Management of Basal Stem Rot (Ganoderma) disease of Coconut using early diagnostic methods.

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Introduction: Basal stem rot or Ganoderma wilt caused by Ganoderma lucidam (Leys). Karst is a lethal disease affecting coconut production in India. Characteristic symptoms of the disease are oozing out of brown liquid from the basal portions of the stem, drooping and drying of leaves starting from lower whorl to top spindle leaf, heavy button shedding, upward extension of stem decay and death of palm. The diseased palms normally die within a period of 6-24 months from the appearance of initial visual symptoms. Integrated disease management practices like root feeding of fungicides 1.3 g aureofingin-sol +105g copper sulphate or 2 ml tridemorph in 100 ml water and soil drenching of 40 litres of 1% Bordeaux mixture, soil application of 5 kg neem cake, 500 g inoculums of Trichoderma harzianum multiplied in 50 kg farmyard manure and banana intercropping are recommended to contain the disease.

Appearance of bleeding patches or exudation of brown liquid from the basal portion of the stem is the initial visual symptom of the disease. By the time of dependable or clear cut visual symptoms appear in the palms, much damage has already been done to the palms. All the recommended disease control measures will be effective, only if the palms are treated in early stages of infection. Hence, developing some methods/ techniques for early identification of basal stem rot (BSR) affected palms will be useful in the management of the disease.

Earlier workers have attempted to develop some methods for the early diagnosis of the disease before visual symptom expression. Preliminary investigations indicated that few chemodiagnostic tests and physiological parameters were found to be useful tools for early detection of coconut diseases. Natarajan et al., reported that biochemical tests like "Iodine-potassium iodide" staining technique, KOH method and EDTA method were useful for early detection of the disease. Physiological parameters like leaf water content, transpiration of root (wilt) disease of coconut caused by Mycoplasma like organisms. In the present study, some promising chemodiagnostic and physiological test have been developed, the methodologies of the diagnostic tests are standardized, procedure for root sampling in the field and the usefulness of early diagnostic tests for the management of the disease have been reported.

➲ Materials and Methods:

Field experiments and laboratory tests have been conducted at Coconut Research Station, Veppankulam during 1995-2000 to find out the promising early diagnostic tests, standardizing their methodologies and to study their utility in disease management under field condition. Various laboratory diagnostic tests viz., estimation of bio chemical constituents, enzyme activities, biochemical tests/ staining techniques using dyes and salts and physiological parameters were performed for the early detection of the disease.

Chemodiagnostic tests Ethylene diamine tetra acetic acid disodium salt (EDTA) method Triphenyl Tetrazolium Chloride (TTC) method and physiological parameters relative water content (RWC) and electrical conductivity (EC) were found to be reliable tests.

➢ Chemodiagnostic tests

- **EDTA (Ethylene diamine tetra acetic acid – di sodium salt) Test:** One gram of chopped root tissue was immersed in 10 ml of 0.3 M ethylene diamine tetra acetic acid – disodium salt solution. The optical density (O.D) values are then recorded in spectronic – 20 colorimeter at 100 nm after one hour of incubation.
- **Triphenyl Tetrazolium Chloride (TTC) test:** One gram of root tissues chopped into small pieces wereimmersed in 10 ml of 0.1% 2,3,5 triphenyl tetrazolium chloride solution for a period of 24 hrs. After incubation, the optical density values were recorded in spectronic – 20 colorimeter at 460 nm.

➢ Physiological parameters

- **Electrical conductivity (EC):** Coconut root tissues (1 gm) were placed in 100 ml beaker containing 40 ml of distilled water. One hour after incubation, the electrical conductivity of the ambient solution was measured in a digital conductivity bridge.
- **Relative water content (RWC):**

The leaf relative water content was calculated as below

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Targid weight} - \text{Dry weight}} \times 100$$

Turgid weight was estimated by taking the leaf sample sin petridishes containing water for four hours.

➢ Root sampling for early diagnostic tests:

Since the coconut tree has extensive root system with plenty of adventitious roots, the root sampling procedure is very important for correct diagnosis with

chemical or physiological tests. Appropriate root sampling alone will give accurate and reliable results in diagnostic tests. So root sampling depth and direction for early diagnostic tests have been standardized.

To find out the optimum depth of root sampling for early diagnostic tests, roots from healthy and diseased palms were collected in the basin area of 1.8 m radius at different depths viz., 5,15,30,45 cm and subjected to the EDTA and TTC tests. Root samples were collected from East, West, North and South directions of coconut palms at 15-30 cm depth to undertake early diagnostic tests.

➢ Field testing of early diagnostic tests:

Two hundred and twenty five apparently healthy palms adjacent to fifty diseased palm were selected from four gardens in the disease endemic areas for field testing of diagnostic tests. Periodical analysis of coconut palms with early detection techniques were attempted to fix up critical values.

➢ Usefulness of diagnostic tests for disease management:

A field experiment was laid out in disease endemic area to find out the utility of the early detection test for the management of the disease. Observations on disease intensity and nut yield were recorded in the trees with and without basal stem rot symptoms and in treated and untreated trees.

➲ Results and discussion:

In the chemodiagnostic tets EDTA and TTC tests, BSR affected root tissues recorded higher optical density (O.D) values that the healthy root tissues. The O.D values increased with increase in disease intensity (**Table 1**). The results of the EDTA test are found to be in accordance with the findings of Kamala Thirumalaiswamy.

The physiological parameter, electrolyte leakage or conductivity (E.C) was higher in root tissues of diseased severity. IN BSR affected coconut palms. The E.C increased with increase in disease severity. In BSR affected coconut palms, the leaf relative water content (RWC) was lesser that the healthy palms. The leaf water content was negatively correlated with disease intensity. The demarcation of healthy and diseased coconut tissues in chemodiagnostic and physiological tests in useful for the early diagnosis of the disease.

Significant differences in O.D values were observed between healthy and diseased tissues collected at various depths in EDTA and TTC tests. However the effect was more pronounced in the roots collected at 15-30 cm depth (**Table 2**).

The results (**Table 3**) indicated that there was no marked difference in the O.D values of healthy and diseased root samples obtained from four different directions. It is concluded that root sampling at 15-30 cm depth from any direction of the palm is found optimum for early detection tests.

Table 1: O.D. values in chemodiagnostic tests and physiological parameters of healthy and BSR affected coconut palms.

Plant Sample	Disease index	Optical density values*		Electrical conductivity (µs S-1)	Relative water content (%)
		EDTA test	TTC test		
Diseases – mild	Below 15	0.28	0.18	92.4	52.4
Diseased – moderate	15-40	0.36	0.24	93.8	50.1
Diseased -severe	Above 40	0.62	0.38	112.0	49.0
Apparently healthy	-	0.12	0.08	73.2	59.7
Healthy	-	0.09	0.06	73.2	59.7
C.D (P=0.05)	-	0.04	0.03	4.80	1.46

* Mean of 10 replications.

Table 2: O.D values of BSR affected and healthy root samples collected at different depths in chemodiagnostic tests.

Sampling Depth (cm)	EDTA test (O.D at 400 nm)*			TTC test (O.D at 460 nm)*		
	Healthy	Diseased	Difference in OD	Healthy	Diseased	Difference in OD
5	0.18	0.53	0.35	0.03	0.19	0.16
15	0.09	0.56	0.47	0.05	0.29	0.24
30	0.07	0.57	0.50	0.04	0.30	0.26
45	0.17	0.50	0.33	0.08	0.22	0.14
Mean	0.13	0.54	0.41	0.05	0.25	0.20

*Mean of 10 replications

EDTA test	C.D (P=0.05)	TTC test	C.D (P=0.05)
Disease	0.04	Disease	0.03
Depth	0.09	Depth	0.06
Interaction	0.18	Interaction	0.11

Table 3: O.D.Values of BSR affected and healthy root samples collected at different directions in chemodiagostic tests.

Sampling Depth (cm)	EDTA test (O.D at 400 nm)*			TTC test (O.D at 460 nm)*		
	Healthy	Diseased	Difference in OD	Healthy	Diseased	Difference in OD
East	0.24	0.44	0.20	0.04	0.24	0.20
West	0.28	0.46	0.18	0.06	0.26	0.20
North	0.24	0.50	0.26	0.05	0.26	0.22
South	0.23	0.46	0.23	0.05	0.27	0.22
Mean	0.25	0.47	0.22	0.05	0.26	0.21

*Mean of 10 replications

EDTA test	C.D (P=0.05)	TTC test	C.D (P=0.05)
Disease	0.05	Disease	0.03
Directions	N.S	Directions	N.S
Interaction	0.08	Interaction	N.S

Table 4: Critical levels of apparently healthy palms in chemodiagnostic and physiological tests.

Diagnostic tests	Test values (range)		Critical level*
	Initial	After 14 months	
EDTA test (O.D at 400 nm)	0.04 - 0.60	0.14 – 0.75	0.20
TTC test (O.D at 460 nm)	0.02 – 0.40	0.08 – 0.70	0.14
E C test (µs S-1)	40.0 - 95.6	54.9 – 108.0	88.0
Relative Water Content (%)	56.0 – 63.5	45.0 – 59.0	50.2

*Critical level at which symptom expression takes place in coconut palms.

Table 5: Usefulness of early diagnostic tests for the management of basal stem rot disease of coconut

Treatments	Disease index*			Mean nut Yield/palm/year*	
	Initial (Aug '98)	Upto Oct 2000	Increase over initial	Pretreatment	Treatment period 1998-2000
Trees without symptoms** -treated	-	3.26	3.26	80	90
-Untreated	-	18.40	18.40	102	83
Trees with symptoms** -treated	12.16	15.80	3.64	104	94
-Untreated	14.12	39.52	25.40	94	67
C.D (P – 0.05)	NS	7.74	-	NS	6

*Mean of six replications

**Trees without symptoms showing high O.D values in early diagnostic tests.

All the above diagnostic tests are dependable, only when they are helpful to contain the disease in the field. In the field experiment on theutility of early diagnostic tests for disease management (**Table 5**), the coconut trees without visual symptoms (but showed higher values than critical level in early detection tests) applied with recommended disease management practices registered a lower diseases index (3.26) as compared to the coconut trees showing visual symptoms with treatment (15.80) and without treatment (39.52). The nut yield was increased in treated coconut palms without symptoms but identified as infected palms by diagnostic techniques. Hence it is concluded that any of these diagnostic techniques can be used for early identification of BSR infected coconut trees in the field and for the most effective disease