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Standardization of Screening Technique for Eucalyptus Canker Disease and Evaluation of Eucalyptus Clones against *Cryptosporiopsis eucalypti*

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A B S T R A C T

The most important diseases of eucalyptus in the plantations are leaf and shoot blight caused by *Cryptosporiopsis eucalypti* and associated with defoliation, dieback and mortality of eucalyptus trees. Disease causes major losses in nurseries and plantations resulting in failure of planting material and reduction of biomass production or loss of germplasm. Pathogenicity test using seedlings have shown that the fungus can infect stems as well as leaves. Isolated organism was identified as *Cryptosporiopsis eucalypti* based on the ITS sequencing and comparing with the NCBI database with more than 98 per cent similarity. 100 clones of eucalyptus were screened in glass house condition. 13 clones showed moderately resistant reaction with disease severity index of 1. 54 clones showed susceptible reaction with disease severity index of 2 and remaining 33 clones showed highly susceptible reaction with disease severity index of 3.

Introduction

Eucalyptus spp. are grown throughout many tropical and sub-tropical regions of the world. They have formed the basis for large-scale plantations and their associated forest product industries, farm and communal plantations and social plantings. In addition, eucalyptus grow well on low fertility, stony or eroded sites and on sloping ground not suited for cultivation of staple food crops (Old *et al.*, 2002).

The Myrtaceae family represents an important source of essential oils with diverse biological activities including bacteriostatic, fungistatic and anti-inflammatory effects. Various Myrtaceae species possess strong antimicrobial potential and their volatile oils are used as antimicrobial and antifungal agents in creams, soaps and toothpastes. Within the Myrtaceae family, the eucalyptus genus has been cultivated and exploited on a large scale for many years. Several species of eucalyptus are used in folk medicine as an antiseptic and against infections of the upper respiratory tract, such as cold, influenza and sinus congestion. Antimicrobial, analgesic and anti-inflammatory properties of *E. citriodora*, *E. globulus* and *E. teretcorni* have been reported from different parts of the world. The leaves of eucalyptus contain about 1.36% essential oil that is predominately citronellal (57%) followed by citronellol (15.89%), citronellyl acetate (15.33%) and other compounds. Due

to its disinfectant action, the essential oil is used externally, applied to cuts and skin infections. Beside antimicrobial activity, the essential oil and its constituents have also been used for their herbicidal and insecticidal properties, as well as in integrated disease management against phytopathogenic fungi (Luqman *et al.,* 2008).

Leaf spot and shoot blight diseases are the main problems for eucalyptus forest plantations. Disease causes major losses in nurseries and plantations resulting in failure of planting material and reduction of biomass production or loss of germplasm. Limited studies are available on eucalypt diseases and pathogens (Sankaran *et al.,* 1995; Keane *et al.,* 2000). Wide range of fungal and bacterial pathogens attack seedlings, saplings and trees of Eucalyptus (Sharma *et al.,* 1985).

C. eucalypti is a host-specific pathogen of Eucalyptus species that occurs over a wide geographical range varying from dry to very humid zones including those in Australia, India, Hawaii (Sankaran *et al.,* 1995). *C. eucalypti* (*Pseudoplagiostoma eucalypti*) has been associated with foliar disease of eucalyptus in many parts of the world, especially on *Eucalyptus camaldulensis*. *C. eucalypti*is known to cause shoot and blight disease in eucalyptusand can also exists as canker pathogen. The disease symptoms in the field is exhibited as canker, gumming *etc*., and *P. eucalypti* was found to be associated with the canker in *E. camalduelsis,* thereby it is evident that this pathogen is causing serious problem in the commercial cultivation. Variation in susceptibility to foliar disease occurs at the family, provenance and sub-species levels offering excellent opportunities for selection of resistant trees. Pathogenicity tests using seedlings have shown that the fungus can infect stems as well as leaves. Stem inoculation may offer opportunities for rapid screening for resistant germplasm (Old *et al.,* 2002). Hence identification of disease tolerant or resistant clones has become inevitable for successful cultivation.

Species of the fungal genus *Cryptosporiopsis* are well known as stem pathogens of woody hosts in temperate regions, including maple, hazel and fruit trees. *C. eucalypti* was first formally described in 1995, but the fungus had attracted attention from eucalypt pathologists considerably earlier. Sankaran *et al.,* (1995) noted that specimens were lodged with the International Mycological Institute (IMI) as early as 1972 from collections made in north east Australia, India and the Hawaiian Islands (Old *et al.,* 2002).

The fungus can be associated with various disease symptoms including leaf spots, shoot blight, cankers on woody tissue, defoliation and even tree death. The leaf spots develop on both sides of leaves and vary in size, shape, and color among eucalyptus species. The fungus proliferates by producing a vast number of spores from conidiomata that develop on infected leaves and shoots. After causing death of shoot tips or small branches, repeated infection can occur over extended periods of time. Leaf blight and other foliar diseases induced by *C. eucalypti* can easily be confused with those caused by other plant-pathogenic fungi, such as *Mycosphaerella* spp. and their anamorphs (Cheewangkoon *et al.,* 2010)

A thorough knowledge of the nature and biology of these agents is thus required. In humid tropics, acacias have become more widely planted than eucalypts, due partly to the high levels of leaf and shoot diseases sustained by Eucalyptus plantations, which depress growth rates and affect product quality. Large numbers of foliar and stem diseases are recorded on eucalyptus, worldwide (Sankaran *et al.,* 1995). The diseases have become more serious due to

continuous monoculturing of same clones affecting the quality of wood.

Materials and Methods

Isolation of *C. eucalypti*

Disease specimens were collected from affected trees for isolation of the pathogen *C. eucalypti* from plantations. The pathogen was isolated from small twigs of infected issue. Small piece of infected tissue was taken and surface sterilized with 0.1 % sodium hypochlorite for 30 seconds and rinsed in sterile distilled water three times continuously and plated on Malt extract agar medium. After isolation, the causal organism (*C. eucalypti*) was identified based on cultural, morphological characteristics and ITS sequencing.

Pathogenicity test

In glass house, eucalyptus clones were planted in pots with sterilized soil supplemented with required nutrients for growth of the plant. The seedlings were allowed for acclimatization in the glass house for 10 days. 15 to 20 days old fungal culture grown on Malt extract agar in a Petri plate was used for stem inoculation. By using sterilized scalpel, Small scalpel cuts were made on stems of the seedlings, to the depth of xylem and fungal disc was taken using cork borer. The fungal disc was placed at the collar region. The inoculation region was covered with para film. Observations were recorded 25-30 days after inoculation.

Molecular characterization of eucalyptus canker causing pathogen

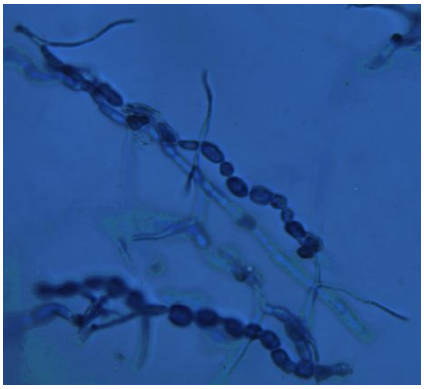
Genomic DNA was extracted following the protocols developed by Murray and Thompson (1980). DNA integrity was checked on 0.8 per cent agarose gel and quantified using Nanodrop®. The extracted DNA was amplified using universal primer set *viz*., forward primer (ITS1: TCC GTA GGT GAA CCT GCG G) and reverse primer (ITS4: TCC TCC GCT TAT TGA TAT GC).

Results and Discussion

Stem canker is an aggressive shoot pathogen. Symptoms of *C. eucalypti* infection developed on both shoots and leaves of eucalyptus. Stem inoculation was initially carried out in order to establish the capacity of *C. eucalypti* to invade wounded and intact stems. Blackening of stem started from the inoculated region after one month of inoculation and spreads towards the foliar region. Leaf spots were initially small, needle-like, and reddish to brown. They enlarge gradually and became circular or oval spots. Drying of leaves started 3-4 months after inoculation. Based on the symptoms ratings were given. Isolated organism was identified as *C. eucalypti* based on the ITS sequencing and comparing with the NCBI database with more than 98 per cent similarity (Table 1–3).

Plate.1 Screening of eucalyptus clones in glass house condition

Plate.2 Cultural and morphological character

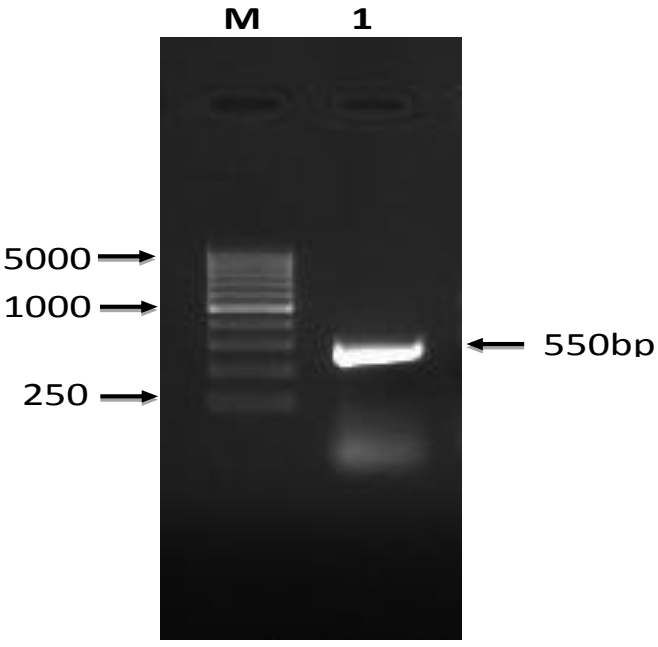


*C. eucalypti*culture

Microscopic observation

Plate.3 Pathogenicity test

Plate.4 PCR amplification of ITS region of *C. eucalypti*



Initial symptom

Absolute control

Stem Canker elongation

Stem canker infected plant

Table.1 Severity index and susceptibility rating for eucalyptus plants in response to the canker pathogen *C. eucalypti*

Symptoms	Severity index (0-3)	Rating
No wilting symptom; leaves unaffected; stem and roots unaffected	0 (0-1)	R
Leaves show turgidity loss and shrunken but not brittle; stem and root remain unaffected	1(1.1-2)	MR
Leaves show turgidity loss, shrunken and drying making them brittle; stem shows drying	2(2.1-3)	S
Leaves completely dried and brittle; stem completely dried	3(3.1-4)	HS

R-Resistant; MR-Moderately Resistant; S-Susceptible; HS-Highly Susceptible

Table.2 Sequences of *C. eucalypti* available with NCBI Genbank

Sl. No.	Identified organism	Accession number of identified organism	Max. identity (%)
1	*Cryptosporiopsis eucalypti*	MH041271	98

Table.3 Screening of eucalyptus clones in glass house condition

Sl. No	Genotypes	Disease recorded	
		Disease score	Disease reaction
1.	1	3	HS
2.	2	3	HS
3.	3	3	HS
4.	4	2	S
5.	5	3	HS
6.	6	3	HS
7.	7	3	HS
8.	9	2	S
9.	10	2	S
10.	11	3	HS
11.	12	3	HS
12.	13	3	HS
13.	14	3	HS
14.	15	3	HS
15.	16	2	S
16.	17	3	HS
17.	18	2	S
18.	19	3	HS
19.	20	2	S
20.	21	3	HS
21.	22	3	HS
22.	23	3	HS
23.	24	2	S
24.	25	3	HS
25.	26	2	S
26.	27	3	HS
27.	29	3	HS
28.	31	2	S
29.	32	2	S
30.	33	2	S
31.	34	3	HS
32.	35	2	S
33.	36	3	HS
34.	37	3	HS
35.	38	2	S
36.	39	1	MR
37.	40	2	S
38.	41	3	HS
39.	42	2	S
40.	43	2	S
41.	44	1	MR
42.	45	3	HS
43.	46	2	S
44.	47	2	S
45.	48	2	S
46.	49	2	S
47.	50	2	S
48.	51	2	S

49.	52	2	S
50.	53	2	S
51.	54	2	S
52.	55	2	S
53.	56	3	HS
54.	57	1	MR
55.	58	2	S
56.	59	1	MR
57.	60	3	HS
58.	61	2	S
59.	62	2	S
60.	63	2	S
61.	64	1	MR
62.	65	3	HS
63.	66	2	S
64.	67	2	S
65.	68	2	S
66.	69	2	S
67.	70	1	MR
68.	71	2	S
69.	72	2	S
70.	73	2	S
71.	74	2	S
72.	75	1	MR
73.	76	2	S
74.	77	3	HS
75.	78	2	S
76.	79	3	HS
77.	80	2	S
78.	81	2	S
79.	82	2	S
80.	83	1	MR
81.	84	2	S
82.	85	2	S
83.	86	1	MR
84.	87	2	S
85.	88	2	S
86.	89	2	S
87.	90	2	S
88.	91	1	MR
89.	92	2	S
90.	93	2	S
91.	94	1	MR
92.	95	1	MR
93.	96	1	MR
94.	97	3	HS
95.	98	2	S
96.	99	3	HS
97.	100	3	HS
98.	101	2	S
99.	102	2	S
100.	316	3	HS

Total of 100 clones were screened in glass house condition. Out of 100 clones 13 clones showed moderately resistant reaction with disease severity index of 1.54 clones showed susceptible reaction with disease severity index of 2 and remaining clones (33) showed highly susceptible reaction with disease severity index of 3.

C. eucalypti is widely distributed in many parts of the tropics and subtropics and has also been collected in several more temperate regions, the pathogen as assumed importance only in parts of India (Sankaran *et al.,* 1995). The association of *C. eucalypti* with significant disease of eucalyptus in Southeast Asia contrasts with lesser symptoms in Northern Australia, Brazil, Japan and New Zealand. Development of canker growth was observed after inoculation of eucalyptus stem with *C. eucalypti* and gradually disease developed towards foliar region and finally complete drying of eucalyptus clones were observed (Old *et al.,* 2002).

References

Cheewangkoon, R., Groenewald, J. Z., Verkley, G. J. M., Hyde, K. D., Wingfield, M.J., Gryzenhout, M., Summerell, B. A., Denman, S., Toanun, C. and Crous, P. W., 2010, Re-evaluation of *Cryptosporiopsis eucalypti* and Cryptosporiopsis-like species occurring on Eucalyptus leaves. *Fungal Divers.*, 44: 89-105. DOI 10.1007/s13225-010-0041-5.

Keane, P. J., Kile, G. A., Podger, F.D. and Brown, B. N., 2000, Diseases and pathogens of eucalypts. CSIRO, Collingwood, Victoria. 565p.

Luqman, S., Dwivedi, G. R., Darokar, M. P., Kalra, A. and Khanuja, S. P. S., 2008, Antimicrobial activity of *Eucalyptus citriodora* essential oil. *Int. J. Essential Oil Therapeutics*, 2: 69-75.

Old, K. M., Dudzinski, M. J., Pongpanich, K., Yuan, Z. Q., Thu, P. Q. and Nguyen, N. T., 2002, *Cryptosporiopsis* leaf spot and shoot blight of eucalypts. *Australasian Pl. pathol*., 31: 337-344.

Sankaran, K. V., Sutton, B. C. and Balasundaran, M., 1995, *Cryptosporiopsis eucalypti* sp. nov., causing leaf spots of eucalypts in Australia, India and U.S.A. *Mycol. Res.* 99 (7): 827-830.

Sankaran, K.V., Sutton, B.C and Minter, D.W. 1995. A checklist of fungi recorded on Eucalyptus. *Mycological Papers* 170. CABI Bioscience, Egham, Surrey. 376p.

Sharma, J. K., Mohanan, C. and Florence, E. J. M. 1985. Occurrence of *Cryphonectria* canker disease of Eucalyptus in Kerala, India. *Ann. Appl. Biol.*, 106: 265–276.

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