

IN VITRO INTERACTION OF ANTAGONISTIC FUNGI WITH *THIELAVIOPSIS PARADOXA*, THE PATHOGEN OF COCONUT STEM BLEEDING DISEASE*

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ABSTRACT

In vitro interaction of three antagonistic fungi, viz., *Aspergillus niger*, *Trichoderma harzianum* and *T. viride* with four isolates of *Thielaviopsis paradoxa* (de Seynes) von Hohnel, the causal agent of stem bleeding disease of coconut was studied. Three methods of inoculation, viz., (1) *T. paradoxa* inoculated 12 hrs before the inoculation of antagonistic fungus; (2) both *T. paradoxa* and antagonistic fungus were simultaneously inoculated, and (3) *T. paradoxa* inoculated 12 hrs after the inoculation of the antagonistic fungus were tested. Minimum growth inhibition of different isolates of *T. paradoxa* was observed when *T. paradoxa* was inoculated 12 hrs before the inoculation of the antagonist and maximum growth inhibition of *T. paradoxa* was observed when *T. paradoxa* was inoculated 12 hrs after the inoculation of the antagonistic fungus. Among the three antagonistic fungi tested, an isolate of *Trichoderma viride* exerted maximum growth inhibition (90%) of the pathogen.

INTRODUCTION

The coconut palm, *Cocos nucifera* Linn. is grown in all the tropical countries. The coconut palm, though hardy in nature, is affected by a number of diseases which are responsible for enormous economic losses. 'Stem bleeding disease' is one such disease of coconut reported from all coconut growing countries. Recently Nambiar et al. (1986) established the pathogenicity of the fungus, *Thielaviopsis paradoxa* (de Seynes) von Hohnel in causing the disease.

T. paradoxa is a weak pathogen. Earlier work (Anonymous, 1976) had reported the presence of other fungi viz., *Aspergillus* sp., *Diplodia* sp., *Fusarium* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp. etc. from the bark and/or soil samples of stem bleeding affected palms. There are many reports where *Aspergillus* sp. and *Trichoderma* sp.

are used as biocontrol agents of plant pathogens. Perhaps the presence of these fungi in the old lesions of affected palms may be a reason why it is difficult to isolate the pathogen from such lesions. Hence *in vitro* studies on the interaction of *T. paradoxa* with *Aspergillus niger*, *Trichoderma harzianum* and *T. viride*, isolated from tissues and soils from stem bleeding affected gardens was taken up in the present study. An understanding of antagonistic fungi may be useful in developing biocontrol measures against the stem bleeding pathogen.

Stem bleeding disease of coconut was first reported from Sri Lanka (Petch, 1906). Sundararaman (1922) reported the disease from India. Now the disease is known to occur in nearly all the coconut growing countries like Philippines (Lee, 1922),

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Malaysia (Sharples, 1923), Andaman Islands (Mitra, 1929), Trinidad (Britton-Jones, 1940) Papua New Guinea (Dwyer, 1953) and Fiji (Mc Paul, 1962), Indonesia (Renard, Brahmana and Rognon, 1984). Ohler (1984) reported *T. paradoxa* (de-Seynes) von Hohnel as a suspected pathogen. Recently, Nambiar et al. (1986) could establish pathogenicity with this fungus. The presence of many fungi viz., *Aspergillus* sp., *Diplodia* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp. etc. from bark and/or soil samples of stem bleeding affected palms has been reported (Anonymous, 1976). In literature many instances are there where *Aspergillus* spp., *Trichoderma* spp. are used as biocontrol agents of plant pathogens. *A. niger* was reported as an antagonist to *Rhizoctonia solani* causing rice sheath blight (Gokulapalan and Nair, 1984) and collar rot of coffee seedlings (Venkatasubbaiah and Safeeulla, 1984). *T. harzianum* was reported as biocontrol agent of many plant pathogenic fungi like *Rhizoctonia solani* affecting coffee seedlings (Venkatasubbaiah, Safeeulla and Somasekhar, 1984), *Cytospora personii* and *C. cineta* causing cankers on peach, plum and cherry trees (Schulz, 1981), *Fomes connatus* in red maple wounds (Smith, Blanchard and Shortle, 1981). *T. viride* as biocontrol agent *Ceratocystis ulmi* causing Dutch elm disease (Ricard, 1983; Webber and Hedger, 1986) and *Pythium* sp. causing root rot of sugarcane seedlings in nurseries (David, 1987).

MATERIALS AND METHODS

Four isolates of *T. paradoxa* isolated from the advancing margins of young lesions from stem bleeding affected palms collected from Pilicode, Kallangai, CPCRI Campus at Kasaragod and Shiria (all in Kerala) were taken for the study and designated as *T. paradoxa* isolate No. 1, 2, 3 and 4 respectively. These isolates showed variability in

the cultural and morphological characters (Gowda, 1987). One isolate of *Aspergillus niger*, two isolates of *Trichoderma harzianum* and three isolates of *T. viride* were tested for their antagonistic reactions with *T. paradoxa*. Direct opposition method used by Webber and Hedger (1986) was adopted for the *in vitro* studies as detailed below:

Individual cultures of *T. paradoxa* and the respective test fungi were established on culture media in petridishes of 9 cm diameter. Using sterile cork borer of 5 mm diameter mycelial discs were cut separately from the edge of the growing cultures of *T. paradoxa* and the test fungi. The discs of *T. paradoxa* and the test fungus were placed (with the fungal side facing the growth medium) at 3.5 cm distance from each other at the centre of the petridish. There were five replications and the dishes were incubated at 23°C in darkness. Radial growth was measured in two opposite directions at 24 hr intervals. From these measurements the percentage of inhibition of *T. paradoxa* caused by each test fungus was found out.

For *T. paradoxa* isolate No. 1, Kirchoff's Agar medium was used while for *T. paradoxa* isolate Nos. 2 and 3, Dextrose Asparagine Phosphate Agar and for *T. paradoxa* isolate No. 4 Potato Dextrose Agar were used since the best growth of the isolates was obtained in the respective media (Gowda, 1987).

The inoculation was done in three different ways:

1. *T. paradoxa* was inoculated 12 hrs before inoculation of the antagonistic fungus;
2. Both *T. paradoxa* and the antagonistic fungus were simultaneously inoculated, and
3. *T. paradoxa* was inoculated 12 hrs after inoculation of antagonistic fungus.

The percentage of growth reduction of *T. paradoxa* 72 hrs after inoculation was determined.

RESULTS AND DISCUSSION

Interaction of *A. niger* with *T. paradoxa*

In the three methods of inoculation tested, inhibition of different isolates of *T. paradoxa* was the best in 72 hrs after inoculation ranging from 13.3% to 30.0% (mean 23.3%) when *T. paradoxa* was inoculated 12 hrs before inoculation by *A. niger* (Table I). In simultaneous method of inoculation, the inhibition percentage ranged from 30.0% to 40.0% (mean 35.0%) while maximum percentage of inhibition was discernible in the third method of inoculation. The percentage of inhibition ranged from 36.7 to 50% (mean 44%) among the different isolates of *T. paradoxa* tested.

Interaction of *Trichoderma harzianum* with *T. paradoxa*

Among the three methods of inoculation tested, inhibition of *T. paradoxa* was minimum (23.3% to 50%) when *T. harzianum* (both isolates) was inoculated 12 hrs after the inoculation of *T. paradoxa* (Table II).

In simultaneous method of inoculation, the inhibition percentage ranged from 43.3

to 60.0 in 72 hrs after inoculation. But maximum percentage of inhibition was observed when *T. harzianum* was inoculated first and *T. paradoxa* was inoculated after 12 hrs (50 to 86.6%).

Interaction of *T. viride* with *T. paradoxa*

Minimum growth reduction of *T. paradoxa* was observed (13.3% to 43.3% for the three isolates of *T. viride*) when *T. paradoxa* was inoculated 12 hrs before *T. viride* (Table III). In simultaneous method of inoculation, growth inhibition of *T. paradoxa* ranged from 26.7% to 66.7% for the three isolates of *T. viride*. However, maximum growth reduction of *T. paradoxa* was found (50% to 90%) when *T. viride* was inoculated 12 hrs before *T. paradoxa* inoculation.

A significant role is played by fungus succession or precedence in development of diseases, survival of the pathogen, damages to the host etc. In the present studies in all the three cases of antagonistic fungi viz., *A. niger*, *T. harzianum* and *T. viride*, maximum percentage of inhibition of *T. paradoxa* was observed when the pathogen was inoculated 12 hrs after the antagonist inoculation. The

Table I. In vitro interaction of *A. niger* with *T. paradoxa**

<i>T. paradoxa</i> isolates	Growth inhibition of <i>T. paradoxa</i> by <i>A. niger</i> (Percentage)		
	A	B	C
No. 1	13.3	36.7	50.0
No. 2	23.3	33.3	39.3
No. 3	30.0	40.0	50.0
No. 4	26.7	30.0	36.7
Mean	23.3	35.0	44.0

A = *T. paradoxa* inoculated 12 hrs before the antagonist

B = *T. paradoxa* and antagonist were simultaneously inoculated

C = *T. paradoxa* was inoculated after 12 hrs growth of antagonist

* Results observed 72 hrs after inoculation of both the fungi

Table II. *In vitro* interaction of *Trichoderma harzianum* with *T. paradoxa**

<i>T. paradoxa</i> isolates	Growth inhibition of <i>T. paradoxa</i> by <i>T. harzianum</i> (Percentage)		
	A	B	C
	<i>T. harzianum</i> isolate No. 1		
No. 1	23.3	50.0	84.0
No. 2	40.0	50.0	80.0
No. 3	40.0	56.7	85.1
No. 4	30.0	46.7	66.7
Mean	33.3	50.8	78.9
	<i>T. harzianum</i> isolate No. 2		
No. 1	23.3	46.7	86.6
No. 2	50.0	50.0	50.0
No. 3	50.0	60.0	62.5
No. 4	26.7	43.3	63.3
Mean	37.5	50.0	65.6

A = *T. paradoxa* inoculated 12 hrs before *T. harzianum*

B = *T. paradoxa* and *T. harzianum* were simultaneously inoculated

C = *T. paradoxa* was inoculated after 12 hrs growth of *T. harzianum*

* Results observed 72 hrs after inoculation of both the fungi

fact that this is only *in vitro* studies calls for further investigations to test whether same results can be applied to *in vivo* conditions also. Venkatasubbaiah and Safeeulla (1984) reported that seed treatment with *A. niger* significantly reduced collar rot of coffee seedlings due to *R. solani*. Similarly Schulz (1981) reported good inhibition of canker caused by *Cytospora personii* on peaches, plum, etc. when the trees were prophylactically treated with *T. harzianum*. Ricard (1983) treated fifteen Dutch elm disease (*C. ulmi*) affected elm trees with *T. viride* pellets and reported that there was no progress of the disease. *Pythium* root rot of sugarcane seedlings could be effectively con-

trolled by incorporation of *T. viride* to seed beds (David, 1987).

This preliminary studies on *T. paradoxa* indicate the possibility of using *A. niger*, *T. harzianum* and *T. viride* as potential antagonists or biocontrol agents to the stem bleeding pathogen in coconut. Studies in this direction are required to screen more biocontrol agents. On the other hand, all fungi antagonistic to the pathogen in *in vitro* need not be antagonistic *in vivo* also, as has been well documented in case of Dutch elm disease (Webber and Hedger, 1986). Hence more studies in this line are required before these antagonists can be employed as biocontrol agents under natural conditions.

Table III. In vitro interaction of *T. viride* with *T. paradoxa**

<i>T. paradoxa</i> isolates	Growth inhibition of <i>T. paradoxa</i> by <i>T. viride</i> (Percentage)		
	A	B	C
<i>T. viride</i> isolate No. 1			
No. 1	16.7	34.5	56.6
No. 2	40.0	60.0	67.9
No. 3	36.6	60.0	90.0
No. 4	33.3	50.0	76.7
Mean	31.6	51.1	72.8
<i>T. viride</i> isolate No. 2			
No. 1	33.3	53.3	90.0
No. 2	40.0	50.0	57.1
No. 3	40.0	66.7	90.0
No. 4	38.0	50.0	80.0
Mean	37.8	55.0	79.3
<i>T. viride</i> isolate No. 3			
No. 1	13.3	26.7	50.0
No. 2	43.3	45.8	50.0
No. 3	40.0	60.0	68.0
No. 4	16.7	33.3	60.0
Mean	28.3	41.5	57.0

A = *T. paradoxa* inoculated 12 hrs before *T. viride*

B = *T. paradoxa* and *T. viride* were simultaneously inoculated

C = *T. paradoxa* was inoculated after 12 hrs growth of *T. viride*

* Results observed 72 hrs after the inoculation of both the fungi

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