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DISTRIBUTION AND RETENTION OF OXYTETRACYCLINE HYDROCHLORIDE INJECTED INTO COCONUT PALMS AFFECTED BY ROOT (WILT) DISEASE*

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ABSTRACT

Oxytetracycline–HCI (OTC) was administered by pressure injector into the trunks of root (wilt) diseased coconut palms. Uptake, translocation and persistence of OTC in various parts of the palms were monitored by microbiological assay of buffer extracts of fresh tissue samples using *Bacillus cereus* subsp. *mycoides* (ATCC 6462). Presence of OTC in the foliage was detected within 24 h after administration. The highest concentration was noticed after 5–6 days. Maximum detectable levels in the range of 3–11 μg/g fresh weight were found in the leaf No. 11. Lower concentrations in the range of 1.0–5 μg/g fresh weight were detected in the leaf No. 1, 6 and 31 after a week of injection. With the onset of senescence, OTC gets redistributed from older leaves to the most actively transpiring leaves. Very little OTC activity was detected in roots, inflorescence, and spear leaf. Detectable levels of OTC was observed for more than 12 weeks in the foliage.

INTRODUCTION

Root (wilt) disease, a non–lethal but debilitating malady of coconut in India, is now prevalent in 4,10,000 ha of coconut plantations in Kerala State, causing an estimated annual loss of about 968 million nuts (Anonymous, 1986). The disease was earlier suspected to be caused by virus (Nagaraj and Menon, 1956). But subsequent studies ruled out the association of virus with the disease (Solomon and Sasikala, 1981). Reports on the association of mycoplasma–like organisms (MLOs) in various parts of root (wilt) affected palms (Solomon, Govindankutty and Neinhaus, 1983) led to experimental treatment of diseased palms with oxytetracycline–HCl to see if mycoplasma etiology could be established through remission of symptoms. In this context, studying the uptake, translocation and persistence of OTC in injected root (wilt) affected palms gained significance. The present paper reports the results concerning residue analysis of OTC in various parts of coconut palm.

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MATERIALS AND METHODS

Application of the OTC

Four 12 – year – old WCT (West Coast Tall) coconut palms in the early stages of root (wilt) disease (disease index score 18 – 23) were injected with 3 g. a. i. of oxytetracycline– HCl (Terramycin, 20% tree formulations; Pfizer Ltd., Thane, India) in 500 ml distilled water each using the pressure injector described by Gopinathan Pillai and Raju (1985) with pressure of 4 kg/cm^2 at 20 cm above ground level.

Method of sampling

The method of leaf sampling was based upon the phyllotaxy of the palms. Beginning with lower most (oldest) frond which was numbered one, each leaf was numbered in a radial spiral up to the crown. Three leaflets were sampled from the middle portion of the leaves numbered 1, 6, 11, 16, 21, 26 and 31 representing the spirally arranged whorl of fronds.

Assay procedure

Uptake, translocation and persistence of OTC were studied by microbiological assay using *Bacillus cereus* subsp. *mycoides* (ATCC 6462) as the test organism. The plant material (5 g) was frozen in liquid nitrogen and pulverized with mortar and pestle. The pulverized material was extracted with 5 ml of McIlvains buffer (pH 4). The extracts were squeezed through a double layered cheese cloth and centrifuged at 10,000 rpm for 10 min. at 10°C. Whatman No. 1 filter paper discs of 5 mm diameter impregnated with 20 μl of the clear supernatent were placed on bacto-antibiotic assay medium No. 3 (Himedia, Bombay) plus 1.5% agar plates seeded with test organism. The inhibition zones of extracts from treated and untreated palms as well as from standard solutions were determined after incubation for 24 h at 30°C. The antibiotic contents of the extracts were estimated by reference to a standard curve. Each test was replicated four times and the antibiotic content was expressed as μg/g fresh weight of plant tissue. Root (wilt) affected palms treated with OTC were monitored for residues present in various parts of the plant at daily intervals upto one week, and thereafter at weekly intervals upto sixteen weeks.

RESULTS

Distribution of OTC

The OTC activity was detected in extracts from leaf tissue within 24 h, reaching maximum concentrations in 5 – 6 days after injection. A week after application, maximum OTC activity (7.0 μg/g) was found in leaf No. 11. Leaf No. 16, 21 and 26 had less (3.6 – 4.7 μg/g) but more or less equal levels. Leaf No. 1, 6 and 31 had lower concentrations (1.0 – 2.2 μg/g) (Fig. 1).

It is evident from Table I that a week after injection, amounts of 0.2 – 0.9 μg/g range were present in the spear leaf, 1.0 – 2.0 and 1.0 – 2.1 μg/g in the fronds of 1 and 6 respectively. The leaf No. 31 had 0 – 5 μg/g and the leaf No. 11, 16, 21 and 26 had the level of 0.5 – 11 μg/g. The root and inflorescence had the levels of 0.0 – 1.2 μg/g range.

Persistence of OTC

The mean residue levels present in foliage of different whorls of crown at

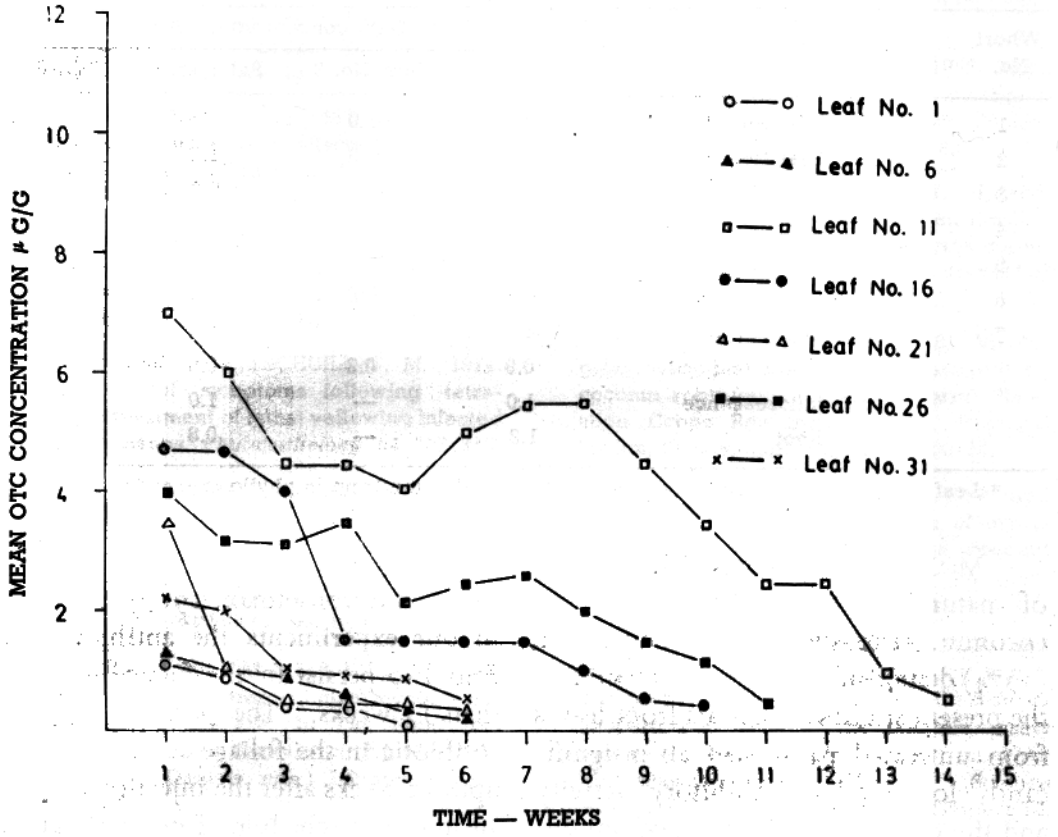
various times after injection are shown in Fig. 1. Leaf No. 1 had a range of 1.02 μg/g during first week to 1.0 μg/g during second week. The residues dropped to 0.5 μg/g during the third week. Leaf No. 6 had the levels of antibiotic ranging from 1.3 μg/g a week after injection, dropping to 1.0 μg/g during second and third weeks and further to 0.5 μg/g during fifth week after administration. Leaf No. 11 had an initial level of about 7.0 μg/g during first week, 6.0 μg/g during second week, dropping to 4.5 μg/g during third week and to 4.1 μg/g during fifth week. Leaf No. 16 had 4.8 μg/g during first and second weeks dropping to 4.0 μg/g during third week and further lowering to 1.4 μg/g during fifth week. Leaf No. 21 had the levels of 3.5 μg/g during first week declining to 2.0 μg/g during second week and to 0.5 μg/g during third and fifth weeks. Leaf No. 26 had the levels of 4.0 μg/g during first week dropping to 3.2 μg/g during second and third weeks and to 2.4 μg/g during fifth week. Leaf No. 31 had the levels of antibiotic ranging from 2.2 μg/g a week after injection to 2.0 μg/g during second week and falling to 0.9 μg/g during third week and 0.8 μg/g during fifth week. OTC persisted for more than 5–10 weeks in leaf No. 1, 6, 16, 21 and 31 and 11 weeks in leaf No. 26 and more than 12 weeks in the leaf No. 11 after initiation of the treatment.

DISCUSSION

Differential chemotherapy along with visualisation of mycoplasma–like organisms (MLO) in tissues are considered as important diagnostic aids in elucidating diseases of suspected mycoplasmal etiology (McCoy and Williams, 1982). Therefore, to determine the effect of the antibiotic in inducing remission of symptoms, it is imperative that the test chemical applied is uniformly distributed within the plant and should be available in all plant parts at reasonable concentrations to induce the desired mycoplasma–static effect.

It is evident from Fig. 1 that maximum concentration of OTC was translocated into the leaf No. 11 (actively transpiring foliage in the crown). Leaf No. 16, 21 and 26 accumulated less but more or less equal levels. However, the leaf No. 1, 6 and 31 had lower concentrations. Similar trend in distribution of OTC and tetracycline in the foliage of treated palms except the senescing fronds was observed in lethal yellowing affected palms by McCoy (1976) and Hunt, Dabek and Schuiling (1974) in Florida and Jamaica respectively. Very little OTC activity was noticed in the non-transpiring tissues of inflorescence and roots. This indicated that the xylem is the pathway for long distance transport of the OTC to the regions of active transpiration and further radial distribution through the inter connecting vascular net work (Zimmermann and Tomlinson, 1974). The results also indicated that OTC levels declined in the leaf No. 11 during the third and fourth weeks and increased again after six weeks of application. (Fig. 1). This was found to be invariably coinciding with the onset of senescence of older leaves. It is surmised that OTC could be redistributed from the senescing fronds to the most actively transpiring leaves accounting for its higher level in these foliage during this period. This also could be

Fig. 1. Mean oxytetracycline (OTC) activity levels in fronds sampled over 16 weeks from four (wilt) affected coconut palms



correlated to the fact that as the leaf grows older there are changes in its metabolism of carbohydrates and proteins and an associated deterioration of the chlorophyll pigments in favour of carotenoids and anthocyanins. As this metabolic shifts takes place, there is gross export of many of the organic and inorganic nutrients from the leaf until abscission interrupts such traffic (Leopald, 1961).

Analysis of OTC concentration in the different whorls of the leaves, roots and inflorescence of the four treated palms (Table I) a week after injection also indicated variation between the palms in the distribution of OTC but on a uniform pattern. The variations between the palms could be either due to the differential metabolic rate, plant size or part of the antibiotic getting inactivated before its absorption in the injection equipment or combination of all these factors. Such a phenomenon has also been observed in the case of lethal yellowing affected palms treated with OTC (McCoy, 1976).

Table I. *Oxytetracycline concentrations in tissues of four coconut palms–1 week after application with OTC*

Whorl No.	Tissue	Mean OTC concentration (μg/g)			
		Palm No. 1	Palm No. 2	Palm No. 3	Palm No. 4
1	Leaf No. 1*	2.0	1.0	1.0	1.1
2	Leaf No. 6	2.1	1.9	1.2	1.0
3	Leaf No. 11	11.0	3.0	8.0	6.0
4	Leaf No. 16	4.9	3.0	5.0	6.2
5	Leaf No. 21	8.0	0.5	1.0	5.0
6	Leaf No. 26	10.0	0.8	2.0	3.5
7	Leaf No. 31	2.5	—	5.0	1.5
	Spear leaf	0.9	0.2	0.5	0.8
	Inflorescence	1.0	—	1.0	0.9
	Root	1.2	—	0.8	0.5

* Leaf No. begins with lower most (oldest leaf) and extends up in phyllotaxic order

McCoy (1976) reported low degree of natural antimicrobial activity in coconut leaf tissues whereas Hunt et al. (1974) demonstrated no such activity. In the present studies the extract from leaves from untreated palms had an insignificantly low level of inhibitory activity and the diameter of the zone of inhibitory activity by standard OTC was unaffected by the presence of extracts from the leaves. Control assays also showed no natural antimicrobial activity from extracts of inflorescence and root.

Persistence of OTC in MLO infected plants for different duration has been demonstrated (Peterson and Sinha, 1977; McCoy, 1976; Dabek and Martin, 1987). In the case of lethal yellowing affected palms, OTC was detectable in the foliage for 6–8 weeks (McCoy, 1976; McCoy, Gwin and Donselman, 1983). However, in our experiments the antibiotic was found to persist in the foliage for more than 12 weeks. The persistence of the antibiotic in the foliage at reduced level upto 12 weeks after the injection suggests an injection schedule of quarterly application for field trials. The present study also suggests that OTC reaches the sink areas where MLO population is abundant (Solomon, Govindankutty and Mathen, 1987).

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