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AN IMPROVED METHOD FOR ISOLATION OF THIELAVIOPSIS PARADOXA FROM STEM BLEEDING AFFECTED COCONUT PALMS

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Stem bleeding of coconut caused by Thielaviopsis paradoxa (de Seynes) Von Hohnel is an important disease affecting coconut in many countries (Menon and Pandalai, 1958; Ohler, 1966, Nambiar and Sastry, 1988). Isolation of the pathogen from diseased tissues on different agar based media has given inconsistent results (Anon., 1976; Anon., 1986). None of the selective media reported for certain related fungi viz., T. basicola (Berk and Br.) Ferr. (David, 1978;Tsao and Bricker, 1966) and Ceratocystis wagnerii Goheen and Cobb (Hicks et al., 1980) proved useful for the isolation of T. paradoxa. Since, standardization of an isolation method is a basic necessity for any study on plant pathogens, an attempt was made to improve upon existing methods.

MATERIALS AND METHODS

Samples were collected from 25 diseased palms near CPCRI, Kasaragod. Small pieces (0.25 x 0.25 cm), cut from margin of infected stem tissues were sterilized with 0.1% $HgCl_2$ for 30 secs and washed thoroughly with sterile water. Two media viz. potato dextrose agar (PDA) and sugarcane juice agar (SJA; 200 ml sugarcane juice, 20 g agar powder and 800 ml distilled water; pH 4.5), with and without addition of antimicrobial agents were compared with a bait method for the isolation of T. paradoxa. Certain antimicrobial agents viz., sodium-diethyl-dithiocarbamate (100 ppm), sodium propionate (1000 ppm), oxbile (1000 ppm), streptomycin (100 ppm), tetracycline (50 ppm) and penicillin (100 ppm) were added to media after screening a number of chemicals for their efficacy (Table 1). Infected bits were kept on agar surface of plates containing different media. Frond pieces (10-12 cm in length) from old coconut leaves were found most effective as bait (Table 2) and were used in subsequent studies. The pieces were inoculated with infected bits by bore-hole method (Nambiar et al., 1985), incubated at 30°C in polythene bags and isolations were made after 10 days of incubation from margin of rotten frond tissues on SJA. Response of T. paradoxa to different media and baiting method was recorded.

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Table 1 Effect of some antifungal compounds, antibiotics and organic dyes on chlamydospore germination and mycelial growth of T. paradoxa.

Chemicals	Chlamydospore germination/ mycelial growth at					
	10 ppm		50 ppm		100 ppm	
	Germi-nation	Growth*	Germi-nation	Growth	Germi-nation	Growth
Antifungal compouds						
Bavistin 50 WP	87.0@	0.0@	86.4	0.0	81.8	0.0
Brassicol 75 WP	15.2	52.5	17.6	45.0	8.9	40.0
Captaf 75 WP	98.6	0.0	94.1	0.0	91.5	0.0
Copper sulphate	100.0	100.0	63.5	91.3	55.0	57.5
Dithane M-45 (75%)	17.8	65.2	0.0	55.1	0.0	50.6
Foltaf 80 WP	0.0	35.0	0.0	28.8	0.0	27.5
Murcuric chloride	17.4	73.0	0.0	37.1	0.0	8.9
Ox-bile	99.1	100.0	86.7	100.	87.6	97.2
Sodium-diethyl-dith-iocarbamate (Technical)	100.0	69.6	100.0	67.4	79.6	65.1
Sodium propionate	98.7	98.6	92.3	97.3	94.5	89.6
Vitavax 75 WP	23.6	73.7	0.0	30.0	0.0	16.3
Antibiotics						
Aureofungin Sol (30%)	33.4	56.3	0.0	28.8	0.0	18.8
Cycloheximide	0.0	37.5	0.0	0.0	0.0	0.0
Mycostatin (4960 units/ mg)	16.8	34.8	0.0	16.8	0.0	6.8
Penicillin G (sodium salt > 1435 units/mg)	100.0	96.3	99.5	92.5	96.4	80.0
Pimaricin (2.5 aqueous susp.)	51.2	31.4	29.7	30.0	0.0	26.6
Streptomycin-sulphate	97.1	97.2	98.2	97.2	100.0	97.2
Tetracycline-hydrochloride	90.4	100.0	87.5	100.0	92.7	100.0
Vancomycin	90.1	75.0	81.3	76.6	79.6	72.4
Organic dyes						
Rose bengal	99.3	25.8	0.0	24.7	0.0	24.5
Melachite green	0.0	21.3	0.0	17.5	0.0	17.5

* Expressed as percentage of growth recorded on control medium

@ Average of three replications

Table 2 : Effect of age on extent of coconut leaf frond tissue colonization after artificial inoculation with T. paradoxa

Leaf position	Extent of colonization* after			
	4 days	8 days	12 days	16 days
2 (upper whorl)	–	+	+++	++++
12 (middle whorl)	–	–	+	++
26 (lower whorl)	+	+++	+++	++++

* –: No decay; + : Decay up to 25%; ++ : Decay from 26 to 50%; +++ : Decay from 51 to 75%; ++++ : Decay 75%

RESULTS AND DISCUSSION

The data on successful isolation of T. paradoxa by different methods are presented in Table 3. The pathogen was isolated from 98.3% infected bits by bait method in comparison to 8.0-19.6% isolation on various media tested. SJA proved slightly better medium over PDA for the purpose. Addition of selected antimicrobial agents to the media did not favour the selective isolation of T. paradoxa. Colonies of many fungi viz., Aspergillus sp., Trichoderma sp., Pestalotia sp., Phoma sp. etc. were noticed in isolation plates of all agar-based media. Such contamination problem could be avoided by using bait-method. The failure of selective media, reported for isolation of related fungi, for obtaining isolation of T. paradoxa can be explained on the basis of observations presented in Table I. Cycloheximide, Brassicol (penta-chloro-nitrobenzene) and mycostatin at 100 ppm and Rose Bengal at 50 ppm inhibited chlamydospore germination or mycelial growth of T. paradoxa or both to appreciable levels. These chemicals have been used in the selective media for T. basicola (David, 1978, Tsao and Bricker, 1966) and C. wagnerii (Hicks et al., 1980) at much higher contrations.

Table 3 : Details of isolation of T. paradoxa from stem bleeding affected coconut palms by using different media/ method

Media/method	No of bits from which T. paradoxa was isolated	No. of palms from which T. paradoxa was isolated
PDA	21* (8.4)#	11(44)
Amended PDA	20 (8.0)	13(52)
SJA	49 (19.6)	22(88)
Amended SJA	28 (10.8)	17(68)
Host bait method	248 (99.2)	25(100)

* 250 bits from 25 diseased palms (10 bits/palm) were used for isolation.
\# Figure in the parenthesis indicate the percent isolation.

CONCLUSIONS

1. The results clearly bring out the advantage of using bait method for isolation of T. paradoxa from stem bleeding affected palms.
2. The method is highly reproduciable and by using it, continous association of T. paradoxa with the diseased palms could be established (Table 3). In past, the inconsistent isolations had created a lot of confusion regarding etiology of the disease (Anon., 1976; Anon., 1986). Thus, this confirms the finding of Nambiar et al. (1985), who successfully reproduced the disease symptoms by inoculating healthy trees with T. paradoxa.

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