mixture rapidly mineralises small quantities of tissue at low temperature.

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VIRUS ASSOCIATED WITH COCONUT ROOT (WILT) DISEASE

ARVIND S. SUMMANWAR, S. P. RAYCHAUDHURI, K. JAGADISH CHANDRA
Division of Mycology and Plant Pathology
NAM PRAKASH
Division of Agricultural Physics, Indian Agricultural Research Institute, Delhi-12
AND
S. B. LAL
Central Coconut Research Institute, Kayangulam, Kerala

INTRODUCTION

THE root (wilt) disease of coconut is causing a serious concern in southern parts of India where coconut has the largest area under plantation.

The disease is characterised by symptoms of 'wilt' accompanied by flaccidity of leaflets and abnormal bending of leaves. The outer whorl of leaves often shows yellowing. The leaves are stunted and the crown is reduced. Ultimately, the malady results in considerable reduction in yield.

Earlier workers, on the basis of their transmission studies, concluded that the disease may be of virus origin.[3,6] Nagaraj and Menon[3] inoculated several plant species belonging to families Palmæ, Graminæ, Solanaceae, Cucurbitaceæ, Leguminoseæ and others. The disease was mechanically transmitted to cowpea, *Vigna sinensis* Endl.[5] Inoculated cowpea seedlings showed distortion and crinkling of trifoliate leaves.[2]

Although the possibilities of the disease to be of virus nature were indicated by earlier workers, no exact cause was yet established. Therefore, work was taken up in this laboratory, employing electron microscopy (em.) technique and infectivity tests to detect the nature of coconut root (wilt) disease.

EXPERIMENTAL : PROCEDURE

Young leaf and root samples were obtained, repeatedly, from severely diseased trees from the Central Coconut Research Station, Kayangulam, Kerala State, where the disease is a major problem. The leaves and roots were macerated separately in a wearing-blender and the material was exposed to low and high

ultra-centrifugation (Beckman Model L) in the following way for purification.

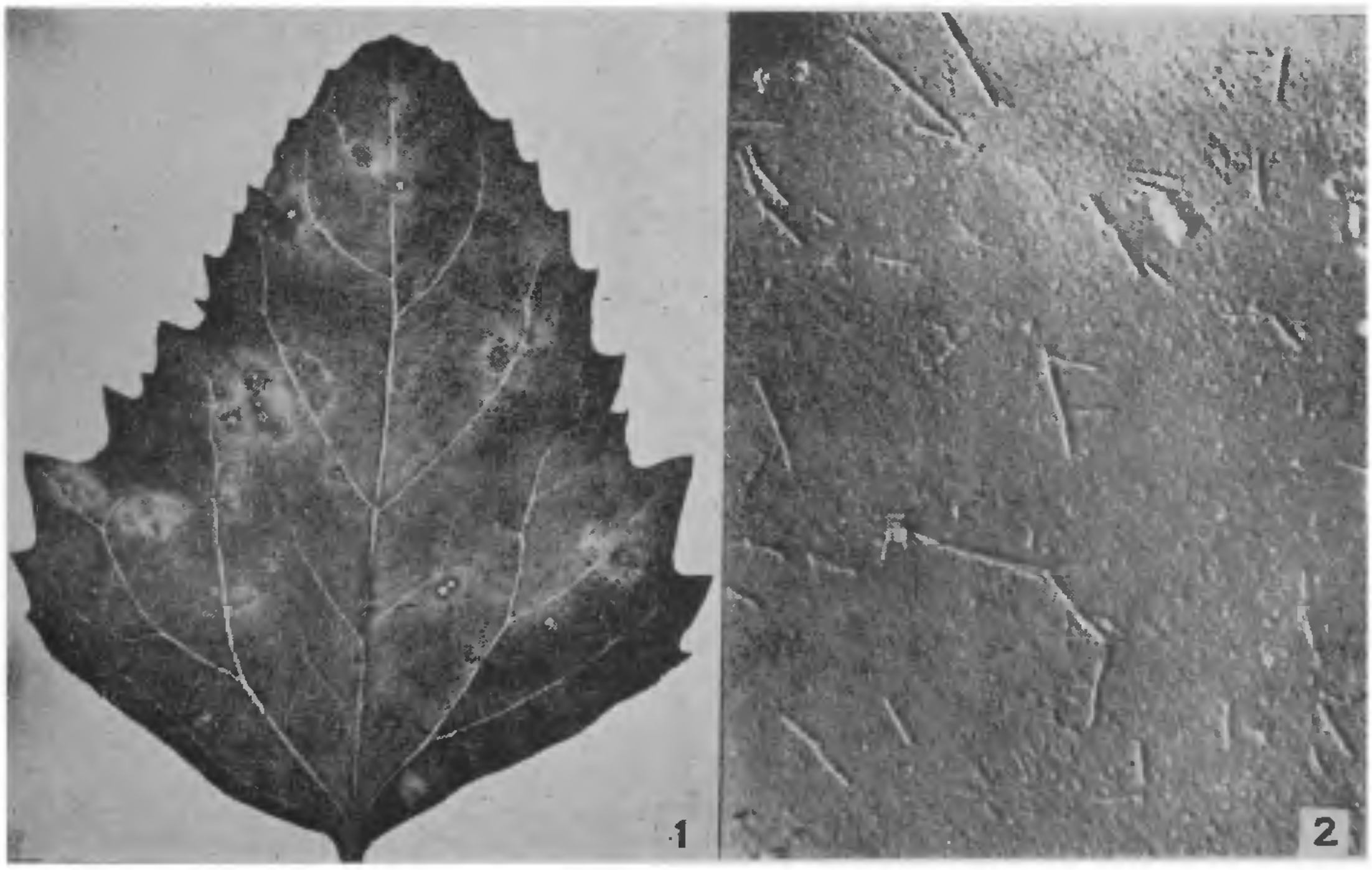
Crude juice
10,000 rpm for 15 min.
Supernatant — Pellet (discarded)
30,000 rpm for 120 min.
Pellet (dissolved in water) — Supernatant (discarded)
20,000 rpm for 20 min.
Supernatant — Pellet (discarded)
40,000 rpm for 100 min.
Pellet (dissolved in water) — Supernatant (discarded)
Filtered through Whatman No. 1 filter-paper
Purified material.

The other lot was sprayed on formvar-coated grids for electron microscopic studies. These were shadowed with gold palladium alloy (60 : 40) at an angle of about 25° and were examined with a Philips 100 Kv electron microscope Model Em 100 operated at 40–80 Kv.

In the same manner, leaves obtained from Calcutta, where the disease does not exist, and copra water obtained from nuts collected from diseased and healthy plants were purified as discussed above for examination under em.

Experimental Results

In infectivity tests with purified materials obtained from diseased leaves and roots, a few



Figs. 1-2. **Fig. 1.** Local lesions on *Chenopodium amaranticolor* caused by coconut root (wilt) disease. Fig 2. Electron micrograph showing rod-shaped particles associated with coconut root (wilt) disease. × 21 000.

The purified materials thus obtained from the diseased leaves and roots were divided into two lots. With the first lot, various herbaceous hosts, *viz.*, *Chenopodium amaranticolor*, *Nicotiana glutinosa*, *N. tabacum*, *Datura stramonium* and cowpea were inoculated under glass-house conditions for infectivity tests. Similarly, infectivity studies on these hosts were undertaken with crude juice obtained from diseased leaves and roots as well as from healthy leaves obtained from Calcutta.

chlorotic lesions appeared on the inoculated leaves of *C. amaranticolor*, a week after inoculation, with a reddish ring at the periphery later (Fig. 1). No infection was observed on several other hosts tested. From lesions thus obtained on *C. amaranticolor*, several *C. amaranticolor* plants were inoculated; virus concentration thus increased and was purified as described above. On examination of this material under em., rod-shaped particles were noticed. The maximum length of the particles

is in the range of 320-360 mμ, the width being 24-25 mμ (Fig. 2).

Repeated examination of the healthy and the diseased samples, coconut leaves and roots, under em., after purification, revealed the presence of rod-shaped particles in the diseased specimens, *i.e.*, leaves and roots, but not in healthy ones. These particles are identical in length and width with those obtained from *C. amaranticolor* in em. studies. However, no *rod-shaped particles were observed in healthy* leaves or in copra water from diseased plants at any time.

Discussion and Conclusion

It has been well established in the present studies, that only the purified materials obtained from diseased leaves and roots are infectious on *C. amaranticolor*. However, crude juice from diseased leaves and roots is non-infectious. Thornberry[7] found that the infectivity of tobacco mosaic virus was directly proportional to the concentration of tannin content. Complete inhibition depends upon *concentration of acid and time of action*. Bawden and Kleczokowski[1] in their studies with strawberry virus found that none of the strawberry viruses are mechanically transmitted because of high tannin contents in the plants. Verma and Raychaudhuri[8] (in press) also found that *in vitro* potato virus X is almost completely inhibited in the presence of tannic acid of 200 ppm concentration. In the present studies, no infectivity is obtained with crude juice extracted from diseased leaves and roots. Only on purification, infectivity as expressed by local lesions, was observed on *C. amaranticolor* which could easily be multiplied on other plants of this species mechanically. The possible explanation lies in the fact that coconut plants also have high tannic content which must be *acting as inhibitor to virus infection* with crude material. But on purification, this inactivator is either eliminated or reduced so as to enable the purified material to express infectivity on *C. amaranticolor*, thus indicating the pathogen to be of virus origin.

The purified material obtained from *C. amaranticolor* and that from diseased coconut leaves and roots, when viewed under electron microscopy, repeatedly, revealed the presence of rod-shaped particles which were never observed with the purified healthy coconut leaf material. It is for the first time that electron microscopy technique has been used for detecting the nature of this disease. The rod-shaped particles observed from purified samples of *C. amaranticolor* and diseased leaves and roots of coconut were identical in length and width. *However, no rod-shaped or any other kind* of particles were observed in healthy leaves of coconut or in copra water obtained from nuts collected from diseased plants at any time. These results, none-the-less, indicate clearly the presence of virus-like particles associated with coconut root (wilt) disease. Electron microscopy is considered to be the most sensitive and sure method over other methods currently operative for the detection of plant *viruses.*[4]

The infectivity tests and electron microscopy technique have now clearly established that coconut root 'wilt' disease is of virus origin. The etiology and other aspects of the disease are being investigated through further work.

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