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CELLULASE ACTIVITY IN THE ROOTS OF COCONUT PALMS AFFECTED BY ROOT (WILT) DISEASE

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ABSTRACT

Cellulase activity was determined in the roots of healthy and root (wilt) diseased coconut palms. Higher activity was obtained in the diseased palms when compared to the healthy ones. The results of the study indicate that cellulase might be one of the factors responsible for the vast decay of roots observed in the diseased palms.

INTRODUCTION

Root (wilt) disease is the most serious malady affecting coconuts in India. Its etiology is unknown till to-date. It is a complex disease possibly caused by the combined effect of pathogens, nutrient imbalance, and water stress. Association of various biological agents like fungi, virus (Lal, Radha, and Shanta, 1970) and bacteria (George, Potty, and Jayasankar, 1976) has been reported. Physiological and biochemical derangements of the diseased palm have also been noticed (Mathew, 1977; Varkey, Michael, and Ramadasan, 1969; Pillai and Shanta, 1965).

The root systems of the diseased palms (Radha and Lal, 1972) are extensively damaged. Because of this, the degenerated root tissues may not be able to discharge their biological functions. The factors responsible for the vast degeneration of roots are not yet fully understood.

Most of the pathogens of higher plants produce enzymes capable of degrading the cell wall polysaccharides. The significance of these enzymes in wilt diseases is not certain (Cooper, Rankin, and Wood, 1978), though they have been implicated to play a major role in the degradation of host cell wall during pathogenesis (cf., Husain and Dimond, 1960; Cooper and Wood, 1973; Wood, 1960; Husain and Kelman, 1958; Husain and Singh, 1968; Kannaiyan, Vidyasekharan, and Kandaswamy, 1975; Mohanty and Addy, 1971).

Only few efforts have been made so far to ascertain the role of degrading enzymes in the root (wilt) disease of coconut. *In vitro* studies carried out with the fungi *Rhizoctonia solani*, *Bipolaris halodes*, and *Pestalotia palmarum* isolated from root (wilt) diseased coconut palms have shown that pectinolytic enzymes are being released into the culture media during the course of their growth (Lily and Jayasankar, 1974). No further information is available. An attempt is therefore made to assay the cellulase activity of coconut roots.

MATERIALS AND METHODS

Root samples from 20 healthy and 20 root (wilt) diseased coconut palms were

used. The palms were of 18-25 years age group and growing in the Institute farm. Four types of root samples were analysed for cellulase activity, viz., healthy roots from healthy palms; decayed roots from healthy palms; apparently healthy samples from root (wilt) diseased palms; and decayed root samples from root (wilt) diseased palms.

Cellulase activity was determined in the root samples according to the method of Pettersson and Porath (1966), modified largely by us to suit the crop.

The reagents used were: acetate buffer pH 5.2; 1.0 per cent carboxy methyl cellulose (CMC) as substrate: 10 gm CMC was dissolved in 900 ml of acetate buffer pH 5.2 *at 50-60°C with occasional stirring.* 20 ml of aqueous merthiolate was added as a preservative and the volume made up to 1 litre with buffer; and dinitrosalicylic acid reagent (DNS): for preparation, *see* Miller, et al. (1960)

Extraction of enzymes. Fresh root samples were washed with double-distilled water and wiped with filter paper to remove adhering water particles. Excluding the root cap region, about 2 cm of the apex of root was sliced. 2 gm of each sample of the tissue was macerated well at 0-4°C with 5 ml acetate buffer pH 5.2, then transferred to centrifuge tubes and kept at 0-4°C for 3 hr with occasional stirring; then centrifuged at 3000 rpm for 5 min., then decanted the clear supernatant, and 0.5 ml of this was used as the enzyme source.

Assay of cellulase activity. 0.5 ml of the above enzyme extract was added to the reaction media composed of 2 ml 1 per cent CMC+1 per cent 1 ml acetate buffer contained in a graduated tube of 20 ml capacity. Simultaneously, control tube containing 3 ml acetate buffer + 0.5 ml enzyme was also taken. The tubes were incubated at 40°C for 24 hr. The reaction was arrested by adding 3 ml DNS reagent to the tubes. The tubes were heated for 15 min in 100°C water bath, then cooled to room temperature and the volume made upto 10 ml. The absorbance was measured in a Klett Summerson colorimeter using green filter.

The protein content of the enzyme extract was determined using Folin-Phenol reagent (Lowry et al., 1951). Cellulase activity was calculated from a standard curve prepared using B-D-Glucose and DNS reagent.

Callulase activity was expressed as: 1 unit=1 mg of glucose liberated/hour/100 mg protein.

Results And Discussion

The results (Table 1) indicate that cellulase was not present in healthy palms, and also in apparently healthy roots of diseased palms. The decayed roots of both healthy and diseased palms exhibited cellulase activity. The activity was more in diseased palms. The enzyme acted in a pH range of 4.9-5.5 with an optimum pH around 5.2. Only negligible activity could be obtained at 30°C. The activity increased with temperture with maximum activity at 40°C. It was not possible to see if the enzyme was host-specific or pathogen–specific. Since activity could be detected in only the diseased roots and not in the healthy roots, it appears that the enzyme is liberated as a result of infection process only. However, the healthy palms also bear a certain number of decayed roots as a result of aging process (Menon and Pandalai, 1960). These decayed roots, where normal metaboilism is deranged may be the sites of infection for the pathogens. Once they have entered the tissue, the pathogens can elaborate the degrading enzymes which ultimately degrade the host cell wall. The hydrolytic products of cellulose released as a result of cellulase action can interfere with the water movement leading

Table I. *Cellulase activity in coconut roots (Mean value taken from 20 palms)*

Sample	Cellulase activity (in units)+	
	Mean	Range
Healthy roots from healthy palms	0	0
Decayed roots from healthy palms	4.5149*	1.14 – 13.40
Apparently healthy roots from diseased palms	0	0
Decayed roots from diseased palms	11.2091*	3.13 – 37.11
SE Difference	2.7056	

+One unit = 1 mg of glucose liberated/hour/100 mg protein

*Significant at P = 0.05.

to a wilting of the palm. An impairment in water uptake by the diseased palm has been noticed by Ramadasan (1970).

Disintegration of plant tissue has been demonstrated with purified preparations of cellulase from *Myrothecium verrucaria* (Cooking, 1960). The hyperactivity of cellulase in diseased palms therefore well supports the degeneration of roots of diseased palms. The free sugars and oligosaccharides formed as a result of hydrolysis of native cellulose can serve as nutrients for the rapid growth and multiplication of the pathogen. Although occlusion in xylem vessels could not be demonstrated in the root (wilt) diseased palms, development of tyloses was noted earlier (Indira and Ramadasan, 1968). The hydrolytic products of cellulose can form insoluble complexes with other macromolecules of the cell which may get deposited in tyloses.

As has been demonstrated with other diseases, it is likely that the pectionolytic enzymes make their primary attack on the polysaccharides permitting the cellulase to further degrade the degenerating tissue (Srivastava, Echandi, and Walker, 1959; Winstead and McCombs, 1961; Norkrans and Hammerstorm, 1965). A comparative study of the degrading enzymes including the arabanases and xylanases will reveal the mode of attack of these enzymes and the role played by each of these in causing root decay observed in root (wilt) diseased coconut palms.

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REFERENCES

MATHEW, C. 1977. Changes in carbohydrate content of coconut affected by root (wilt) disease. *J. Plant. Crops* **5**: 84-88.

COOKING, E. C. 1960. A method for the isolation of plant protoplasts and vacuoles. *Nature.* **187**: 962-963.

COOPER, R. M., RANKIN, B., AND WOOD, R. K. S. 1978. Cell wall degrading enzymes of vascular wilt fungi. *Physiol. Pl. Pathol.* **13**: 101-134.

COOPER, R. M. AND WOOD, R. K. S. 1973. Induction of synthesis of extra cellular cell wall degrading enyzmes in vascular wilt fungi. *Nature* **246**: 309-311.

PETTERSSON, G. AND PORATH, J. 1966. A celluloytic enzyme from *Penicillium notatum*. pp. 603-607 *in Methods in Enzymology* Vol. 8 (Eds. EF. Neufeld and V. Ginsburg). Academic Press, New York.

HUSAIN, A. AND DIMOND, A. E. 1960. Role of cellulolytic enzymes in pathogenesis by *Fusarium oxysporum f. Lycopersici*. *Phytopathology* **50:** 329-331.

HUSAIN, A. AND KELMAN, A. 1958. Role of pectic and cellulolytic enzymes inpathogenesis by *Pseudomonas solanacearum*. *Phytopathology* **48:** 377-386.

HUSAIN, A. AND SINGH, G. P. 1968. Role of enzymes in pathogenesis by *Fusarium lateritium* F. *cajani*. *Indian Phytopathol.* **21:** 361.

INDIRA, P. AND RAMADASAN, A. 1968. A note on the anatomical derangement in the root tissue of root (wilt) diseased coconut palm. *Curr. Sci.* **37:** 290.

KANNAIYAN, J., VIDYASEKHARAN, P., AND KANDASWAMY, T. K. 1975. Production of pectolytic and cellulclytic enzymes by bajra ergot fungus. *Indian Phytopathol.* **28:** 123-124.

LAL, S. B., RADHA, K., AND SHANTA, P. 1970. Etiology of root (wilt) disease of coconut palm. pp. 662-669 *In* Plant Disease Problems (Eds. S. P. Raychaudhury. Proc. Ist International Symposium on Plant Pathology). Indian Phytopathological Society, IARI, New Delhi.

LILY, V. G. AND JAYASANKAR, N. P. 1974. Pectionolytic properties of certain fungi associated with diseased coconut palms. *J. Plant. Crops* **2:** 17-19.

LOWRY, O. H., ROSEBROUGH, N. S., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with Folin-Phenol reagnet. *J. biol. Chem.* **193:** 265-275.

GEORGE, M., POTTY, V.P., AND JAYASANKAR, N. P. 1976, Association of *Enterobacter* with coconut root (wilt) disease. *Curr. Sci.* **45:** 677-678.

MENON, K. P. V. AND PANDALAI, K. M. 1960. The Coconut Palm A Monograph 384 pp. Indian Central Coconut Committee, Ernakulam.

MILLER, G. L., BLUM, R., GLENNON, W. E., AND BURTON, A. L. 1960. A reagent for the colorimetric determination of free sugars. *Anal. Biochem.* **2:** 127.

MOHANTY, P. K. AND ADDY, S. K. 1971. Production of pectolytic and cellulolytic enzymes by *Glomerella cingulata in vitro* and *in vivo*. *Indian Phytopathol.* **24:** 690-693.

NORKRANS, B. AND HAMMARSTROM, A. 1963. Studies on growth of *Rhizinaundulata* Fr. and its production of cellulose and pectin decomposing enzymes. *Physiol. Plantarum* **16:** 1-10.

PILLAI, N. G. AND SHANTA, P. 1965. Free amino acids of coconut palm affected by root (wilt) disease. *Curr. Sci.* **34:** 636-637.

RADHA, K. AND LAL, S. B. 1972. Diagnostic symptoms of root (wilt) disease of coconut. *Indian J. Agric. Sci.* **42:** 410-413.

RAMADASAN, A. 1970. On the nature of wilt in the root (wilt) disease of coconut palm. In Plant Disease Problems. pp. 670-675 (Ed. SP Ray chaudhuri). Indian Phytopathological Society, IARI, New Delhi.

SRIVASTAVA, D. N., ECHANDI, E. AND WALKER, J. C. 1959. Pectolytic and cellulolytic enxymes produced by *Rhizopus stolonifer*. *Phytopathology* **49:** 145-148.

VARKEY, T., MICHAEL, K. J., AND RAMADASAN, A. 1969. Note on the investigations on the nitrogen and phosphorus metabolism of coconut palm affected by root (wilt) disease. *Indian J. Agric. Sci.* **39:** 25-26.

WINSTEAD, N. N. AND McCOMBES, C. L. 1961. Pectionolytic and cellulolytic enzyme production by *Pythium aphanidermatum*. *Phytopathology* **51:** 270-273.

WOOD, R. K. S. 1960. Pectic and cellulolytic enzymes in plant disease. *Ann. Rev. Plant Physiol.* **11:** 299-322.