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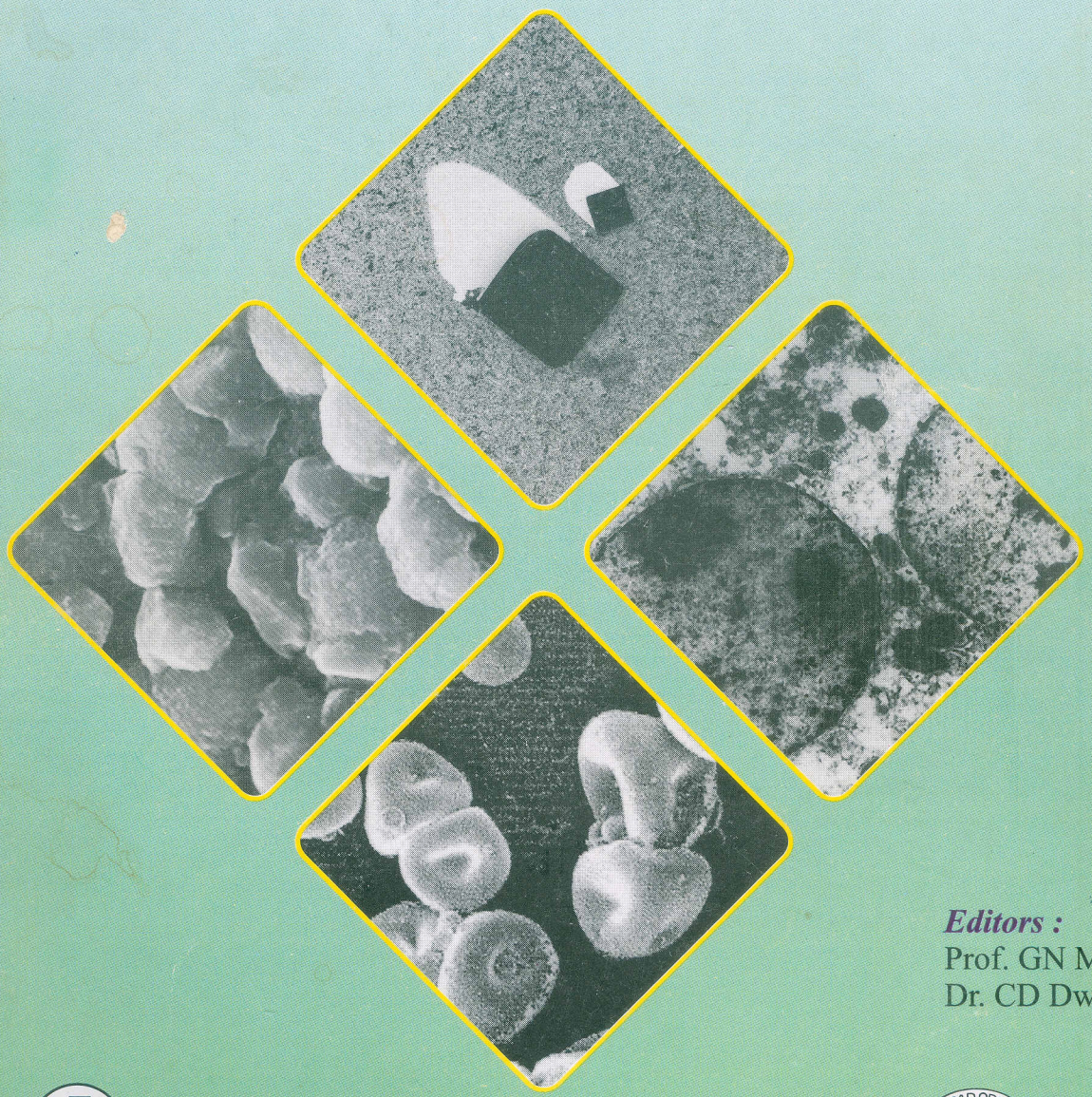

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ULTRA-STRUCTURAL STUDIES IN ESTABLISHING THE ETIOLOGY OF YELLOW LEAF DISEASE OF ARECANUT AND THE VECTOR ROLE OF THE PLANT HOPPER, PROUTISTA MOESTA (WESTWOOD)

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Yellow leaf disease (YLD) is a serious non lethal disease affecting arecanut palms in South India. Although Nayar and Selisker (1978) reported the presence of mycoplasma-like organisms (now referred as phytoplasma) in the sieve elements of diseased palms, the etiology of the disease continued to be uncertain. Systematic electron microscopic studies done since then has established the constant association of phytoplasma with the disease. Locating phytoplasma in the salivary glands of the plant hopper, proutista moesta offered required acquisition and incubation period (A+IP) on YLD areca palms and in the areca seedlings fed by infective hoppers established the vector role of the insect. The ultra-structural studies have thus aided in establishing the etiology and vector of the disease.

INTRODUCTION

Yellow leaf disease is the major disease of arecanut in Kerala, Karnataka, Maharashtra and Tamil Nadu. It is a non-lethal but debilitating disease. Apart from yellowing which is the characteristic symptom, the endosperm of the nuts in affected palms exhibit blackish discoloration having higher arecoline content. This renders the nuts unfit for human consumption.

Nayar and Selisker (1978) reported the presence of phytoplasma in yound sieve elements of areca palms declining with YLD in Kerala and Karnataka States. However, this needed further confirmation as only limited number of samples had been studied. The insect vector of the disease also had not been identified. This paper describes the ultra structural studies of the YLD areca palms and the insect vector in establishing the phytoplasmal etiology and the vector role of proutista moesta.

MATERIALS AND METHODS

Plant Tissues : YLD juvenile arecanut palms and adult palms and healthy palms from different locations in Kerala and Karnataka States and arecanut seedlings inoculated with infective plant hoppers and the uninoculated seedlings were sampled. In the case of destructive sampling, sub apical meristem, petiole of developing leaves, spear leaf,

rachilla and tender roots were sampled. In non destructive sampling tender root and rachilla were sampled.

The plant tissues were fixed in cold 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.2, post fixed in 2% osmium tetroxide in phosphate buffer, dehydrated in graded alcohol series followed by changes in acetone and embedded in Spurr's resin.

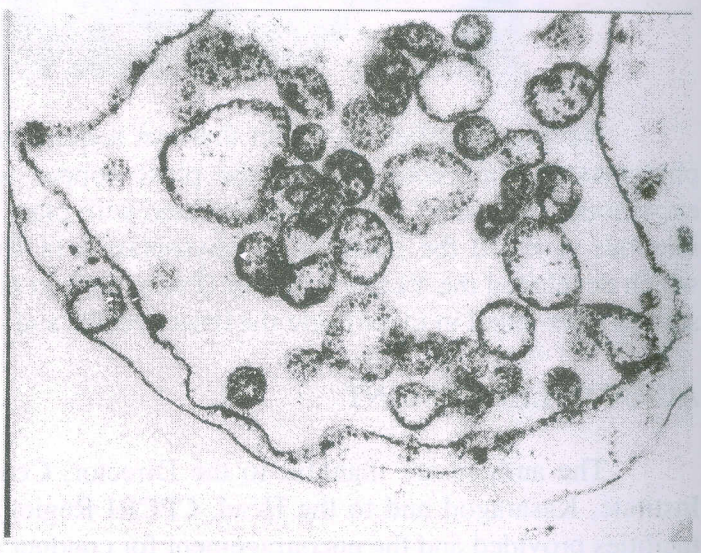
Insect Tissues : Laboratory reared freshly emerging adult plant hoppers were confined to tender leaves of YLD arecanut palms to render them infective by offering different A+IP (Ponnamma, Rajeev and solomon, 1991). The insects were recaptured and the salivary glands with head capsules were dissected and fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer pH 7.4 containing 0.17M sucrose at 40°C. Samples were post-fixed in 2% Osmium tetroxide and processed as in the plant tissues and embedded in Spurr.

Serial ultra-thin sections of the plant and insect tissues were made in LKB ultratome IV. Ultra thin sections of 600-700 A° were packed in 200 mesh uncoated copper grids and stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined under Carl Zeiss EM 109 Turbo transmission electron microscope operating at an accelerating voltage of 50 KV.

RESULTS AND DISCUSSION

Ultra thin sections of the plant tissues revealed the presence of phytoplasma in sieve tubes of YLD areca palms and were totally absent in healthy palms. The organisms were bound by a triple layered unit membrane having less dense fibrillar nuclear area and peripherally distributed ribosomes constituting the electron dense area. Pleomorphic forms ranging from spherical, oval and elongated beaded structures were observed (Figure 1). The size of phytoplasma ranged between 250-500 nm. They were often positioned close to the sieve tube wall and also found traversing through the sievepore. Phytoplasmas were found in increasing numbers in the juvenile tissues in the following order sub-meristem, root, rachilla and petiole of developing leaves. They were found in fewer numbers, lacking internal contents characteristic of degenerated forms were observed in yellowed mature leaves. The

Figure 1 : Phytoplasma in sieve tubes of YLD of arecanut

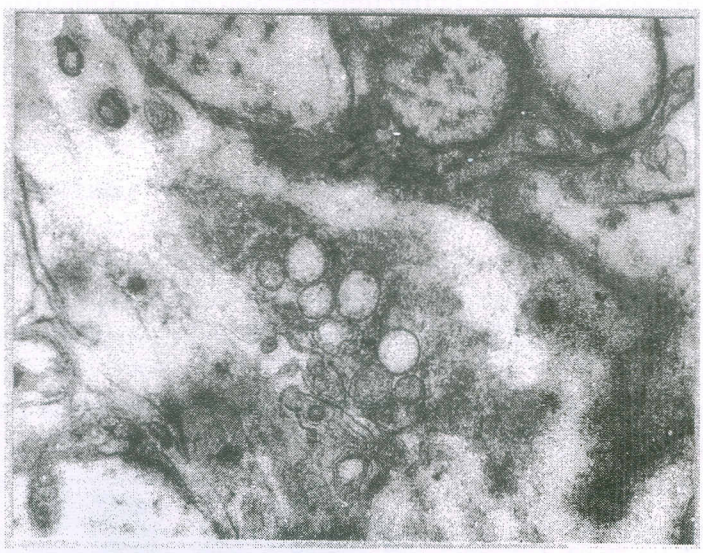


distribution of the organism was sparse. Only a few sieve tubes in a vascular patch contained them (Solomon, 1991). Occasionally the phytoplasma had hyperinfection by a tailed bacteriophage. The exact role of the bacteriophage vis-vis symptom development is not known.

Phytoplasma had been consistently observed in all the 75 diseased palms studied and were absent in the 60 healthy palms examined. The palms sampled were from different locations in Kerala and Karnataka states and of various age groups. The ultrastructural studies have thus established the constant association of phytoplasma with YLD.

EM examination of the ultra thin sections of the plant hopper revealed the presence of phytoplasma in the acini of the salivary glands of hoppers offered A+IP of more than 30 days on YLD palms (Figure 2). However, the organisms were not observed in hoppers not fed on YLD affected palms and also in insects offered less than 30 days A+IP (Ponnamma et al., 1991).

Figure 2 : Phytoplasma in the salivary gland tissues of proutista moesta



Having identified on insect that can acquire and sustain the multiplication of phytoplasma, the vector role of the plant hopper was assessed in a transmission experiment. Arecanut seedlings inoculated with plant hoppers and sampled at regular intervals revealed the presence of phytoplasma in five out of six inoculated plants which developed the YLD symptoms (Ponnamma et al., 1997). Thus the ultastructural studies have aided in identifying the insect vector and confirming its vector role.

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