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STEM BLEEDING DISEASE OF COCONUT: REPRODUCTION OF SYMPTOMS BY INOCULATION WITH *THIELAVIOPSIS PARADOXA* *

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ABSTRACT

Samples of diseased tissues were collected from coconut palms affected by the stem bleeding disease, growing in a number of locations in Karnataka, Kerala and Goa. The fungus *Thielaviopsis paradoxa* was consistently isolated from all locations. Characteristic symptoms of stem bleeding, in the form of rusty brown discolouration of bark, were reproduced in all the 11 inoculated palms located in Kasaragod, Appangala and Vittal. Inoculum comprised small pieces of coconut rachilla, used as a substrate, which were completely covered by an actively sporulating mycelium of *Thielaviopsis paradoxa*. These bits were introduced in bore-holes made on palm trunks. Symptoms appeared at inoculated sites within 2 weeks (at Appangala) to 8 weeks (Kasaragod and Vittal). The organism was re-isolated from the tissues taken from the inoculated palms.

INTRODUCTION

Stem bleeding disease of coconut palms is known to occur in nearly all the countries which grow coconut (Menon and Pandalai, 1958; Ohler, 1964). The disease has been attributed to several factors, possibly due to the many types of stem bleeding observed (Menon and Pandalai, 1958; Nagarajan, 1985). Mathew and Ramanandan (1980) stated that neither soil and pH nor soil conductivity were directly related to the incidence of stem bleeding disease. The consensus of opinion is that *Ceratostomella paradoxa* (de seynes) Dade, or *Thielaviopsis* (de Seynes) van Hohnel (the imperfect stage of the fungus), is involved in the disease. Being a wound pathogen, the fungus was considered only as an incitant and not as the primary cause (Briton-Jones, 1940). Petch (1908) found that the fungus could infect through normal growth cracks present on the trunk. However, the role of the fungus in reproducing typical symptoms following artificial inoculation has not been established conclusively so far by earlier workers (Ohler, 1984; Davis, Sudasrip and Darwis, 1985). In previous attempts by artificial inoculation, only two out of the 25 inoculated palms showed disease

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symptoms (Anon., 1971), and did not yield any consistent results. This paper describes an experiment wherein characteristic symptoms of stem bleeding could be consistently reproduced on artificial inoculation with isolates of *Thielaviopsis paradoxa* in three different locations.

MATERIALS AND METHODS

Samples of diseased tissues were collected from stem bleeding affected palms, growing in a number of locations in Karnataka, Kerala and Goa. Small tissue pieces from the advancing margins of the lesions were plated on potato dextrose agar (PDA) after surface sterilization with 0.1% mercuric chloride, and incubated at room temperature. Fungi belonging to a number of genera were isolated from samples collected in different places. Thus *Thielaviopsis* sp. (from Kasaragod, Vittal and Appangala), *Fusarium* sp. (Kasaragod and Appangala), *Alternaria* (Kasaragod), *Chaetomium*(Vittal) were isolated.

Thielaviopsis sp. was consistently isolated from all locations from young lesions only. This, together with the fact that all preliminary attempts of proving pathogenicity by inoculation with isolates of *Alternaria, Chaetomium* etc. were inconclusive, prompted us to use *Thielaviopsis* sp. alone in subsequent experiments.

Cultures of the fungus initially grown on PDA were multiplied on host tissues. For this, either small pieces of young rachilla (10–15 mm long) or peeled leaf petiole bits (10 mm – 15mm long) were used. These were placed in conical flasks with a small quantity of water, autoclaved and then seeded with PDA-grown culture of *Thielaviopsis* sp. Optimum temperature of incubation was found to be around 22 °C, at which all the host tissues were thoroughly covered with actively sporulating mycelium within 4–5 days. Another substrate, which was found useful in promoting good growth and sporulation, was the petiole bits (10–15 mm long) of umbrella grass (*Cyperus* sp.)

For inoculation trials, a total of 16 healthy palms (10 at Kasaragod, 3 at Appangala, and 3 at Vittal) ranging in age from 10 to 45 years were selected. On each palm at one or more sites on the trunk, boreholes were made using an augur. These bore-holes were about 10 mm wide and 10 to 15 mm deep. These inoculation sites were located at heights ranging from 25 to 100 cm above the ground. At Vittal, the inoculation was done differently: a 1.5 × 1.5 cm patch of bark was removed from the trunk, the inoculum (fungus grown on leaf petiole bits) was placed next to underlying exposed tissue and the patch of bark replaced. At Kasaragod in 5 palms out of a total of 10 palms the bore holes were filled up by placing into each one a piece of leaf petiole of *Cyperus* containing the inoculum while the remaining 5 palms formed the control. The debris extruded out at the time of boring the holes was used in covering the hole mouth at the site of inoculation and a piece of ordinary sticking plaster was kept over this to keep the inoculum intact. Uninoculated bore-holes were similarly treated (but without inoculum) to serve as controls, in the remaining palms. At Appangala, the coconut rachilla containing the inoculum

was used for inoculation. At Vittal and Appangala, because of paucity of palms, inoculation was done on one side of the trunk at the rate of 2–3 holes per trunk while untreated control was maintained on the other side of the trunk. The inoculation was done during November 1985 at all places.

RESULTS AND DISCUSSION

Characteristic rusty brown discolouration of the bark was noticed at inoculated sites within 2 weeks (Appangala) to 8 weeks (Kasaragod and Vittal) of inoculation in case of all the 11 inoculated palms. This discolouration was not confined to the actual point of inoculation only but spread several centimetres around it in every direction. The characteristic brown patch was visible from a distance. A brownish liquid oozes out from the point of inoculation as well as from the growth cracks around the inoculated area, which subsequently dries up to form a rusty deposit. At uninoculated sites (control), only the fringe of the bore-hole site had turned brown. At Appangala, copious exudation of a brown liquid was noticed with beads of gum hanging at the site of inoculation. No such extensive gummy exudation was seen at the other two locations, though it was present in a milder form. Perhaps the low temperature (16.5 – 21 °C mean) prevalent at Appangala at the time of inoculation (November 1985) would have predisposed the palms to become highly susceptible to the infection. The gummy exudate on examination showed the presence of spores of *Thielaviopsis paradoxa*.

Table I. *Lesion size at different depths in the trunk of coconut palms artificially inoculated with* T. paradoxa.

Palm No.	Days after inoculation	Depth of lesion (mm)	Lesion size (mm)
		KASARAGOD (45-yr palms)	
1	90	12	68×27
		18	Very faint discolouration
2	90	5	35×18
		12	No discolouration
3	90	6	43×31
		14	30×20
		18	No discolouration
4**	90	10	50×27
		15	No discolouration
		VITTAL (15-yr old palms)	
1*	90	0	180×95
		10	102×75
		20	55×35
		25	20×18
		APPANGALA (10-yr old palms)	
1	51	5	152×31
		30	220×42
		70	140×25
		110	30×20
2	81	5	110×60
		30	100×40
		70	20×3
		110	0
3	112	5	180×40
		30	280×60
		70	230×40
		110	110×30

* Out of 3 palms only in one palm the lesion site was exposed to study the pattern of the lesion size. Other palms are under observation.

** Out of five palms only in four the lesion site was exposed to study the pattern of the lesion size. Other palm is under observation.

When the bark was removed so as to expose the underlying tissues, decay of the tissue was found to have penetrated deeper at the inoculated sites. The zone of decay was in the form of a crater, *i. e.*, wider at its mouth and progressively narrowing with depth (Table I). The depth to which the lesion stain was noticed also varied in different locations. While at Kasaragod and Vittal the lesion depth was 16 mm on an average, it was 100 mm at Appangala when examined 3 months after inoculation. It is likely that the low temperature prevalent at Appangala is responsible for this.

Thielaviopsis sp. was re-isolated from the tissues taken from advancing margins of lesions resulting from artificial inoculation. Briton-Jones (1940) opined that fluctuating levels of moisture and nutrients in the soil led to more growth cracks, thereby paving the way for the fungus. Potty and Radhakrishnan (1978) implicated a deficient supply of nitrogen or increased phosphorus in disease incidence. However, the present investigation comprised those palms which were manured regularly and received a balanced fertilizer dose. Reproduction of symptoms in such palms has clearly brought out the primary role of *Thielaviopsis* in stem bleeding disease of coconut.

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