

Know about phytoplasma as pathogen in relation to coconut diseases and with reference to root wilt

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Introduction

Crop plants are often affected by diseases resulting in huge losses in their production and productivity. Various biological agents such as fungi, bacteria, viruses, phytoplasmas, etc. cause diseases in plants with diverse symptoms. The coconut palm is one of the most useful trees to mankind having multiple uses. However, this palm is also affected by various pathogens leading to different diseases which may be systemic (involving the whole palms) or localized/restricted to specific part such as leaf, stem, fruit, etc. Among the pathogens, the phytoplasma is a unique organism and probably less familiar to common man. It was therefore felt that it would be useful to provide more information on this group of pathogens to enable coconut growers.

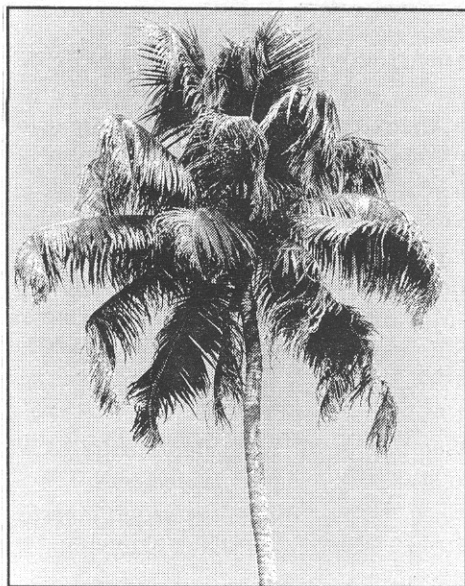


Fig : 1 A typical root wilt affected coconut palm

Phytoplasmas as Plant Pathogens

Japanese Scientists (Doi, Teranaka, Yora and Asuyama) in 1967 discovered a new group of organisms, associated with certain plant diseases and such organisms were referred to as mycoplasma-like organisms (MLOs) as they resembled Mycoplasma. By then Mycoplasmas were known as pathogens of human beings and animals. Following this, accelerated research had led to the discovery of the association of MLOs with a vast number of plant diseases in different parts of the world. As on today over 300 plant diseases are implicated to be incited by MLOs. In fact, several diseases which were originally believed to be of viral aetiology turned out to be due to MLOs. All these MLOs are currently referred to as 'Phytoplasmas', as accepted by the 'International Organization for Mycoplasma' in 1994.

The phytoplasmas are cell wall-free prokaryotic organisms which are distinct from bacteria and are found (restricted to) in the phloem sieve tubes of the vascular bundles of affected palms. These organisms are fastidious bodies. Unlike bacteria, fungi, etc. they are not cultivable in the laboratory in cell-free culture media and for this reason, identification of MLOs in the past had been very cumbersome. These organisms are ultramicroscopic and hence cannot be seen even by powerful research microscope. These are pleomorphic, (variable in shape and size) and are observable through Electron microscope (EM). There are certain

supplementary techniques also available for detection of phytoplasmal infections. The phytoplasmas are transmissible from infected plants to healthy plants through insect vectors and the organisms usually induce systemic symptoms-phytoplasma induced diseases are commonly referred to as 'yellows'. The plants affected by phytoplasmal diseases respond to broad spectrum antibiotics like Oxytetracycline Hydrochloride. They prevent the multiplication of phytoplasmas resulting in temporary remission in the symptoms. There are certain plant mycoplasmas with helical morphology which are referred to as Spiroplasma and these are cultivable in cell-free media. The non-helical forms are not yet amenable to cultivation in cell-free medium.

The spiroplasmas are thus distinct from phytoplasmas. The animal mycoplasmas (which are cultivable) and phytoplasmas have been proved to be genetically unrelated.

The phytoplasma parasitises after its entry into the plant, induce specific (systemic) symptoms and hence the infected plant suffers. The phytoplasmas can be transmitted from infected host to recipient host not only through insect vectors but also by using phanerogamic parasites such as dodder. Indicator plants such as periwinkle can also be infected in this way. As phytoplasmas are not-cultivable and hence they cannot be distinguished based on morphological features, attempts to distinguish them based on their genetic material using molecular techniques. As on to day, 75

phytoplasmas are distinguishable in this way and they have been classified into 20 phylogenetic groups (sub-clades).

Phytoplasmas in Coconut Diseases

In the past three decades, several coconut diseases of uncertain aetiology have been studied and association of phytoplasma has been reported in many of them. The world distribution of phytoplasma associated coconut diseases is presented in *Table 1*.

Symptoms of these diseases are widely documented in various countries. The classical lethal yellowing and - like diseases cause rapid death of palms and infected palms usually die within 3-7 months of the initial symptoms. The early symptoms are: shedding of immature nuts, blackening of immature inflorescences, necrosis of spindle leaves and death of root tips. Discolouration and necrosis of leaves usually start with the oldest leaf and progress to the younger leaves; mid whorl leaves occasionally show early discolouration. With the progress of foliar discolouration (yellowing) the spindle leaves cease to expand and become necrotic. From the tissues of the spindle leaf, a foul smell emanates and ultimately the growing point is affected, and crown falls-off. All the listed diseases could be generally placed under 'yellows'. A notable point is that the phytoplasma associated diseases of coconut in India (Root wilt and Tatipaka), are non-lethal but debilitating, whereas, all other phytoplasma diseases in the world are lethal *i.e.*, the infected palms die within a short span of time as spelt out. Non-lethal nature of these diseases (in India) could be an exception rather than a rule. Non lethal nature of certain phytoplasma diseases of other crop plants are also known. Nevertheless, in phytoplasma infected coconut palms severe disturbances occur in the phloem transport and several physiological/bio-chemical

changes take place (as for example in stomatal regulation, hormonal imbalances, respiratory rate, vascular sap constituents etc.); their trend often varies from one disease to another.

Among the enlisted coconut diseases, phytoplasma was first discovered in the Caribbean lethal yellowing (LYD) infected palms in 1972 and the causative organism was observed under EM (reported identically in 1972 from three independent laboratories). Phytoplasma as the causal agent of other coconut diseases came to light subsequently and the list is likely to extend. As an inducer of systemic diseases, the phytoplasma bodies are

present in different parts of the affected palm, even while restricted to phloem sieve - elements. Usually very high population of the bodies are found in the root tips, base of spindle, young inflorescences, etc. (sink sites).

As coconut phytoplasmas are also non-cultivable and cannot be distinguished morphologically, molecular diagnostic techniques have to be used for the characterization and differentiation. For example, phytoplasmas of the diseases in Tanzania and Kenya (East Africa) are shown to be genetically similar (but that differed from Cape St. Paul wilt and Awka wilt pathogens of West Africa); Caribbean phytoplasma is

Table 1. Phytoplasma diseases of coconut distributed in different parts of the world.

Sl. No.	Name of diseases	Geographical distribution	Insect vector
1.	Lethal yellowing	Caribbean Island (Bahamas, Cayman Islands, Cuba, Dominican Republic, Haiti, Jamaica); USA (South Florida & South Texas), Mexico, Belize, Honduras	<i>Myndus crudus</i>
2.	Blast, dry bud rot	Ivory Coast (West Africa (also suspected in South American countries and Indonesia)	<i>Recilia mica</i>
3.	Cape St. Paul wilt disease	Ghana (West Africa)	<i>Myndus adiopo-dumeensis</i>
4.	Kaincope	Togo (West Africa)	Not Known
5.	Kribi disease	Cameroon (West Africa)	Not Known
6.	Awka wilt/Bronze leaf wilt	Nigeria (West Africa)	Not Known
7.	Lethal diseases	East African countries <i>viz.</i> , Tanzania, Kenya and Mozambique	<i>Diastrombus mkurangai</i>
8.	Coconut stem necrosis*	Malaysia, Sumatra (Indonesia)	Not Known
9.	Root wilt Disease	South-East Asia (India (Kerala and Tamil Nadu states) (South Asia)	<i>Stephanitis typica</i> and <i>Proutista moesta</i>
10.	Tatipaka disease	India (Andhra Pradesh) (South Asia)	Not Known

*Current evidence points to the possible association of *Phytoplasma* in Natuna wilt and Kalimantan wilt diseases also in Indonesia.

more closely related to East African than West African phytoplasma. Hence the recent molecular techniques revealed that pathogens of lethal diseases of coconut in the Caribbean and Africa are not genetically identical; pathogens of West and East African diseases are also not identical. Phylogenetic analysis has also confirmed that phytoplasmas of lethal diseases of coconut (from USA, Mexico and Africa) form a separate cluster within the phytoplasma clade and West African organism formed a new sub-clade within this cluster - differing from East African one.

As no conventional protection measures are available for managing the phytoplasma infected plants at field level, coconut phytoplasma diseases control mostly depends on integrated management with a thrust on the exploitation of host genetical resistance against the pathogen and rehabilitation of plantations by regulatory measures. Since the phytoplasmal diseases are infectious, potentially dangerous, strict avoidance of their introduction to hitherto unrecorded regions/countries is of paramount importance.

Phytoplasma in Root Wilt Disease

The century old root wilt disease (RWD) of coconut is prevalent endemically in eight southern districts of Kerala State. The latest estimation is that the RWD has affected 24.05% out of 102 million palms in this endemic region. The spread of RWD to northern districts (even the northern most District of Kasaragod) of Kerala and also the border districts of adjoining Tamil Nadu has been detected. Recently, incidence of RWD in epidemic form in Cumbum valley of Tamil Nadu has been brought out.

It may be recalled that RWD is a slow decline, debilitating disease in contrast to lethal yellowing and similar diseases in Caribbean Islands, Africa etc. Flaccidity (ribbing of middle whorl

leaves) is the most consistent visual symptom of RWD. Others are yellowing and marginal necrosis of older leaves; whitening - softening and weakening of spindle leaves (Fig. 1).

Various biological agents, physiological reasons, soil/geochemical factors, etc. were discussed in the context of the cause of the disease. Since no success was met, it was felt that it is a systemic disease (not a disorder) and hence the involvement of an ultra - microscopic biological agent (mostly a phloem bound pathogen) cannot be ruled out. Thus presence of phytoplasma in RWD affected palms was observed by 1983. Constant association of the organism with the disease has since been made out.

Pleomorphic nature of RWD associated phytoplasma with its trilamellar membrane, DNA strands and ribosomes (internal organelles) resembling any other phytoplasma has been confirmed (Fig. 2.). A point need to be noted is that particularly in coconut, the overall concentration of phytoplasmal bodies is low (in any relevant disease) in contrast to other phytoplasmal diseases of crop plants; also the distribution of coconut phytoplasma in the vascular bundles of the palm is sparse, uneven and hence the organism may not be found in all vascular bundles of infected palms. Since the situation is similar in RWD, detection of phytoplasma in the diseased palms is often passed through with difficulties.

Physiological/biochemical changes in RWD affected palms have been found out; internal and external symptomatological features are also evident.

Since the disease has been endemic for long in the eight southern districts of Kerala, involvements of geochemical factors were proposed. However, involvement of such factor (s) as the causal or predisposing factor (s) of the disease is not available. Predisposing factors of plant diseases are those which might prepare the ground for the incidence of disease. However such factor(s) alone cannot merit the disease. Hence soil/geo-chemical factors in relation to RWD has to be viewed with due care.

The RWD phytoplasma has been transmitted from diseased to healthy palms by insect vectors *viz.*, lace bug (*Stephanitis typica*) and plant hopper (*Proutista moesta*) and the RWD symptoms have been reproduced; presence of phytoplasma in the recipient plants is confirmed. The organism also has been transmitted from RWD infected palms to periwinkle (*Catharanthus roseus*), an internationally accepted indicator plant, through a parasitic dodder (*Cassytha filliformis*) in line with such other plant diseases. Intensive efforts on *in vitro* culturing of RWD associated phytoplasma (in several serum enriched media, phloem sap based media and also in embryonated hen's eggs) pointed more to the non-cultivable, highly fastidious and filterable nature of the organism.

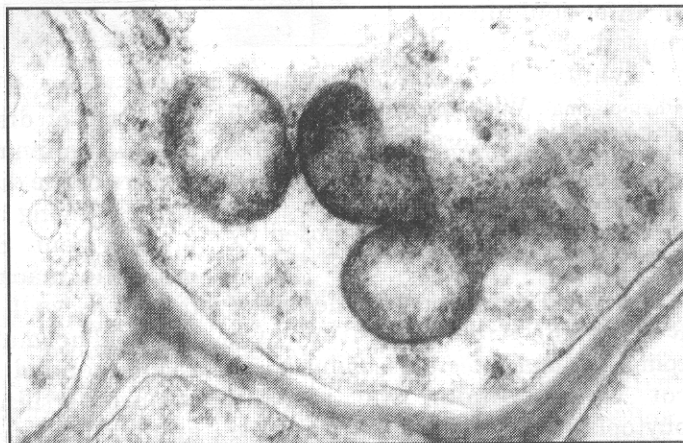


Fig : 2 Phytoplasma bodies in phloem sieve - tubes of root wilt infected palm as under Electron Microscope (E.M.). The organism colonize the host only in the sieve - tubes.

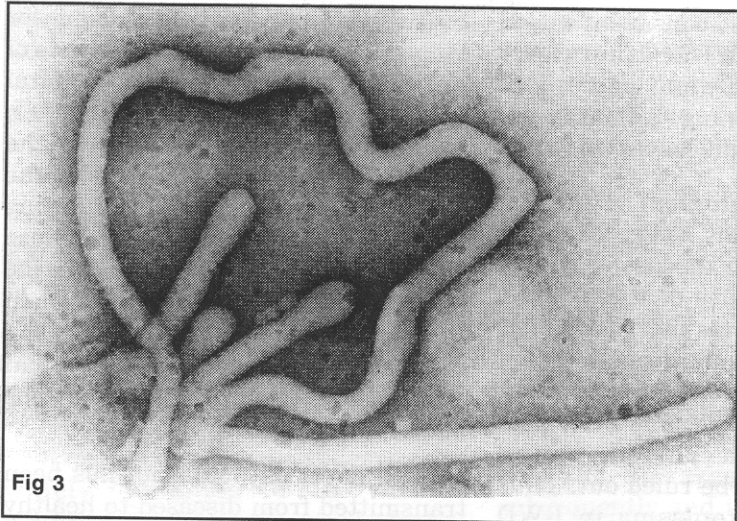


Fig 3

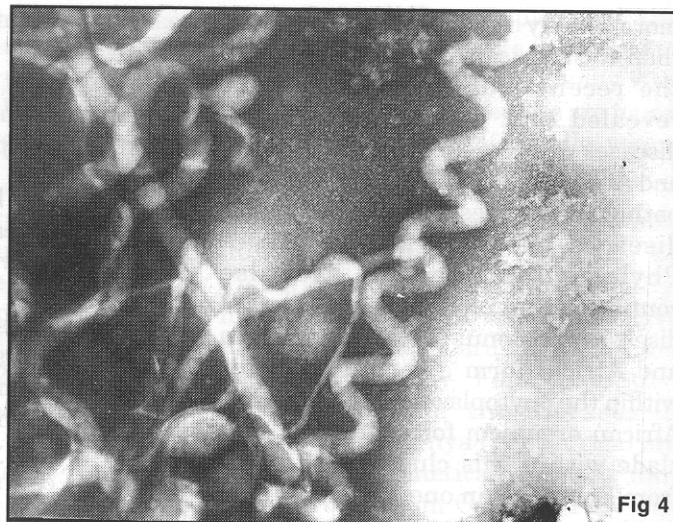


Fig 4

Spiroplasmas were not found associated with RWD (see Figs. 3 to 5 for glimpses on *Spiroplasma* cultured from other plant source). Molecular studies of RWD associated phytoplasma is in progress. RWD affected palms responded to the antibiotic, Oxytetracycline Hydrochloride and temporary remission in the disease symptoms was observed. From all such evidences it could be decided that RWD is an infectious disease caused by a non-cultivable biological agent - "Phytoplasma". With no other organism or factor, the disease symptoms have been so far reproduced. RWD is a slow spreading-infectious disease. The infected palms can be diagnosed by serological methods.

Figs. 3 to 5 : Feature of Spiroplasma under E.M. Cultured in cell-free medium from sesamum plants infected with phyllody disease, by the author and associate. Note the spiral structure of the organism. Such Spiroplasma was found neither associated with root wilt nor cultured from the diseased palms. Hence root wilt associated organism is identified as non-spiroplasmal i.e., phytoplasma which is non-cultivable.

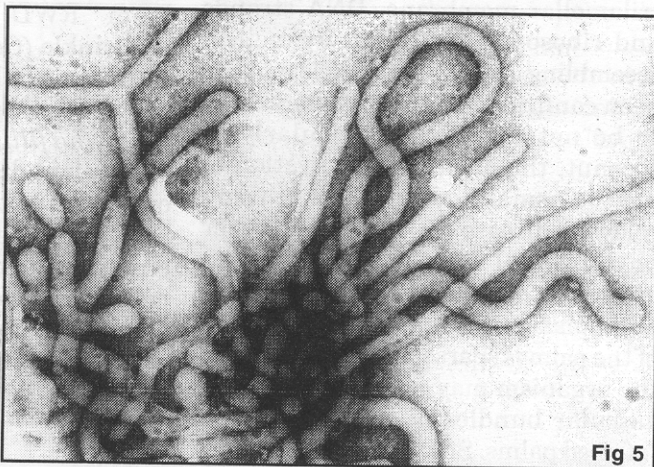


Fig 5

An added significance in the case of RWD is the superimposition of a complex fungal infection leading to leaf rot which render the already phytoplasma infected palms to a phase of fast decline and deterioration (Fig.6). While RWD is caused by phytoplasma, the leaf rot is due to

fungi. Hence, RWD - leaf rot complex is a case of distinct phytoplasma - fungal combination. The redeeming feature of the disease is its non-lethal nature, giving scope for sustainable management. Strategies have been evolved for managing the disease in the mildly as well as severely affected areas. The management practices recommended are: Application of balanced fertilizers, control of leaf rot, application of farm yard manure, irrigation during summer, provision of drainage, growing green manure crops, raising intercrops, removal of disease

advanced (uneconomic) palms and replanting with elite seedlings, removal of diseased juvenile palms, canopy restricting to harvest maximum sunlight and in mildly affected areas - removal of all diseased palms to eliminate the foci of infection.

Understanding RWD and adopting integrated management measures by growers would go a long way in improving coconut production/productivity in a sustainable manner.

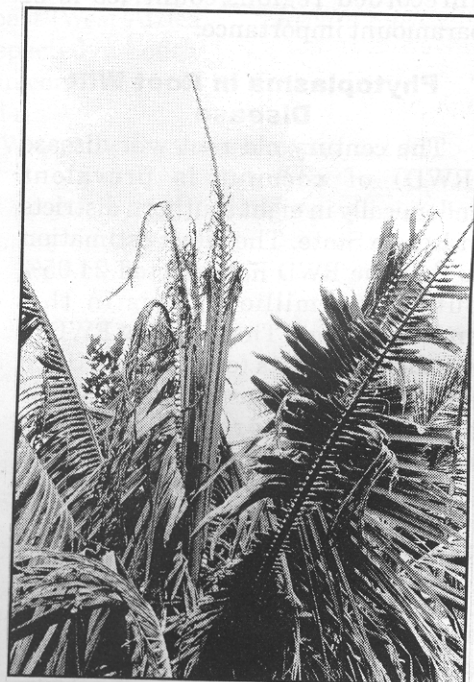


Fig 6 : Leaf rot symptoms in crown of root wilt affected palm