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Leaf Spot Diseases of Waterhyacinth in Sri Lanka

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ABSTRACT

Several leaf spot diseases of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) were observed in the North Western and Western Provinces of Sri Lanka. Five pathogenic fungi capable of producing leaf spots were isolated. Of these, *Myrothecium roridum* and *Cercospora piaropi* have been previously recorded on waterhyacinth. The other isolates, namely, *Curvularia tuberculata, Septofusidium elegantulum* and *Phaeotrichoconis crotalariae* were not previously recorded on waterhyacinth.

Key words: pathogen, biocontrol, fungi antagonism, synergism, pathogenicity, *Cercospora.*

INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) which is believed to have been introduced to Sri Lanka as an ornamental plant (5), has now become a serious pest in waterways, ponds and slow running streams in the coastal districts of North Western, Western and the Southern Provinces (6). Local diseases of waterhyacinth have not been documented and there are no reports of pathogenic fungi isolated from waterhyacinth in this country. This paper records the pathogenic and nonpathogenic fungi associated with leaf spots of waterhyacinth. It also reports three new leaf spot diseases of this plant.

METHODS AND MATERIALS

Waterhyacinth leaves with leaf spots were collected from eight different sites in the North Western and Western Provinces of Sri Lanka. They were transported to the laboratory in polythene bags. For isolating pathogenic fungi, leaves with spots were cut into small pieces, surface sterilized with 0.1% $HgCl_2$ for 20 seconds, rinsed thoroughly in four changes of sterile distilled water and dried with sterile filter paper. The leaf pieces were then cut into smaller pieces and plated on Czapek dox or potato dextrose agar supplemented with 25 ppm streptomycin and incubated at 28 to 30 C. The fungi that grew were isolated, and pure cultures were obtained from single spores or hyphal tips and maintained on potato dextrose agar in tubes.

Fungal isolates were tested to establish their pathogenicity on waterhyacinth leaves following Koch's postulates. Waterhyacinth plants were maintained in 0.75 x 1.0 x 0.5 m concrete tanks filled with tap water. Healthy plants with five leaves were selected; two leaves other than the youngest and the oldest served as test leaves and the third leaf served as a control. The inoculum consisted of 1 cm^2 blocks of potato dextrose agar carrying mycelium and/or spores of the test fungus. These were rubbed on four 0.5 cm^2 areas on the ventral surface near the leaf base. The area of inoculation was damaged by pricking with a sterile needle before inoculation. Leaves used as controls were trated similarly, but without fungi. After inoculation, each leaf was covered with a polythene bag to maintain humidity. These plants were transfered to open tanks and after 24 hrs, the polythene bags were removed and observations continued for a period of 5 to 6 weeks. Reisolations were done as described above.

RESULTS AND DISCUSSION

Forty different kinds of leaf spots on waterhyacinth from eight different sites investigated yielded 70 fungal isolates which were found to belong to 15 species (Table 1). In pathogenicity tests, only five species were found to be capable of producing leaf spots on healthy leaves. Two of these species, *Myrothecium roridum* Tode ex Fr. and *Cercospora piaropi* Tharp have been previously recorded on waterhyacinth (2,7).

Our isolate of *M. roridum* produced leaf spots within five days after inoculation. The first symptom developed three days after inoculation. It took the form of a narrow brown band which spread from the point of inoculation to the leaf tip. Development of leaf symptoms slowed down after about one week and the disease spread to neighbouring plants after 5 to 6 weeks (Figure 1A).

Cercospora piaropi isolated in this study grew well in the agar media used; and our culture of *C. piaropi* was the first one deposited at the Commonwealth Mycological Institute (CMI) (personal communication). The first symptom which developed five days after inoculation was a browning in the region of inoculation, and spot development occurred for two weeks after which infection ceased to spread.

The other three pathogenic fungi, *Curvularia tuberculata* Jain, *Septofusidium elegantulum* (Pidlopl.) W. Gams, and *Phaeotrichoconis crotalariae* (Salam & Rao) Subram., have not been recorded previously as causing diseases of waterhyacinth. In pathogenicity tests, they were less virulent than *M. roridum* (Table 1).

Curvularia leaf spots were oval, yellowish brown to black, 3 to 5 mm in size and were found scattered over the leaf surfaces of waterhyacinth collected at Kelaniya, Wattala and Bingiriya. The causative fungus, identified as *Curvularia tuberculata* Jain (IMI 261801) (1), produced brown oval leaf spots on artificial inoculation (Figure 1C). In culture, the fungus produced straight or flexous mononematous conidiophores about 20 μm in length bearing terminal solitary conidia (Figure 2A). These conidia measured from 21 to 54 (36) μm in length to 12 to 23 (18) μm in breadth and were obovoid, straight and dark brown in color with end cells paler than others.

Under field conditions, one or more nonpathogenic fungi like *Curvularia lunata, Penicillium oxalicum, Cephalosporium* sp., *Fusarium* sp. and pathogenic fungi like *C.*

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TABLE 1. FUNGI OBTAINED FROM WATERHYACINTH LEAF SPOTS, THEIR LOCATION, % FREQUENCY OF OCCURRENCE AND PATHOGENICITY.

Fungus	Location[a]	Number of isolates	% Frequency of occurrence[b]	Pathogenicity[c]
Cephalosporium sp.	1,2,3,4,5,6,8	9	22.5	–
Penicillium oxalicum Currie & Thom	1,3,6,8	8	20.0	–
Cercospora piaropi Tharp (IMI 261803)[d]	2,4,8	8	20.0	++
Curvularia lunata (Wakker) Boedijn (IMI 264391)	2,3,4,5,8	7	17.5	–
Curvularia tuberculata Jain (IMI 261801)	1,2,8	6	15.0	+
Phaeotrichoconis crotalariae (Salam & Rao) Subrm. (IMI 261804)	3,4,6,8	5	12.5	+
Sterile fungus I	1,3,8	5	12.5	–
Idriella lunata Nelson & Wilhelm (IMI 264393)	1,3	4	10.0	–
Mucor sp.	1	4	10.0	–
Septofusidium elegantulum (Pidlopl.) W. Gams (IMI 261800)	1,7,8	3	7.5	++
Myrothecium roridum Tode ex Fr. (IMI 261802)	1,3	3	7.5	+++
Fusarium sp.	1,3	3	7.5	–
Alternaria sp.	1,4	2	5.0	–
Aspergillus sp.	4	2	5.0	–
Neurospora sp.	1	1	2.5	–

[a] 1—Wattala, 2—Kelaniya, 3—Bellanwilla, 4—Moratuwa, 5—Panadura, 6—Madampe, 7—Chillaw, 8—Bingiriya

[b] $\%\text{frequency of occurrence} = \frac{\text{number of spots yielding the isolate}}{\text{total number of spots examined}} \times 100$

[c] – negative, + low, ++ medium, +++ high

[d] The IMI numbers are the numbers assigned to cultures of these fungi deposited by us at the Commonwealth Mycological Institute, Kew, England.

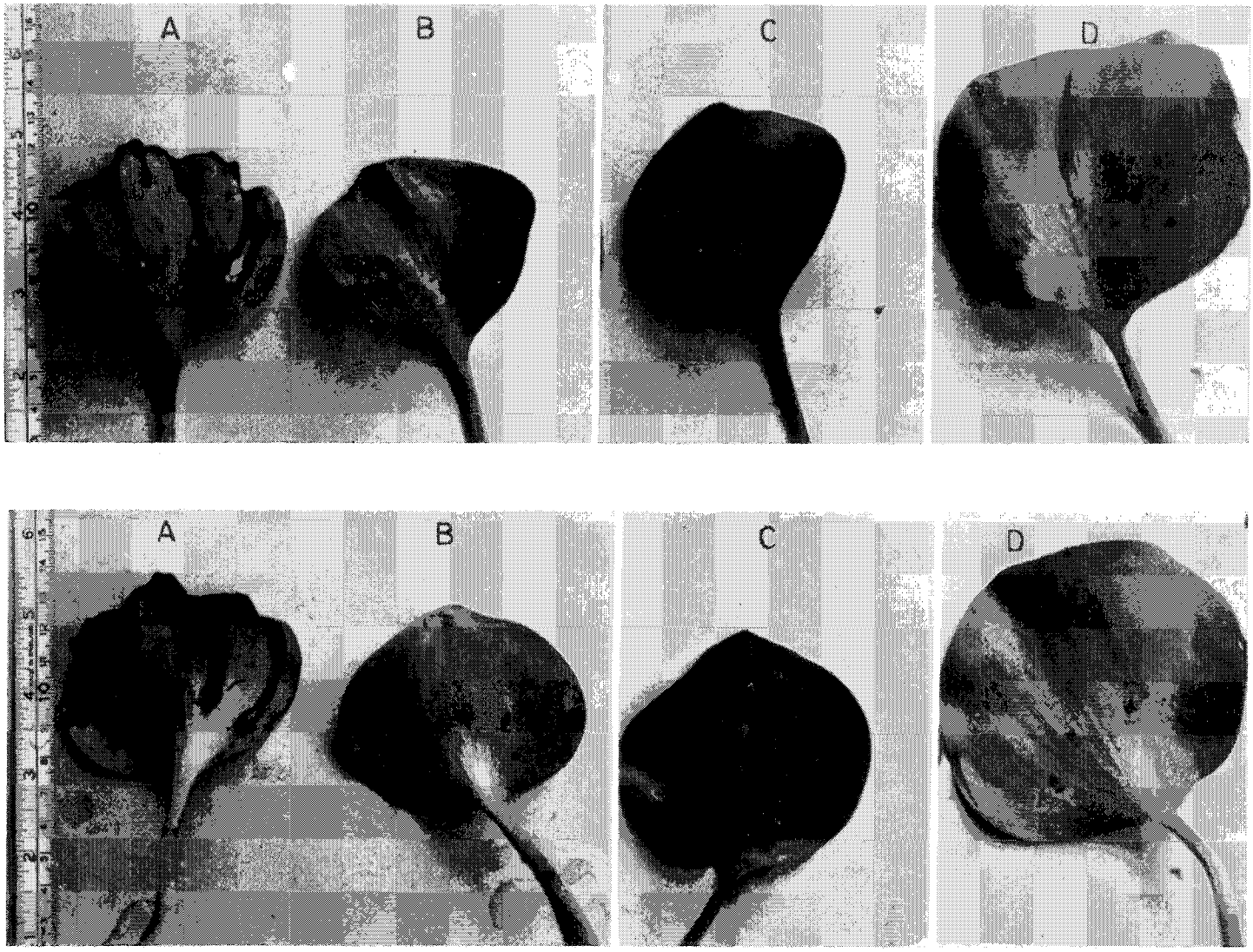

Figure 1. Leaf spots which developed on waterhyacinth (*Eichhornia crassipes*) leaves 25 days after inoculating the lower surface with (A) *Myrothecium roridum*, (B) *Septofusidium elegantulum*, (C) *Curvularia tuberculata* and (D) *Phaeotrichoconis crotalariae*. (Upper row—dorsal surface of the leaf and lower row—ventral surface of the leaf).

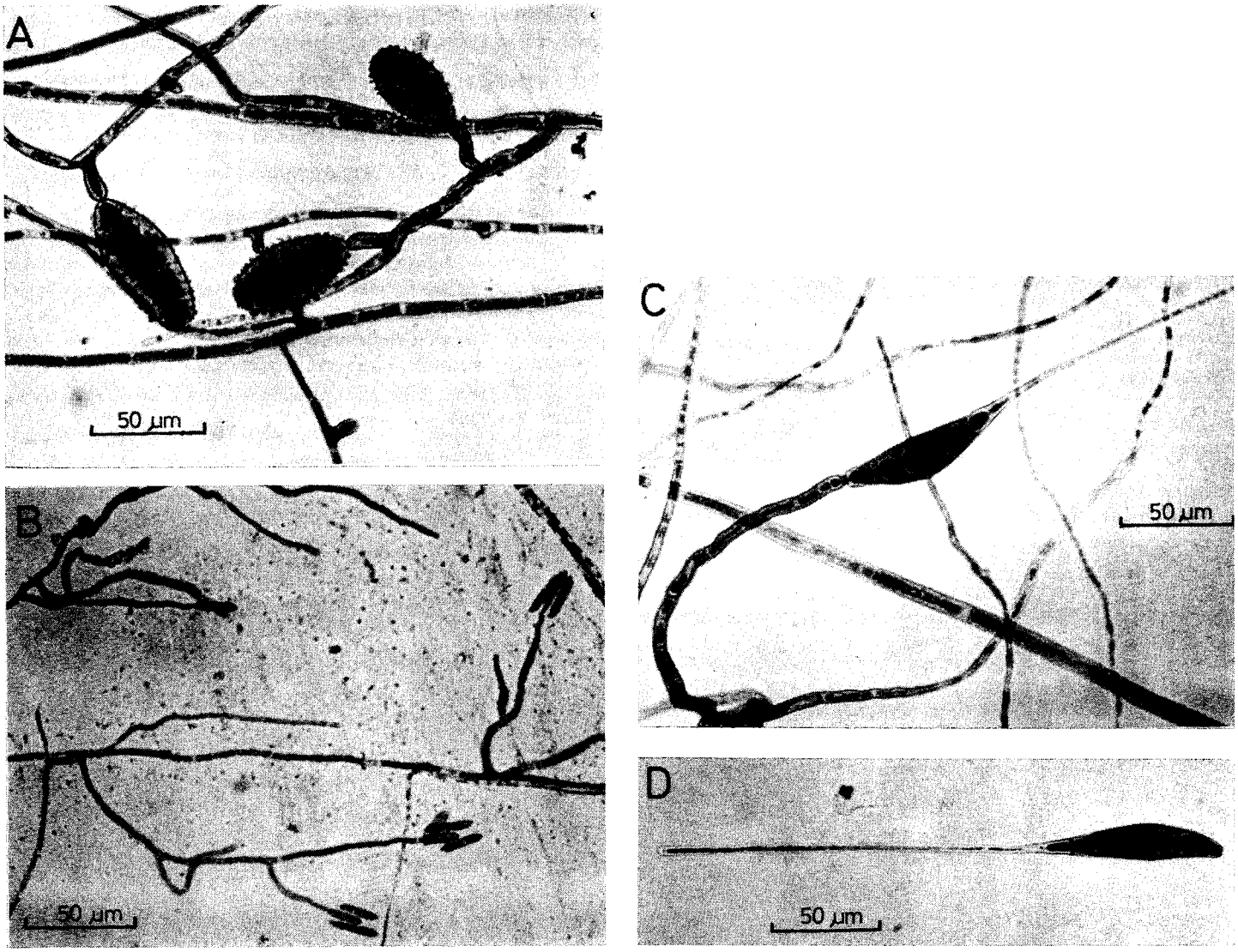

Figure 2. Conidiophores and conidia from Czapek dox agar slide cultures fixed in 1% cotton blue lacto-phenol. (A) *Curvularia tuberculata*, (B) *Septofusidium elegantulum* and (C & D) *Phaeorichoconis crotalariae*.

piaropi and *P. crotalariae* were associated with *C. tuberculata* leaf spots.

Septofusidium leaf spots were recorded from Wattala, Chillaw and Bingiriya areas. They were oval and brown, 2 to 10 mm in size and had a characteristic yellowish brown band, comparable to that formed by *M. roridum*, spreading from the spot to the margin of the leaf.

Smaller leaf spots yielded a fungal isolate which was identified as *Septofusidium elegantulum* (Pidlopl.) W. Gams (IMI 261800) (3). In larger leaf spots, *S. elegantulum* was associated with the pathogenic *P. crotalariae*, and the nonpathogenic *P. oxalicum* and another sterile fungus. Pathogenicity tests with *S. elegantulum* alone produced brown oval spots about 3 mm in size with a yellow band spreading from the spot towards the leaf margin (Figure 1B). In culture, *S. elegantulum* formed branched septate conidiophores which produced weak chains of 2-celled phialoconidia ranging from 9 to 15 (12.5) µm in length to 2.7 to 3.2 (3.0) µm in breadth (Figure 2B). *S. elegantulum* has been reported to be a hyperparasite of other fungi on leaves (3). However, positive pathogenicity by pure cultures of the present isolate showed it to be a primary pathogen of waterhyacinth leaves.

Phaeotrichoconis leaf spots were encountered at Bellanwila, Moratuwa, Madampe and Bingiriya areas. These were circular spots that spread over the leaf surface and ranged from 2 to 5 mm in size and yellow to black in color. They yielded an isolate identified as *Phaeotrichoconis crotalariae* (Salam & Rao) Subram. (IMI 261804) (1) capable of producing blackish, circular and small leaf spots on artificial inoculation (Figure 1D). In culture, mononematous, unbranched, septate, flexous or straight conidiophores about 130 µm in length were formed. These bore solitary, obclavate, septate, brownish conidia with a long hyaline beak and a dark scar at the point of attachment (Figure 2C & D). The body of conidia were 4 to 6 septate, 66 to 94 (77) µm long, 12 to 23 (16) µm thick at the broadest part. The beaks were 30 to 125 µm long and 1.0 to 2.5 µm broad.

Under field conditions, some of the *Phaeotrichoconis* leaf spots were associated with one or more of the nonpatho-

genic *P. oxalicum, C. lunata, Cephalosporium* sp. and *Alternaria* sp., and the pathogenic *S. elegantulum* and *C. tuberculata.*

Association of more than one pathogenic fungus or the association of pathogenic fungi and nonpathogenic fungi with a single waterhyacinth leaf spot is noteworthy. Nonpathogenic associates, such as *P. oxalicum, C. lunata* and *Fusarium sp.* were already recorded as local leaf surface inhabitants of waterhyacinth (4). Hence, such associations suggest the possible formation of disease complexes which could exhibit various degrees of synergism and/or antagonism. In certain instances, only nonpathogenic fungi were isolated from some leaf spots. This may be due to extreme cases of antagonism where the primary pathogen was completely replaced by nonpathogens. Alternatively, these leaf spots may be caused by fungi which did not readily grow on the media used.

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