity or flooding there was a rapid accumulation of ABA in their leaves.

In the present study the ABA content in the root (wilt) affected palms is significantly greater than that in healthy and apparently healthy palms. This may be due to an impaired water uptake by plants leading to an increase in leaf water deficit. This imbalance can occur either due to waterlogging when the root is unable to function efficiently under anaerobic condition or due to the degeneration of the root tips by the disease. ABA is found to promote senescence in plant parts. Wareing and Hillman (1967) reported that senescence of isolated excised leaf discs of Acer pseudoplatanus is promoted by ABA. This may be the reason for the increased senescence seen in the diseased palms which is

expressed by the yellowing and flaccidity of the leaves. It is found that stunting of plants by various diseases and also wilting are accompanied by an accumulation of ABA ( Pegg and Selman; 1959) (Steadman and Sequeira, 1969) (Mizrahi et al.1970). The stunting syndrome seen in root(wilt) affected palms such as reduction in size and number of fronds, leaflets etc. might be due to the accumulation of ABA.

### Root

Generally roots do not attain high levels of ABA reached by stems and leaves but like leaves they are believed to be able to synthesize ABA atleast in small amounts during stress conditions. (Walton et al.1976). In the present study there is significant increase in ABA levels in the roots of diseased palms when compared to healthy and apparently healthy palms. Within the diseased palms the concentration of ABA increased as the disease intensified. When the root system of the palms become waterlogged the hormonal balance within the plant is drastically disturbed. This will lead to reduction in stem elongation and leaf expansion. Root growth will also be inhibited.

Studies on the water relations of the diseased palms revealed that absorption of water was very much reduced in diseased palms (Davis, 1964). The uptake and upward transport of water through the trunk in diseased palms was found to be 35 per cent less than that of the healthy palms (Ramadasan, 1964). Significant increase in transpiration rate was noticed in the diseased palms as compared to that in healthy palms. This will lead to an increase in leaf water deficit which in turn result in accumulation of ABA. The increase in ABA level may also be induced by toxic or foreign substance produced in the roots such as ethanol (Mayak and Halevy, 1972).

#### Nut (Total endosperm)

ABA level is found to rise sharply and then fall during the development of seed in a variety of plants like wheat, soybean, pea, barley, cotton, tomato and pear (Walton, 1980). The highest concentration of ABA in the embryo occur at a time when their dry weight is increasing rapidly. Milborrow and Robinson (1973) reported that both wheat endosperm and embryos are able to incorporate  $C^{14}$  - mevalonic acid into ABA and that the developing seeds have the capacity to metabolize ABA

at various stages of their development. Radely (1976) found that during the development of wheat grains, the period of rapid water loss after starch formation is preceded by an increase in ABA content. Gillard and Walton (1976) reported that determination of endogenous ABA and dihydrophaseic acid concentrations in the liquid endosperm of Eastern wild cucumber seed suggested the involvement of ABA -hydroxylating and pyruvic acid reducing enzymes in metabolizing ABA in the intact seed.

Addicot and Lyon (1969) reported that the level of ABA in the fruits increase during maturation and ripening in certain sp. He suggested that a relatively high endogenous GA content and relatively low level of ABA and ethylene promote growth and prevent ripening during the maturation period and the ripening process is correlated with increase in ethylene and ABA and decrease in gibberellins.

In the present study a significant increase in ABA has been observed in the nuts of root(wilt) affected palms over healthy and apparently healthy palms. A drastic reduction in yield of the affected palms as reported by Radha et al. (1962) was noticed here also.

The higher accumulation of ABA might be the reason for the abnormal shedding of immature nuts and female flowers associated with the root(wilt) disease complex. Thus it is seen that there is a definite cycle of cause and effect and finally the disease intensifies and result in complete deterioration of the palm.

The comparatively low yield in apparently healthy palms when compared to healthy palms revealed that the palm had already the attack of the disease though no visual symptoms are seen.

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## SUMMARY

## SUMMARY

This study was undertaken to find out the physiological bases for the pattern of degeneration and destruction of root(wilt) affected coconut palms. Healthy, apparently healthy and root(wilt) affected palms at five different intensities of the disease were selected for the study. A biochemical analysis of leaf, root and developing nut of the different types of coconut palms was carried out to make the following estimations.

### 1. Estimation of gibberellins

The gibberellins were extracted from the leaf, root and nut of the different types of palms following the procedure of Radely (1958) and the activity of GA like substance was evaluated on the basis of cucumber hypocotyl elongation bioassay (Reeve and Crozier, 1975).

In the case of leaf, root and nut there was a significant reduction in the level of gibberellins in the root (wilt) affected palms when compared to healthy and apparently healthy palms. Within the diseased palms also the level of GA like substance declined as the disease intensified. Between healthy and apparently healthy

palms also there was a significant difference. The physiological explanation for the alterations in the hormone content and activity in the light of earlier work done are discussed.

## 2. Estimation of abscissic acid

ABA like inhibitors were extracted from the leaf, root and developing nut of the different types of palms according to the procedure of Linton et al. (1971) and the activity of ABA like inhibitors was assessed by the straight growth wheat coleoptile bioassay (Nitch, 1956 and Rudricki, 1969).

There was a significant increase in the ABA level in the leaf, root and nut of the root(wilt) affected palms when compared to healthy and apparently healthy palms. Within the diseased palms also the ABA level increased as the disease intensified. Between healthy and apparently healthy palms also there was a significant difference. The physiological and biochemical explanations for the change in ABA levels and activity on the basis of earlier observations are discussed.

A correlation study of the disease with the yield of the palms was also undertaken. The study revealed a significant reduction in nut yield in the diseased palms from those of healthy and apparently healthy palms. The comparatively low yield in the apparently healthy palms when compared to healthy palms is also physiologically explained.

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\* Originals not seen.

## **APPENDICES**

Appendix I (a)

Gibberellin content of leaf of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm

ANOVA

| Source    | SS        | df | MS       | F        |
|-----------|-----------|----|----------|----------|
| Treatment | 86827.493 | 6  | 14471.25 | 801.73** |
| Error     | 216.65    | 12 | 18.05    |          |

\*\* Significant at 1 per cent level

Appendix I (b)

Gibberellin content of root of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm

ANOVA

| Source    | SS        | df | MS       | F        |
|-----------|-----------|----|----------|----------|
| Treatment | 111034.91 | 6  | 18505.82 | 351.62** |
| Error     | 631.59    | 12 | 52.63    |          |

\*\* Significant at 1 per cent level

Appendix I (c)

Gibberellin content of nut of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm.

ANOVA

| Source    | SS       | df | Ms      | F        |
|-----------|----------|----|---------|----------|
| Treatment | 51688.73 | 6  | 8614.79 | 336.25** |
| Error     | 307.47   | 12 | 25.62   |          |

\*\* Significant at 1 per cent level

Appendix II (a)

ABA content in the leaf of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm.

ANOVA

| Source    | SS       | df | MS     | F      |
|-----------|----------|----|--------|--------|
| Treatment | 2311.402 | 6  | 385.23 | 71.6** |
| Error     | 64.51    | 12 | 5.38   |        |

\* Significant at 1 per cent level

Appendix II (b)

ABA content in the root of healthy, apparently healthy and various intensities of root(wilt) affected coconut palm

ANOVA

| Source    | SS       | df | MS     | F       |
|-----------|----------|----|--------|---------|
| Treatment | 3415.134 | 6  | 569.19 | 42.04** |
| Error     | 162.526  | 12 | 13.54  |         |

\* Significant at 1 per cent level

Appendix II (c)

ABA content in the nut of healthy, apparently healthy and various intensities of root(wilt) affected coconut palms

ANOVA

| Source    | SS       | df | MS     | F       |
|-----------|----------|----|--------|---------|
| Treatment | 3737.297 | 6  | 622.88 | 53.74** |
| Error     | 139.133  | 12 | 11.59  |         |

\*\* Significant at 1 per cent level

### Appendix III

Yield of nuts from healthy, apparently healthy and various intensities of root(wilt) affected coconut palm, for the last five years.

#### ANOVA

| Source    | SS       | df | MS      | F        |
|-----------|----------|----|---------|----------|
| Treatment | 9497.096 | 6  | 1582.85 | 65.288** |
| Error     | 581.856  | 24 | 24.244  |          |

\*\* Significant at 1 per cent level

**PHYSIOLOGICAL AND BIOCHEMICAL BASIS  
OF DEGENERATION IN ROOT (WILT)  
AFFECTED COCONUT PALM**

**BY  
SHEELA CHERIAN**

**ABSTRACT OF THE THESIS  
SUBMITTED IN PARTIAL FULFILMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE IN AGRICULTURE  
FACULTY OF AGRICULTURE  
KERALA AGRICULTURAL UNIVERSITY**

**DEPARTMENT OF AGRICULTURAL BOTANY  
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## ABSTRACT

Several studies on the physiological and biochemical aspects of the root (wilt) disease have been carried out and conclusions have been drawn. A recent study comprising of the estimation of protein, non-protein amino acids and cytokinin from the leaf, root and developing nut of healthy, apparently healthy and root (wilt) affected coconut palm was carried out in this laboratory (unpublished). The chlorophyll content of leaves was also estimated.

The present study comprised of estimation of gibberellins and abscissic acid from the leaf, root and nut of healthy, apparently healthy and five different intensities of root (wilt) affected coconut palms. The results of the biochemical analysis show that in the root (wilt) affected coconut palms there are alterations or deviations in normal metabolic activities from that of healthy palms. Earlier work done has shown a reduction in cytokinin content in the root(wilt) affected palm in addition to protein and chlorophyll content. The present study has revealed a deficiency of gibberellins another important growth regulator and an accumulation of the growth inhibitor, abscissic acid in the root(wilt) affected palms. The sites of synthesis and / interconversion of gibberellins are damaged together with

the impairment of the translocation system.

Increased respiration, lowered  $\text{Co}_2$  fixation and severe damage of the shoot system in the later stages are in brief, the internal and external expressions of the root (wilt) syndrome. Thus the shoot and root system which are interdependant are unable to function in harmony leading to a failure in their growth and development. An accumulation of ABA along with a reduction in gibberellin level might be the reason for the stunting syndrome seen in root(wilt) affected palms.