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Induction of Fruiting and Sporulation in an Attenuated Culture of *Ascochyta cypericola* Causing Leaf Blight of *Cyperus rotundus* L.

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Abstract: An attenuated *Ascochyta cypericola* culture that has lost the ability to produce pycnidia and conidia on serial subculturing is made to produce pycnidia and conidia by supplementing Czapek-Dox agar medium with aqueous green leaf extracts of *Cajanus cajan*, *Parthenium hysterophorus*, *Catharanthus roseus* and its natural host *Cyperus rotundus*.

Key words: *Ascochyta cypericola*, *Cyperus rotundus*, conidia, pycnidia and attenuated culture

INTRODUCTION

Upadhyay *et al*. (1991) isolated a new pathogen called *Ascochyta cypericola* from severely diseased leaves of purple nutsedge *Cyperus rotundus* L. and claimed it as a potential mycoherbicide in the control of the noxious weed. A culture of *A. cypericola* isolated from diseased leaves collected from fields around Visakhapatnam after 6 or 7 subcultures has failed to produce pycnidia on Potato dextrose agar medium which was shown to be a better medium among others tested (Narayana Rao and Ratnakumar, 1997). Since spores are required in attempts to evaluate as a potential mycoherbicide, studies were conducted to induce sporulation in the attenuated culture.

MATERIALS AND METHODS

Agar media such as Potato dextrose, Potato sucrose, Czapek-Dox, Oatmeal, Richard's, Sabouraud's, Starch, Carrot, Asthana and Hawker's, Czapek-Dox+5 g L^{-1} Yeast extract or Malt extract or Beef extract, or Peptone and *C. rotundus* leaf decoction, were attempted to induce fruiting and conidia.

For *C. rotundus* leaf decoction about 50 g of green leaves were dried at 60°C for 2 days. The dried leaves were placed in 100 mL-distilled water and boiled for 30 min. The resultant decoction was incorporated into Czapek-Dox agar. Since on inoculated plates of the above media incubated in light, though there was luxuriant growth in all but in none pycnidia were produced. Hence, the aqueous green leaf extracts of *C. rotundus*, *Cajanus cajan*, *Parthenium hysterophorus* and *Catharanthus roseus* were incorporated into Czapek-Dox agar medium at concentrations as shown in Table 1. For green leaf extracts 60 g of washed cut leaves were taken in 100 mL MD, macerated in a mortar and pestle using acid washed sand. The macerated tissue was squeezed through muslin and the resultant green extract was centrifuged at 2500 rpm for 20 min. The light brown supernatant was incorporated into the medium. These supplemented media were sterilized in an autoclave. Triplicates for each were maintained and inoculated with pinpoint inoculum from the attenuated culture. The inoculated plates were placed in 7 h light of a specially made light chamber with 27.432 μE m^{-2} sec^{-1} photon flux density at the plate's surface at a temperature of 32±2°C.

Table 1: Sporulation of *Ascochyta cypericola* on Czapek-Dox agar incorporated with leaf extracts

Extract (%)	No. × 10^4/plate			
	C. rotundus	*C. cajan*	*P. hysterophorus*	*C. roseus*
0.0	0.0	0.0	0.0	0.0
2.5	225.0	50.0	25.0	45.0
5.0	150.0	82.5	75.0	45.0
10.0	1500.0	45.5	25.0	25.0
20.0	1200.0	25.0	37.5	42.5
30.0	1050.0	100.0	32.5	32.5

Liner growth measurements were taken on alternate days till 8 days by which day the colonies have filled the plates. The incubation period was extended further and around 20 days of incubation since pycnidia started appearing the incubation time was further extended to 30 days as more pycnidia appeared. Then to each plate 25 mL of distilled water was added and brushed when then the conidia ooze out of the pycnidia. The resultant was filtered through a single layer of muslin. And as spores were present the sporulation was estimated using a Thoma Haemocytometer.

RESULTS AND DISCUSSION

There was no difference in growth of extract-supplemented media when compared with each other and

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Table 2: Sporulation of *Ascochyta cypericola* on Czapek-Dox agar incorporated with tuber extract of *Cyperus rotundus*

Extract (%)	No. × 10^4/plate
0.0	0.0
2.5	437.5
5.0	450.0
10.0	562.5
20.0	412.5
30.0	500.0

the un-supplemented. Regarding sporulation (Table 1), spores were produced in all the concentrations more than the Czapek-Dox without the extracts. Better sporulation occurred in the medium supplemented with extract of *C. rotundus*. Maximum sporulation occurred in 10% concentration. Though sporulation was induced it was only to a lower degree in *C. cajan*, *P. hysterophorus* and *C. roseus* supplemented media (Table 2).

Pathogenic fungi often lose the ability to sporulate in culture on serial sub-culturing. Difficulty was experienced in obtaining sporulation and maintaining sporulating ability of many *Ascochyta* isolates (Person, 1961). To sporulate the attenuated *A. cypericola* when 14 diverse media were tried, no medium could induce pycnidia and conidia production. However, supplementing Czapek-Dox agar with yeast, Malt and Beef extracts only could induce incipient pycnidia. These incipient pycnidia were sterile only. The aqueous leaf extracts of some poaceae enhancing sporulation of *Pyricularia* sp. has been reported by Narayana Rao *et al.* (1972). Malleswara Rao and Narayana Rao (1981) reported the stimulation of sporulation in *Pyricularia* sp. by leaf extracts of *Commelina benghalensis*.

In our own laboratory, Lakshmi (1997) showed that *C. cajan*, *P. hysterophorus*, *C. roseus* enhancing manifold the sporulation of *Helminthosporium maydis*. Hence, when aqueous leaf extracts of these plants are incorporated into Czapek-Dox agar ample spores could be produced to the maximum extent by *C. rotundus* extract. Even tuber extracts of *C. rotundus* could induce sporulation amply in the attenuated culture (Table 2). Evidently the leaf extracts might be containing sporulation-inducing constituents. *C. rotundus* being the natural host of *A. cypericola* should be containing potential substances inducing sporulation which to be elucidated. The same results were obtained when the experiment was repeated thrice.

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