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NOTES

Some Leaf Surface Fungi of Waterhyacinth in Sri Lanka[1]

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INTRODUCTION

Leaf surface constitutes a distinct microhabitat that under normal conditions is inhabited by a large and varied assemblage of microorganisms. The most numerous among them are bacteria followed by yeasts, filamentous fungi and actinomycetes in that order (5). Factors affecting fungal populations on aerial plant surfaces and the adaptations of these fungi to an epiphytic mode of life have been reviewed (2). Leaf surface microorganisms have been observed to fix atmospheric nitrogen, degrade plant waxes, produce growth regulators, compete with pathogens and stimulate phytoalexin production (6). This brief report summaries some of the preliminary observations regarding the leaf surface fungi of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms). Further work is in progress to determine the ecological role if any, of the principal isolates.

MATERIALS AND METHODS

Twenty samples each from tender, mature, moribund and dead waterhyacinth leaves from an abandoned paddy field and a swampy area were removed at fortnightly intervals between May-October 1980. In both sites waterhyacinth occurred in association with *Azolla and Salvinia,* and being shaded, received maximum sunlight.

The optimum diameter of the leaf discs that would yield the maximum number of fungal isolates without occluding slow growing forms was determined in a preliminary experiment and was found to be 5mm. For isolating fungi, a modification of Harley and Waid (4) serial washing technique was used. Two 5mm diameter discs were cut from each of the 20 leaves sampled, using a sterile cork borer. These were placed in 50ml sterile distilled water contained in a 250ml screw capped medicine bottle and washed by shaking at 400 oscillations/minute in a BTL flash shaker for 2 minutes. One ml teepol (detergent) was added to the first wash. The detergent increased the wettability of the leaf discs and enhanced the washing efficiency. To estimate the minimum number of washings required to dislodge the detachable propagules from leaf discs, decanted water from 1st, 5th, 10th, 15th, 20th, 25th, 30th and 35th washings were collected, appropriately diluted and plated on Czapek dox agar plates. The number of propagules dislodged by each washing was estimated by the number of colonies developing on each plate. Twenty washings in the case of tender and young leaves and 25 washings in the case of mature and moribund leaves was found to be sufficient to remove most of the detachable propagules from their surfaces. Washed leaf discs were dried with sterile filter paper, plated on Czapek dox agar at pH 7 containing 40 ppm streptomycin and incubated at 28-30 C. The plates were periodically examined for 14 days and fungi appearing on them isolated. The frequency of occurrence (= isolation) of each fungus was estimated in the following manner:—

$$\text{Frequency} = \frac{\text{number of leaf discs colonized by the fungus}}{\text{total number of leaf discs examined}} \times 100$$

RESULTS AND DISCUSSION

From the total of 17 different fungi obtained, a larger number were present on mature and moribund leaves than on tender leaves (Table 1). This agrees with similar observations on leaves of *Beta vulgaris* (5) and certain other tropical plants (9). The higher frequency of fungi on older leaves appears to indicate continuous deposition of fungal spores on the leaf surfaces and the establishment of some of these spores in the presence of leaf leachates and exudates.

Penicillium oxalicum, Curvularia lunata, Fusarium sp. and sterile fungus I occurred on a larger number of fortnightly samples at a higher mean % frequency than *Trichoderma viride, Myrothecium roridum, Aspergillus niger* and species of *Helminthosporium, Mucor, Penicillium, Aspergillus, Cunninghamella* and *Cladosporium* (Table 1). The higher frequency of the former group of fungi possibly indicate their ability to grow extensively on healthy leaf surfaces. According to Leben (7), these isolates can be considered as 'residents' and the rest of the isolates as 'casuals.' Amongst the resident fungi *P.oxalicum* has not been previously recorded on waterhyacinth while *C. lunata, Fusarium roseum* and *Curvularia* sp. have been recorded and are classed as weak or minor pathogens (3).

Amongst the casuals. *T. viride, M. roridum, A. niger* and *Helminthosporium* sp. were more frequent in their occurrence (Table 1) and records of these genera on waterhyacinth has already been reviewed (3). The presence of pathogenic *M. roridum* as a prolific casual on healthy undamaged leaf surfaces in the present study suggests its ability to grow in a nonaggressive manner and survive on leaf surfaces before invading them as pathogens. Many pathogenic fungi show this kind of epiphytic growth prior to invasion of hosts (8); and further studies on this aspect of *M. roridum* could perhaps determine the nature of interaction between the saprophytic and parasitic phases of this organism.

Less prolific casuals such as, *Mucor, Penicillium, Asper-*

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TABLE 1. MEAN PERCENT FREQUENCY OF LEAF SURFACE FUNGI ON TENDER, MATURE, MORIBUND AND DEAD WATERHYACINTH LEAVES FROM TWO SITES.

Fungal isolate	Leaf stage:	Tender		Mature		Moribund		Dead	
	Site:	A[1]	B[2]	A	B	A	B	A	B
Penicillium oxalicum		24(12)[3]	10(10)	54(12)	44(12)	68(12)	55(11)	55(11)	85(12)
Curvularia lunata		10(9)	14(8)	27(9)	41(12)	37(9)	59(12)	35(10)	72(12)
Fusarium sp.		10(6)	5(2)	12(6)	15(12)	27(10)	41(11)	41(8)	53(11)
Sterile fungus I		21(9)	14(6)	39(10)	56(11)	21(9)	60(9)	23(11)	43(7)
Trichoderma viride		—	10(9)	5(2)	27(10)	7(2)	10(3)	10(2)	—
Myrothecium roridum		20(1)	—	12(1)	8(1)	36(1)	—	92(1)	—
Helminthosporium sp.		5(2)	7(6)	10(1)	5(1)	10(6)	—	—	—
Aspergillus niger		4(2)	7(3)	8(5)	5(1)	13(3)	—	—	—
Aspergillus sp. II		7(8)	5(1)	30(1)	—	25(1)	—	—	—
Aspergillus sp. III		5(3)	—	15(1)	5(1)	—	—	—	—
Mucor sp.		—	—	—	5(1)	30(8)	—	7(6)	—
Penicillium sp. III		—	—	25(1)	6(3)	25(2)	—	—	—
Sterile fungus II		—	—	—	30(1)	—	30(1)	—	12(1)
Sterile phycomycete		—	—	—	60(1)	—	15(1)	—	40(2)
Aspergillus clavatus		—	—	5(1)	—	40(1)	—	—	—
Cunninghamella sp.		—	—	—	5(2)	5(1)	—	—	—
Cladosporium sp.		—	—	—	—	5(1)	—	—	—

[1]Abandoned paddy field
[2]Swampy area
[3]The numbers in brackets give the number of fortnightly samples on which the isolates were present out of a total of 12 samples

gillus, Cunninghamella and *Cladosporium* observed in the present study may be considered to have arisen from their propagules that were deposited from the air and failed to get dislodged in the serial washing procedure.

Most of the leaf surface fungi found on waterhyacinth in the present study have also been reported on the leaves of *Salvinia molesta* growing under similar environmental conditions (10). However, as already pointed out (9), they are different from the mycoflora normally encountered on leaf surfaces of plants growing in temperate climates including waterhyacinth (1). A further understanding of the probable interaction of these leaf surface fungi or their metabolites with potential pathogens would be useful in the biocontrol of waterhyacinth by fungal pathogens.

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