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ACKNOWLEDGEMENTS. We thank Prof. V. S. Rama Das for providing fluorescence spectrophotometer. Financial assistance in the form of fellowship to K.K. from UGC and Research Associateship to M.V.S. from CSIR, Government of India, is gratefully acknowledged.

Received 12 January 1998; revised accepted 17 September 1998

## Foliar levels of rare earth elements and thorium in coconut palm in relation to root (wilt)-disease

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Concentrations of major nutrients and micronutrients, rare earth elements (REEs), Th and nutrient/REE ratios in the leaves of diseased and apparently healthy coconut palms of the root (wilt)-disease-affected tract and healthy palms of the disease-free tract, covering three major soil types of Kerala, namely alluvial (entisols), laterite (ultisols), and sandy (entisols), were examined in relation to the disease. Accumulation of major nutrients, especially K, was generally observed in the leaves of diseased palms. Mg content of leaves of palms growing on laterite soil in disease-affected tract was lower than that of palms

in the disease-free tract. The leaf concentrations of La, Ce, Pr, Nd, Sm, Gd and Th did not show significant differences between healthy and diseased palms. The only exception to this trend was Th whose concentration was less in the diseased than in apparently healthy palms growing on laterite soil in the disease-endemic area than that in the healthy palms of the disease-free area. Some of the essential plant nutrients (EPN)/La, and EPN/Ce ratios were significantly different in palms of the disease-affected tract compared to that in palms of the disease-free tract indicating imbalances in the relative concentrations of EPNs and REEs. These results call for more detailed study of the geochemical differences between the disease-affected and disease-free tracts for identification of the soil chemical factors associated with the incidence of the disease.

COCONUT root (wilt)-disease has a history of over a century. It was first reported in 1874 in Erattupetta of Meenachil taluk in Kottayam district of Kerala State. It is a debilitating disorder affecting about 0.41 million hectares of coconut gardens in the State, causing an estimated yield loss of 968 million nuts annually<sup>1</sup>. Presently, the disorder is seen mostly in the central and southern districts of Kerala.

Over the past few decades, considerable research has been done on various aspects of the root (wilt)-disease. Etiology of the disease has been examined from several angles namely, bacteriological, fungal, virological, nematological, and nutritional aspects. The work of Solomon *et al.*<sup>2</sup> revealed the presence of mycoplasma-like organisms (MLOs) in the phloem element of root (wilt)-disease affected coconut palms. Valiathan *et al.*<sup>4</sup> reported that presence of high Ce and low Mg levels in leaves may be responsible for the incidence of the disease. They also proposed a common geochemical basis for endomyocardial fibrosis in human beings and root (wilt)-disease of coconut palm. Their study was based on analysis of a few leaf samples collected from healthy and diseased palms. In view of the abundance of monazite deposits (the source of rare earth elements and Th) in Kerala soils, the possibility of absorption of these elements, especially Ce, in large amounts by coconut palms cannot be overlooked. The present study was therefore taken up to examine in detail the levels of rare earth elements (REEs) and Th in coconut palms growing in disease-affected and disease-free tracts of the State.

Based on the prevalence of the disease<sup>1</sup>, the districts of Kollam, Pathanamthitta, Idukki, Kottayam, Alappuzha and Ernakulam, and parts of Thrissur district, were considered disease-affected areas. The districts of Thiruvananthapuram, Palakkad, Malappuram, Wayanad, Kozhikkode, Kannur, Kasaragod, and parts of Thrissur were considered disease-free areas. Leaf samples were collected from coconut palms growing in root (wilt)-endemic areas and root (wilt)-free areas, repre-

three major soil types, namely alluvial (Entisols), laterite (Ultisols), and sandy (Entisols). From the disease-endemic areas, samples were collected from the disease-affected palms, and healthy palms.

The healthy palms in disease-affected area are designated as apparently healthy palms. From the disease-free tract, samples were collected from healthy palms. From each garden selected in the disease affected tract, samples were collected separately from five diseased and five apparently healthy palms, and pooled to form a sample for each category. Leaflets were collected from the frond arrived at by the formula  $[(N/2) + 1]$ , where  $N$  is the total number of fronds present on the crown of the palm. Altogether 238 samples were collected from all over Kerala. The particulars of the samples collected under each category of the palms are given in Table 1. Oven-dried (75°C) and powdered leaf samples were analysed for major nutrients and micronutrients, namely N, P, K, Ca, Mg, S, Fe, Mn, Cu and Zn. Nitrogen was analysed by Kjeldahl method. The other nutrient elements were analysed after digesting the samples with nitric-perchloric acid mixture (2:1 v/v). Phosphorus in the digest was estimated by vanadomolybdophosphoric yellow colour method; potassium, using a flame photometer; and sulphur by turbidimetric method. Calcium, Mg, Fe, Mn, Cu, and Zn were determined using an atomic absorption spectrophotometer.

REEs, namely La, Ce, Pr, Nd, Sm and Gd were analysed using inductively coupled plasma-mass spectrometer (ICP-MS) at the Materials Chemistry Division, Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam. The acid digests of leaf samples prepared by the same method as given above were used for the analysis of REEs also. The detection limit for all the rare earths were 60 ng g<sup>-1</sup>.

Thirty leaf samples (10 of each, representing diseased, apparently healthy, and healthy palms) were also analysed for Th by instrumental neutron activation analysis (INAA) at the Health Physics Division, Bhabha Atomic Research Centre, Mumbai. The leaf samples were heat-sealed in polythene containers along with Th standard. These samples were then irradiated in a neutron flux of  $10^{13}$  n cm<sup>-2</sup> s<sup>-1</sup> in Apsara Research Reactor for a period of 7 h. The samples were then cooled to allow the interfering activities generated from other elements present in leaves such as <sup>24</sup>Na from sodium, <sup>42</sup>K from potassium, <sup>56</sup>Mn from manganese, and <sup>38</sup>Cl from chlorine, to decay. The samples were cooled for a total period ranging from 12 to 15 days. The samples and the irradiated standards were then counted for the characteristic gamma rays emitted from <sup>233</sup>Pa, the radionuclide produced from Th. <sup>233</sup>Pa has a half-life of 27 d and emits gamma radiation of 311.8 keV. The minimum detection limit for Th by this method is 1.5 ng. The irradiated samples and elemental standards were counted for 4 h each, using 54 CC

Hyperpure Germanium (HpGe) detector coupled to 4084 channel, multichannel analyser to measure the gamma activity. From the count rates, the quantity of Th present in the samples were determined in accordance with the count rates obtained for the Th standard.

Leaf nutrient levels in coconut palms in relation to root (wilt)-disease are given in Table 2. Soil-wise differences were noticed in nutrient levels in palm leaves in relation to the disease. Thus in alluvial soil, the diseased palms accumulated more K than apparently healthy and healthy palms, while both diseased and apparently healthy palms had lower Zn content in their foliage than the healthy palms of the disease-free tract. Palms growing on laterite soil in the disease-affected tract had higher contents of P and Cu than healthy palms of the disease-free tract while Mg content of leaves of these palms was less than in healthy palms. In sandy soil, diseased palms accumulated more N, K, S and Cu than healthy palms. In these cases also, barring K, the differences between diseased and apparently healthy palms were not significant. The higher content of Cu in the foliage of palms of the disease-affected tract could be due to the frequent spray of Bordeaux mixture by the farmers of this area. The accumulation of major nutrients and lower levels of Mg in root (wilt)-affected palms was also reported earlier<sup>7</sup>. Greater accumulation of K was consistently observed in diseased palms irrespective of the soil type. This warrants detailed study.

Concentrations of REEs and Th in palm leaves in relation to the disease are presented in Table 3. The differences in individual REE concentrations among diseased, apparently healthy and healthy palms were not statistically significant for alluvial and sandy soils. However, in laterite soil, leaf concentration of Gd was significantly less in both disease-affected and apparently healthy palms of the diseased tract than in palms of the healthy tract. The total concentrations of the REEs and Th did not show significant differences between diseased and healthy conditions.

Notwithstanding the similarity observed in the leaf composition of REEs (except in the case of Gd) among healthy, apparently healthy, and diseased palms, essential plant nutrient (EPN)/La and EPN/Ce ratios were worked out to examine whether there is any imbalance in their relative concentrations with respect to the disease. The

Table 1. Details of leaf samples collected

Soil type	D	AH	H	Total
Alluvial	32	21	29	82
Laterite	36	30	34	100
Sandy	21	18	17	56
Total	89	69	80	238

D, diseased; AH, apparently healthy; H, healthy.

other REEs were not considered for computation of the ratios because their concentrations in many samples were below the detection limit. The EPN/REE ratios given in Table 4 indicated that leaf Ca/Ce, Mg/Ce, Mn/Ce, Zn/La and Zn/Ce ratios were significantly less in palms (both diseased and apparently healthy palms) growing on alluvial soil in the disease-affected tract than in healthy palms growing on the same soil in the disease-free tract. However, in laterite soil, these ratios failed to indicate significant differences. On the other hand, some other ratios, namely N/La, N/Ce, K/La, K/Ce, Ca/La, S/La, Fe/La and Mn/La were found to vary significantly. The palms of the healthy tract had lower K/La, Fe/La and Mn/La ratios compared to the palms (both diseased and apparently healthy) of the disease-affected tract. Palms growing on sandy soil showed a similar trend with respect to K/La and K/Ce ratios as the palms growing on laterite soil, with higher ratios registered by palms growing in disease-endemic areas. On the other hand, Mg/La and Mg/Ce ratios showed a similar trend as in palms growing on alluvial soil, with palms of the disease-free tract registering higher values. However, the differences were not statistically significant.

Significant differences observed in EPN/REE ratios

between palms of the healthy tract and those of the disease-affected tract (both diseased and apparently healthy palms) growing on alluvial soil, and a similar trend (although statistically not significant) observed in palms growing on sandy soil suggest imbalances in EPN/La and EPN/Ce concentrations in the palm. Even though the individual REE concentrations (except that of Gd) did not vary significantly, the trends observed with the ratios of Ce and La with nutrient elements are suggestive of La- and/or Ce-induced nutritional imbalances. These results agree with that of Valiathan *et al.*, who found that higher leaf Ce levels were associated with low Mg levels. At the same time, it is not possible to consider the individual REE concentrations as toxic because in several other plants much higher foliar levels than those observed in the present study have been reported with apparently no adverse effects<sup>8</sup>. Further, soil application of REEs has been reported to enhance growth and productivity of crops<sup>9</sup>, and La and Ce have been listed as beneficial elements for crop plants<sup>10</sup>. Studies of soil REE levels in relation to cadang-cadang disease of coconut palm in the Philippines have also failed to indicate toxicities of these elements in the incidence of the disease.

Table 2. Nutrient composition of coconut palm leaves in relation to root (wilt)-disease

Nutrient	Alluvial			Laterite			Sandy		
	AH	H	D	AH	H	D	AH	H	D
N (%)	1.73	1.66	1.66	1.94	1.85	1.77	1.94a	1.84ab	1.66b
P (%)	0.16	0.14	0.15	0.16a	0.14b	0.15ab	0.17	0.15	0.14
K (%)	1.61a	1.14b	1.15b	1.84a	1.60a	1.23b	1.43a	1.04b	0.83b
Ca (%)	0.28	0.30	0.34	0.35a	0.29b	0.34a	0.34	0.38	0.41
Mg (%)	0.27	0.26	0.33	0.25a	0.22a	0.31b	0.24	0.28	0.26
S (%)	0.18	0.17	0.15	0.18	0.18	0.18	0.23a	0.22a	0.15b
Fe (ppm)	296	268	341	321	237	264	365	310	284
Mn (ppm)	117	131	132	172	126	158	98	104	95
Cu (ppm)	8.4	10.8	7.0	7.1a	6.6a	4.3b	9.4a	7.7ab	5.4b
Zn (ppm)	29.8	36.4	62.2	38.6	30.4	43.2	33.3	40.3	31.0

D, diseased; AH, apparently healthy; H, healthy.

Means followed by the same alphabet under a soil type are not statistically different at  $P=0.05$ .

Table 3. Concentrations of REEs (ppm) and Th (ng g<sup>-1</sup>) in coconut palm leaves in relation to root (wilt)-disease

Element	Alluvial			Laterite			Sandy		
	D	AH	H	D	AH	H	D	AH	H
La	1.893	2.099	2.096	2.654	2.489	4.026	0.530	0.596	0.414
Ce	2.358	2.971	2.286	3.574	2.777	3.355	0.907	0.993	0.875
Pr	0.403	0.437	0.362	0.532	0.483	0.841	0.125	0.149	0.054
Nd	1.644	1.752	1.317	2.191	1.994	3.723	0.474	0.534	0.351
Sm	0.180	0.220	0.129	0.244	0.212	0.407	0.054	0.062	0.022
Gd	0.233	0.280	0.298	0.295a	0.246a	0.613b	0.031	0.048	0.034
Total	6.712	7.759	6.488	9.490	8.201	12.964	2.121	2.383	1.749
Th*	30.5	32.4	23.9						

D, diseased; AH, apparently healthy; H, healthy.

Means followed by the same alphabet under a soil type are not statistically different at  $P=0.05$ .

\*Means of 10 values; irrespective of the soil type.

Table 4. Ratios of concentrations of essential plant nutrients and rare earth elements in coconut palms in relation to root (wilt)-disease

Ratio	Alluvial			Laterite			Sandy		
	D	AH	H	D	AH	H	D	AH	H
N/La	3.08	2.24	3.22	1.30ab	1.88b	0.74a	5.80	6.06	7.46
N/Ce	1.71	1.44	2.15	0.86ab	1.23b	0.68a	3.28	3.57	3.64
P/La	0.28	0.19	0.30	0.11	0.15	0.06	0.57	0.50	0.60
P/Ce	0.16	0.11	0.20	0.07	0.10	0.06	0.32	0.30	0.29
K/La	3.15	1.87	2.01	1.16a	1.52a	0.50b	4.23	3.80	3.71
K/Ce	1.74	1.08	1.40	0.76ab	1.03a	0.47b	2.40	2.23	1.76
Ca/La	0.46	0.35	0.75	0.22ab	0.27a	0.15b	1.05	1.27	1.82
Ca/Ce	0.26a	0.23a	0.49b	0.15	0.18	0.14	0.60	0.75	0.90
Mg/La	0.43	0.33	0.57	0.18	0.20	0.13	0.69	0.93	1.25
Mg/Ce	0.25a	0.21a	0.41b	0.12	0.13	0.12	0.39	0.55	0.61
S/La	0.30	0.22	0.31	0.12a	0.18b	0.07a	0.65	0.70	0.67
S/Ce	0.17	0.14	0.20	0.08	0.11	0.07	0.37	0.41	0.32
Fe/La	566	359	622	223a	253a	111b	993	884	1233
Fe/Ce	312	216	391	123	161	102	548	523	597
Mn/La	139	119	199	110a	103a	55b	236	281	366
Mn/Ce	82a	74a	128b	60	64	54	125	162	165
Zn/La	48a	44a	103b	23	30	17	112	104	130
Zn/Ce	28a	30a	68b	14	19	16	61	60	63

D, diseased; AH, apparently healthy; H, healthy.

Means followed by the same alphabet under a soil type are not statistically different at  $P=0.05$ .

Recent report of greater accumulation of REEs in the roots than in the tops of plants<sup>11</sup> demands verification of such a preferential distribution of these elements in coconut palm. Localization of Al, Mn, Cu and Co ions in the roots of root (wilt)-diseased palms has been reported<sup>12</sup>. Besides these, higher concentration of Ni and Sr in root tissues of the diseased palms compared to that of healthy palms also indicated a tendency for localization of certain elements or hindered translocation in the diseased palms<sup>13</sup>. Further studies are therefore required to bring out the role of REEs, if any, in the incidence of root (wilt)-disease.

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ACKNOWLEDGEMENTS. We thank Dr A. M. Michael, former Vice Chancellor, Kerala Agricultural University; Dr M. S. Valiathan, Vice Chancellor, Manipal Academy of Higher Education, and Dr Placid Rodriguez, Director, Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, for their interest in this project and for their encouragement. Our thanks are also due to Dr S. Krishnamony, Head, Health Physics Division, Bhabha Atomic Research Centre, Mumbai, and Dr G. Periaswamy, Head, Materials Chemistry Division, IGCAR, for providing analytical facilities.

Received 27 December 1997; revised accepted 21 September 1998