Yellow Leaf Disease of Arecanut. III-Root Region Microflora and Involvement of Toxin

B. M. Bopaiah*

Abstract

The root region microflora of disease early, disease advanced and apparently healthy palms from yellow leaf disease affected areas and healthy palms from disease free areas of Chikmagalur, Shimoga and Dakshina Kannada districts of Karnataka State were enumerated. The bacterial population was more in the disease affected area as compared to healthy area. But the fungal flora were less in the disease affected area of Aranthodu (Sullia) and Sagar (Shimoga) as compared to the disease free area. Qualitatively the microflora did not differ much in the healthy and disease affected palms and no selective proliferation of micro-organisms occurred in either healthy or disease affected palms. The study has indicated the possible involvement of a phytotoxin in the disease.

Introduction

Yellow leaf disease (YLD) of arecanut is prevalent in Kerala, Karnataka, Tamil Nadu and parts of Maharashtra. In Kerala State the disease is prevalent in 35 - 40 per cent of the area under arecanut. The disease is present in parts of Chikmagalur, Shimoga and Dakshina Kannada districts of Karnataka State. Though research on this disease was initiated in 1959, the etiology of the disease is not clearly understood. (Rawther, 1976; Nayar, 1976; Koshy et al., 1976; Mohapatra et al., 1976 and Mathai, 1976). In the present study, the rhizosphere microflora of yellow leaf disease affected and healthy palms were enumerated with a view to investigate the proliferation or inhibition of specific micro-organisms in the root region of YLD affected palms.

* Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, Karnataka

Materials and Methods

Sampling of rhizosphere soil

The samples were collected from the basin of areca palms at a distance of 30 - 60 cm from the bole and at 0 - 30 cm depth, according to the procedure of Bopaiah and Koti Reddy, (1982). Rhizosphere samples were collected from the disease affected areas of Koppa - Sringeri (Chikmagalur), Sagar (Shimoga) and Aranthodu-Sullia (Dakshina Kannada). Sampling was done from 3 palms each of 3 different groups of palms based on the disease intensity, viz., apparently healthy, disease early stage and disease advanced stage. The samples representing the healthy palms were collected from Vittal (Dakshina Kannada) and Tirthahalli (Shimoga) for comparison. The above studies were carried out during 1979 and 1980.

Dilution plating technique was employed for the enumeration of microflora (Allen, 1957). The population of bacteria, fungi and actinomycetes were counted using Nutrient Agar (NA), Martin's Rose Bengal Agar (MRBA) and Kustar's Agar (KA) respectively. The Warcup soil plate method was followed to enumerate the mycelial form of fungi in the root region (Warcup, 1950). The fungi isolated were identified by microscopic examination and bacterial and actinomycetes isolates were identified up to generic level following colony morphology, cultural, physiological and biochemical tests (Buchanan and Gibbons, 1974).

Roots from yellow leaf disease affected and healthy palms were collected for toxin extraction and they were chilled under deep freeze to facilitate maceration of the tissue. The crude toxin was extracted following method of Rai and Strobel(1969). Biological assay for the crude toxin was conducted by treating tomato leaves (7.5 cm trifoliate leaves) with 0.5 mg/ml concentration of compound as described by Strobel (1967).

Results and Discussion

The bacterial population in the root region of YLD affected and healthy palms are presented in Table 1. There was a significant difference in the bacterial counts between disease affected and healthy palms. However, within the diseased area the bacterial flora of apparently healthy palms and palms showing different intensities of disease (early to advanced YLD) did not vary much. Qualitatively bacteria did not differ much between the healthy and YLD affected palms. The bacteria inhabiting the root region of healthy and YLD affected palms are *Bacillus* sp., *Arthrobacter* sp., *Micrococcus* sp. and *Pseudomonas* sp.

The fungal flora was considerably less in Sullia and Sagar area (diseased) as compared to healthy areas of Vittal and Tirthahalli (Table 2). No difference in the fungal population was observed among the apparently healthy and diseased palms. Though severe root rotting of diseased palms was reported from Kerala (Rawther, 1976) such root rotting was not observed in Sullia and Sagar area. The dilution plating method yielded *Trichoderma harzianum, T. viride, Aspergillus niger, A. sydowi, A. fumigatus* and *A. tamari* in larger proportion. The other genera of fungi recorded were *Penicillium, Mucor, Rhizopus, Fusarium, Alternaria* and *Cylindrocarpon* species in both diseased and healthy palms. *Trichoderma harzianum, T. viride, Curvularia* sp., *Fusarium* sp. and *Cladosporium* sp. were observed in Warcup plates also.

Table 1. Bacterial population in the root region of yellow leaf disease affected and healthy areca palms [10^5]

Location	Healthy	Apparently healthy	Initial stage of YLD	Advanced stage of YLD
Sullia*	—	17.12	17.20	14.96
Koppa - Sringeri*	—	23.23	21.40	21.26
Sagar*	—	27.40	28.50	28.00
Vittal**	13.50	—	—	—
Tirthahalli**	10.40	—	—	—

*Yellow leaf disease affected area **Healthy (Disease free) area

Not much variation was noticed in the actinomycetes population in the sample collected from different areas except the rhizosphere samples of Vittal (Table 3). The actinomycetes belonged to the genera *Streptomyces, Nocardia, Micromonospora* and *Actinomyces.*

The soil pH, root exudate, soil organic matter and season are some of the factors which influence the microflora in the root region. The enumeration of the micro-organisms in the root zone of arecanut palms at Palode (South Kerala) revealed a higher level in the disease as compared to healthy palms (Anonymous, 1964). In the present investigation, the difference was observed in the quantitative bacterial population. No difference in the qualitative micro-flora was noticed in the YLD affected root regions.

The crude toxin obtained from the roots of yellow leaf affected palms resulted in reversible wilting of tomato leaves. The roots of healthy palms though yielded small quantity of toxin precipitate did not show any wilting. The tomato cuttings were treated with an aqueous solution (0.5 mg/ml) of toxin and the wilting was recorded when the margin of leaves become flaccid. Wilting symptom was observed in 2 min. This shows that in yellow leaf disease affected palms there is an accumulation of toxin like substances. Further investigations are needed to characterise the toxin principle.

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Table 2. Fungal population in the root region of yellow leaf disease affected and healthy areca palms [10^3]

Location	Healthy	Apparently healthy	Initial stage of YLD	Advanced stage of YLD
Sullia*	—	10.67	16.83	15.00
Koppa - Sringeri*	—	20.33	15.40	23.50
Sagar*	—	8.50	12.16	10.16
Vittal**	25.33	—	—	—
Tirthahalli**	24.00	—	—	—

*Yellow leaf disease area **Healthy area

Table 3. Actinomycetes population in the root region of yellow leaf disease affected and healthy areca palm [10^4]

Location	Healthy	Apparently healthy	Initial stage of YLD	Advanced stage of YLD
Sullia*	—	4.00	4.06	3.00
Koppa - Sringeri*	—	3.33	1.00	1.00
Sagar*	—	3.75	2.00	2.20
Vittal**	10.33	—	—	—
Tirthahalli**	2.70	—	—	—

*Yellow leaf disease affected area **Healthy area

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Discussions

Abi Cheeran:

How did you separate the toxin from plant sample?

B M Bopaiah:

Toxin is precipitated using ethanol as solvent and centrifuged. The precipitate is passed through Dowex column to get the crude toxin.