Journal of Plantation Crops 20 (Supplement) : 69-72

VARIATION IN CULTURAL CHARACTERS AMONG ISOLATES OF *THIELAVIOPSIS PARADOXA*, CAUSAL AGENT OF STEM BLEEDING DISEASE OF COCONUT*

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INTRODUCTION

Stem bleeding disease is one of the diseases of coconut reported from all coconut growing regions. Nambiar *et al.* (1986) reported *Thielaviopsis paradoxa* (de Seynes) Von Hohnel as the causal agent of the disease. The fungus multiplied in the infected stem tissues and soil debris and produces endoconidia and/or chlamydospores. In studies with different isolates of the pathogen, some variations were noticed in cultures. This promoted us to investigate in detail the variation among different isolates of *T. paradoxa*, since such a study has not been taken up so far in this fungus. In this paper results of studies on the variation in cultural, morphological and spore production characters of five different isolates of *T. paradoxa* are reported.

MATERIALS AND METHODS

Tissue samples collected from young lesions on the stem of affected palms were used for isolating *T. paradoxa* on potato dextrose agar (PDA). The isolates were collected from five different coconut gardens one each situated at Kayangulam, Pilicode, Kallangai, CPCRI Kasaragod campus and Shiria, all in Kerala. The isolates were designated as *T. paradoxa* isolate No. 1, 2, 3, 4 and 5 respectively.

This isolates were grown on six different media viz. Potato Dextrose Agar (PDA), Corn Meal agar (CMA), Oat Meal agar (OMA), Coconut Tissue Extract agar (CTEA), Oak Wilt Agar-A (OWA) and Kirchof's Agar (KA) and incubated at 23°C. There were four replications. The colony diameter was measured at 24 hr. intervals for a period of 3 days, and formation of endoconidia and chlamydospores was observed. The colony and hyphal characters were studied by growing all the isolates on PDA.

RESULTS

In vitro growth of the five isolates of *T. paradoxa* was compared on six different media on the 3rd day of incubation (Table I). The colony diameters of *T. paradoxa* isolate Nos. 1, 3 and 4 were different from that of isolate Nos. 2 and 5 in that latter isolates grew faster. Maximum growth was seen with isolate No. 2, while isolate No. 1 showed poor growth on all media tested.

Studies on colony character of different isolates on PDA (Table II) showed that isolate Nos. 1, 2, 3 and 5 had circular colonies as compared to irregular colony with wavy margin in the case of isolate No.4. Isolate No. 5 produced a fruity smell while other isolates did not. Due to this smell, this culture attracted fruit flies (*Drosophila sp.*) to the culture plates. The hyphal colour was brown in isolate Nos. 1 and 4, hyaline in isolate Nos. 3 and 5 and light grey in No. 2. There is no much variation in the hyphal diameter in different isolates.

Among the five isolates of *T. paradoxa*, only isolate No. 5 produced endoconidia on all the culture media tested (Table II) while other isolates did not produce endoconidia. The conidia of isolate No. 5 are terminal, hyaline and rectangular measuring 3.6 x 7.2 to 18.0 µ.

All the five isolates of *T. paradoxa* produced

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Table I. ***In vitro*** **growth of different isolates of *T. paradoxa* on six culture media (Mean of 4 replications)***

Culture media	Colony diameter of T. paradoxa isolates (in cm)					
	Isolate No. 1	No. 2	No. 3	No. 4	No. 5	Mean
PDA	0.6	9.0	3.6	3.4	9.0	5.1
CMA	0.6	9.0	6.5	3.7	7.3	5.4
OMA	0.5	9.0	6.4	4.1	5.2	5.0
CTEA	0.5	9.0	7.4	4.0	8.0	5.8
OWA	0.6	9.0	7.3	4.1	6.0	5.4
KA	0.6	9.0	8.7	5.5	7.0	6.2
Mean	0.6	9.0	6.7	4.1	7.1	5.5

* The observation was recorded on the 3rd day of incubation

PDA = Potato Dextrose Agar
CMA = Corn Meal Agar
OMA = Oat Meal Agar
CTEA = Coconut tissue extract Agar
OWA = Oak wilt Agar - A
KA = Kirchof's Agar

Table II. Colony and mycelial characters of different isolates of *T. paradoxa**

Colony/Mycelial character	Isolates of *T. paradoxa*				
	No. 1	No. 2	No. 3	No. 4	No. 5
Colony margin	CE	CE	CE	IW	CE
Culture colour (Top)	Grey	Grey	White	White	Grey in centre white periphery
Odour	Nil	Nil	Nil	Nil	Fruity smell
Mycelial colour	Brown	Light grey	Hyaline	Brown	Hyaline
Hyphal diameter (μ)	1.8-3.6	1.8-3.6	1.5-3.0	1.8-3.6	1.8-3.6
Endoconidia	Absent	Absent	Absent	Absent	Present
Chlamydospores	Present	Present	Present	Present	Present

* The growth on PDA was recorded on the 3rd day of incubation
CE = Circular and entire, IW = Irregular, wavy

chlamydospores on all the six media used (Table III). Isolate No. 2, 3 and 5 took 6, 7 and 3 days respectively for production of chlamydospores on different media whereas isolate No.4 took 5 days except in the case of coconut tissue extract agar (CTEA) with 9 days. In the case of isolate No. 1 the chlamydospores were produced very late, the duration ranging from 9 to 30 days on different media.

All the isolates produced brown coloured chlamydospores which are oval to oblong or spherical (Table IV). The chlamydospores are intercalary and terminal in isolate No. 5. Chlamydospores were smaller in size in isolate No. 1 (3.6 x 9.0 μ) and larger

Table III. Chlamydospora formation in different isolates of *T. paradoxa* on different media

Culture media	Days taken for different isolates for chlamydospore production					
Isolate	No. 1	No. 2	No. 3	No. 4	No. 5	Mean
PDA	9	6	7	5	3	6.0
CMA	20	6	7	5	3	8.2
OMA	9	6	7	5	3	6.0
CTEA	30	6	7	9	3	11.0
OWA	25	6	7	5	3	9.2
KA	15	6	7	5	3	7.2
Mean	18	6	7	5.7	3	7.9

Table IV. Morphological characters of chlamydospores in different isolates of *T. paradoxa*

Character	Isolates of *T. paradoxa*				
Isolate	No. 1	No. 2	No. 3	No. 4	No. 5
Colour	Brown	Brown	Brown	Brown	Brown
Shape	Oval	Oval	Oval to spherical	Oval to oblong	Oval
Position	Terminal and Intercalary	Intercalary	Intercalary	Intercalary	Terminal
Size (in μ)	3.6 x 9.0	7.2 x 25.2	10.8 x 21.6	7.2 x 19.8	7.2 x 16.2

in isolate No. 3 (10.8 x 21.6 μ).

DISCUSSION AND CONCLUSION

The variations in *in vitro* growth of different isolates of *T. paradoxa* on different culture media indicates that the nutritional requirements are different for different isolates. Similar phenomenon has been reported in the case of *Colletotrichum falcatum* on sugarcane (Chona and Srivastava 1960) and *C. gloeosporioides* in cacao (Chandra Mohan *et al.*, 1987). In the present study *T. paradoxa* isolate No. 2 grew very fast on all culture media while isolate No. 1 showed poor growth. More studies are needed to know the actual requirement of different nutrient factors by different isolates. A comparison of more isolates from a wide range of localities, where the disease is prevalent, will be required to understand the variation in disease incidence.

Among the five isolates, isolate No. 5 gave a fruity odour to the culture and this attracted *Drosophila* flies to the culture. This isolate produced abundant endoconidia and chlamydospores which are terminal (Tables III and IV). The chlamydospores are produced very early (in 3 days). The occurrence of large number of terminal conidia and chlamydospores coupled with the factor that insects are attracted towards the culture on account of fruity odour perhaps has a direct bearing on the dispersal of the pathogen. The flies visiting the diseased lesions might help in disseminating the pathogen as a passive carrier. Insect dissemination of *Ceratocystis ulmi* causing Dutch Elm Disease has been reported (Collins, 1941). It may be mentioned that often insects like *Diocalandra* sp., *Xyleborus* sp. have been found to infest old decayed lesions (Menon and Pandalai, 1960). Further studies are needed in this line.

Whether these isolates are really different strains differing in virulence can be determined only by further

studies by inoculation techniques. Under field conditions it has been noticed that the garden from which isolate No. 5 collected had severe disease incidence. In case less virulent isolates are present, there is a possiblity of using them as biocontrol agents by protecting the plant against more virulent isolates. Weber and Stahmann (1966) reported such a phenomenon in sweet potato varieties susceptible to *Ceratocystis fimbriata*. This aspect also needs further studies in stem bleeding as it will have great field application.

ACKNOWLEDGEMENT

The authors are thankful to Dr. M.K. Nair, Director, CPCRI, Kasaragod for providing facilities for the conduct of this work. The first author wishes to thank the U.G.C. for the award of fellowship for his M. Phil. course of Mangalore University during which this work was undertaken.

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