EARLY DETECTION OF *GANODERMA* INFECTION IN COCONUT AND ARECANUT

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Detection of 'root rot' or *anabe* disease infection in its early stages is difficult in coconut because of the time lag between infection and expression of visible symptoms. At present, the disease is diagnosed only on the basis of visual symptoms like yellowing of leaves, wilting and in advanced stages gummosis at the basal portion of the trunk. This often results in difficulty in adopting control measures at the appropriate time. As such, the necessity for detecting the disease in its early stages was felt. Kumar (1970) has, outlined some of the techniques for obtaining antigen specific antibody for detecting plant pathogenic fungus.

The culture of *Ganoderma* used in the study was isolated from infected roots of coconut palms and was grown for 40 to 45 days in Waksman's liquid medium at room temperature (28 ± 2°). The mycelium was retained by filtering and dried at 40°C and 600 to 700 mg of mycelium was ground in 15 to 20 ml physiological saline (PBS) and filtered through seitz filter. The filtrate was made up to 20 ml and this formed the antigen. Two ml of antigen was emulsified with equal volume of Freund's complete adjuvant (Difco) and injected intramuscularly thrice a week in 1.5 to 2 kg albino rabbits. The rabbits were bled after 12 days of last injection when the agglutination of the serum was at its maximum. The serum was separated by slow speed centrifugation (7000 rpm). Equal volume of antiserum + 0.15 m NaCl were mixed and to it, saturated solution of ammonium sulphate (3.2N) pH 7.0 was added drop by drop in quantity equivalent to the total volume of the mixture. The suspension was centrifuged at 6000 to 8000 rpm for 15 to 20 minutes. The resultant precipitate of antibodies was dissolved in 2 ml tris-HCl buffer (pH 7.2) and reprecipitated until the precipitate was absolutely white. Then the pH of the

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solution was raised to 8.5 with 0.5 m carbonate-bi-carbonate buffer (pH 9.0) before stirring it with Fluorescein Isothiocyanate (FITC) which was added to the solution at the rate of 50 mg per g of protein for four to five hours. The preparation was then eluted with Tris HCl buffer on the sephadex G-25 column to remove unconjugated dye.

Root sections of disease affected, apparently healthy and healthy coconut palms were cut and were flooded with one to two drops of FITC conjugated antiserum and left in the moist chamber for three to four hours at room temperature. After draining the unconjugated antiserum, the sections were washed with Tris-HCl buffer and mounted on buffered glycerin and examined under fluorescence microscope. The root sections of healthy palms showed pale green fluorescence in the xylem and phloem cells. However, the apparently healthy and diseased root sections showed additional apple green fluorescence in the xylem and phloem cells. No such intense green fluorescence areas could be made out in the xylem and phloem cells of healthy root sections. Kumar *et al.* (1975) observed apple green fluorescent dots in the greening affected citrus leaf sections when they were stained with FITC conjugated antiserum. Out of the samples examined 60 to 62 per cent of the root samples showed *Ganoderma* infection and the rest were found to be healthy (Table 81.1). The first report on the use of fluorescent antibody technique for detection of plant pathogenic fungi is by Beno and Allen (1964) on *Puccinia sorghi* and on *Polyporus tomentosis* by Kumar and Patton (1964). Price (1970) could successfully stain conidia of *Spherotheca pannosa* in a mixture of conidia and the fungus.

Table 81.1: No. of root samples examined for *Ganoderma* infection

Host	*Total No. of samples examined*	*No. of root samples showing disease infection*	*No. of root samples that did not carry the infection (healthy)*
1. Coconut	50	31	19
2. Arecanut	50	30	20
Total	100	61	39

The work described here was carried out as a necessity to investigate the disease infection in question, as the routine cultural methods failed to detect the fungus. Since the causal organism has a long incubation period, a technique of this nature which is very sensitive in detecting root infections by *Ganoderma* even before visible symptoms can appear, will be very much helpful to adopt disease control measures.

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