fungi are not distinct species, but are synonyms of *C. zonatum*. Attempts to locate Sawada's original description of *C. zonatum* have not as yet been successful, but a comparison of those for *C. fici* (9) and *C. eichhorniae* (5) reveals that they are nearly identical. Additional work is underway to test the pathogenicity of the Louisiana and Florida isolates on coffee and to obtain cultures of *C. zonatum* from Costa Rica for comparison.

The possibility of using *C. zonatum* as a biological control agent of waterhyacinth does not seem favorable at the present time. Studies indicate that the fungus is restricted to the leaves and petioles of the plant and that even on these its progress is not rapid. It does not seem capable of killing that plant nor of seriously hindering its prolific growth. In those areas where the disease was first noted it did not appear to be causing significant damage as many of the plants were uninfected. The possibility exists, however, that applications of the fungus at high concentrations could cause significant damage and this area is now being explored.

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Pathogenicity of Fungi and Bacteria from India to Hydrilla and Waterhyacinth[1]

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INTRODUCTION

Surveys were made in southern India from November 1971 to January 1972 in a search for plant pathogens as possible biocontrol agents of hydrilla (*Hydrilla verticillata* Royle) and other aquatic plant problems in Florida. Hydrilla has existed much longer in India than in Florida and, being a cosmopolitan plant of warm temperate to tropical regions (7), occurs extensively throughout India. For this reason, India was considered to be one of the places where chances are good for finding suitable biocontrol agents of hydrilla. While on this tour, pathogens of waterhyacinth (*Eichornia crassipes* (Mart.) Solms.) were also collected. Several promising pathogens were isolated in India on this tour from diseased plants and were brought to this country in strict compliance with quarantine regulations as specified by the U. S. Department of Agriculture and Florida Department of Agriculture and Consumer Services. About 100 cultures of fungi and bacteria were obtained and studied using the quarantine facilities available at the Plant Pathology Department of the University of Florida.

MATERIALS AND METHODS

Isolation of pathogens and culturing. Plant pathogens were isolated from specimens of hydrilla and waterhyacinth that had lesions, rots, and browning. Specimens of some diseased plants were cut into small pieces, surface-sterilized with 0.1% aqueous solution of mercuric chloride for 1.0 min and rinsed thoroughly with sterile water. These pieces were then plated on potato dextrose agar (PDA) or nutrient agar (NA) in petri plates and incubated at 25 C. Any emergent fungi and bacteria were isolated. Other diseased specimens were incubated in moist chambers to allow the slow-growing parasites to

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emerge from tissues prior to isolation by transferring them to PDA plates. Pure cultures of fungi and bacteria were maintained respectively on PDA and NA in tubes.

Transport of pathogenic microorganisms. Sealed cultures were transported in an impact resistent plastic box until they reached their destination in Gainesville. These cultures were opened for study only when inside the quarantine glasshouse at the University of Florida.

Pathogenicity trials. The fungal and bacterial isolates collected in India were tested to establish their pathogenicity to hydrilla and waterhyacinth following Koch's postulates. These tests were conducted exclusively in the quarantine glasshouse maintained at 26 to 30 C and under approximately 1000 ft-c of light intensity. Any remains of cultures, test plants, water, and glassware used inside the isolation chamber were sterilized in an oven at 150 C for an hr before being taken outside the facility.

Pathogenicity tests on hydrilla were conducted in an assembly shown in Figure 1. A 25 by 150 mm test tube was filled with distilled water, and covered with six layers of cheesecloth. A 30 ml beaker was inverted over the mouth of the tube. The whole assembly was pre-sterilized before introducing a sprig of hydrilla (about 100 mm long) with an active apical bud. The hydrilla sprig was washed with five changes of sterile water before being introduced into the tube. One day later, each sprig was inoculated with a 1.0 ml suspension of bacteria washed from a NA culture or with a 5 mm^2 agar-block with a fungus. Control hydrilla sprigs received 1.0 ml washings from noninoculated NA tubes or a piece of noninoculated PDA. Results were recorded over a 4-week period.

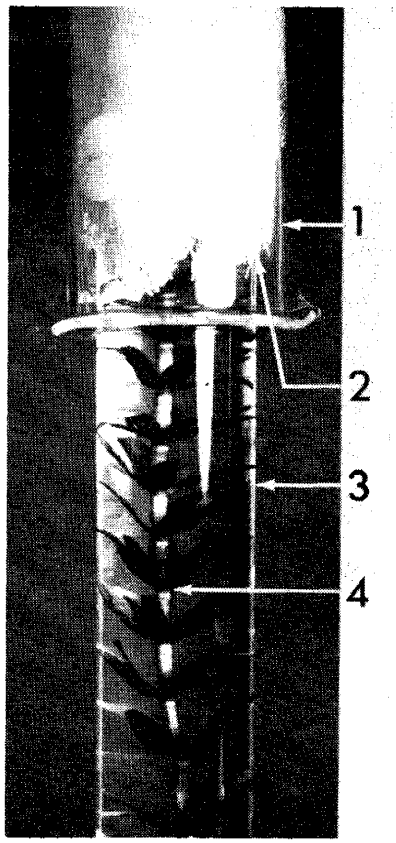

Figure 1. Assemble for testing pathogenicity of microorganisms to hydrilla. 1. A 3-ml beaker; 2. Six folds of 50 by 50 mm cheesecloth; 3. Test tube 25 by 150 mm with sterilized water; 4. About 100 mm long shoot of hydrilla with an active apical growing tip.

Waterhyacinth plants were maintained in 20-cm diameter pots lined with plastic bags and filled with tap water. Since the parasites were originally isolated from leaves, this organ of the plant was chosen for inoculations. Fungi and bacteria from waterhyacinth were placed on leaf areas rubbed with 600-mesh carborundum powder. Four leaves per plant were inoculated, each at two spots, one on each leaf surface. The inoculum consisted of a drop of a dense suspension of either fungal spores (and/or mycelia) or bacterial cells. Control plants were treated similarly and inoculated with a drop of sterile water. After inoculation, each plant and its pot were covered with a thick plastic bag to maintain high humidity. The plants were observed over a period of 4 weeks.

For reisolation of fungi and bacteria, inoculated plant parts were surface-sterilized with 10% Chlorox for 30 sec (hydrilla) or 1 min (waterhyacinth), washed repeatedly with sterilized water and plated on PDA or NA plates.

RESULTS

A list of fungal genera, types of bacteria, and number of isolates obtained from surface-sterilized hydrilla and waterhyacinth samples are presented in Table 1. The number of isolates under each genus included variants of the same species of pathogen, or different species of the genus or both. Fungi collected from hydrilla included *Pythium, Sclerotium, Cephalosporium* and *Fusarium*, species of which are significant pathogens of terrestrial plants. The fungi from waterhyacinth included species of *Alternaria, Cephalosporium, Fusarium, Myrothecium* and *Rhizoctonia*.

Forty fungal and fifteen bacterial isolates were tested for pathogenicity to hydrilla. Among the fungal isolates

Table 1. A List of Indian Fungal and Bacterial Isolates Obtained from Hydrilla and Waterhyacinth Samples.

Hydrilla	Number of isolates	Waterhyacinth	Number of isolates
Fungi			
Aspergillus	3	*Alternaria*	4
Cephalosporium	1	*Aspergillus*	1
Chaetophoma	2	*Aureobasidium*	1
Fusarium	3	*Cephalosporium*	2
Gliocephalis	1	*Curvularia*	1
Gliocladium	1	*Fusarium*	2
Oidiodendron	1	*Gonotobotrys*	1
Penicillium	3	*Myrothecium*	5
Periconia	1	*Nigrospora*	1
Phoma	2	*Phoma*	1
Pythium	1	*Pythium*	2
Sclerotium	2	*Rhizoctonia*	1
Stigmina	1	*Stagonospora*	1
Trichoderma	1	*Trichoderma*	1
Unidentified	17	Unidentified	8
Total	40	Total	32
Bacteria			
Gram negative rods	6	Gram negative rods	2
Gram negative spheres or ovoids	9	Gram negative spheres or ovoids	4
Total	15	Total	6

TABLE 2. INDIAN FUNGI AND BACTERIA THAT DAMAGE HYDRILLA.

Organism	Degree of damage	Number of isolates
Aspergillus sp.	*a	1
Pythium sp.	***	1
Sclerotium sp.	***	2[b]
Trichoderma viride Pers. ex Fr.	*	1
Penicillium spp.	**	3[c]
Unidentified (Mycelia Sterilia?)	*	1
A gram negative, rod-shaped bacterial isolate	*	1
Total		10

a* = Yellowing of a portion of the plant; ** = general yellowing of the entire plant; *** = severe discoloration of the plant and decomposition of portions.
[b]The two *Sclerotium* isolates belonged to the same species.
[c]Isolates of *Penicillium* represented three different species.

tested, *Pythium* sp. and *Sclerotium* sp. were significantly damaging to the test hydrilla plants (Table 2). A severe discoloration on portions of test plants was caused by *Sclerotium* and *Pythium* (Figure 2). About 3 weeks after inoculation, such discolored portions became readily detached from the rest of the plant. Sometimes, green lateral shoots appeared from portions of such discolored plants. However, the growth of such side shoots was slow compared to controls. Generally, the fungal mycelia were seen growing over the yellow and discolored portions of plants.

Four other fungi as well as a bacterium, induced yellowing of hydrilla plants. In this category were *Aspergillus* sp., *Penicillium* spp., *Trichoderma viride* Pers. ex Fr., an unidentified fungus (Mycelia Sterilia ?), and a gram negative rod-shaped bacterium isolate number 58).

Thirty-two isolates of fungi were tested on waterhyacinth, of which ten proved to be highly pathogenic (Table 3). None of the six bacterial isolates tested was significantly pathogenic. Several of the non-pathogenic fungi and bacteria, however, caused necrosis at the site of inocula-

TABLE 3. FUNGI PATHOGENIC TO WATERHYACINTH

Organism	Degree of Pathogenicity	Number of isolates
Alternaria eichhorniae	*a	4
Myrothecium roridum	***	5
Rhizoctonia solani	***	1
Total		10

a** = spreading but not extensive necrosis on inoculated leaves; *** = spreading and extensive necrosis, resulting in further spread of disease to noninoculated leaves.

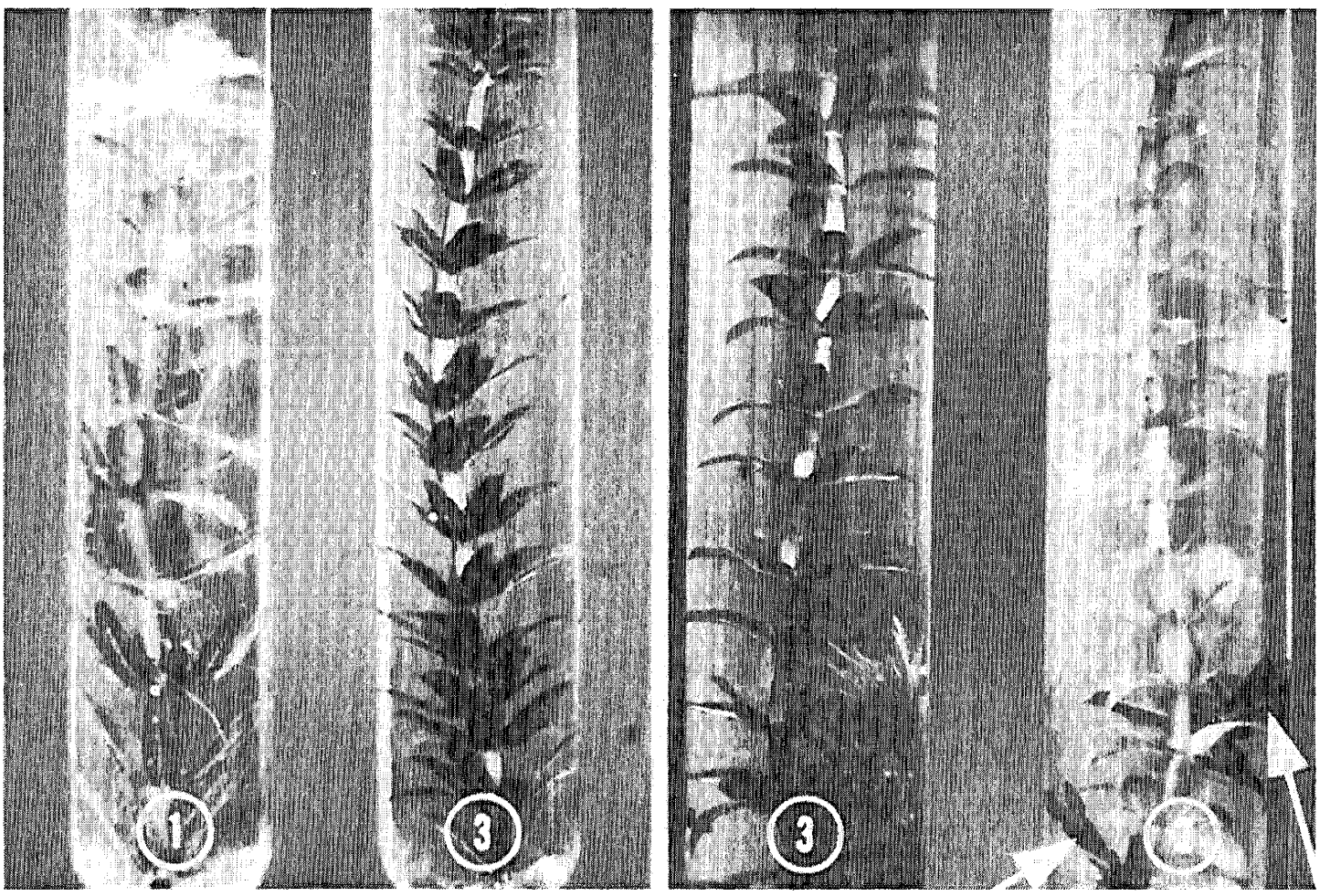

Figure 2. Damage to hydrilla caused by *Sclerotium* and *Pythium* species. 1. Hydrilla with discoloration, especially of the growing tip induced by *Sclerotium*. 2. Damage due to *Pythium*. Arrows point to the development of green axillary shoots. 3. Noninoculated controls.

tion due to wound infection. Certain other isolates necrosed the tissue around the site of inoculation, spreading to only 3 to 6 mm from the wound. Three isolates of *Fusarium* spp. belonged to this category and were relatively avirulent in these tests. The most pathogenic isolates, however, caused extensive necrosis that progressed over the 4-week period. The ten most pathogenic cultures were identified as isolates of *Alternaria eichhorniae* Nag Raj & Ponnappa, *Myrothecium roridum* Tode ex Fr., and *Rhizoctonia solani* Kuehn. (Figure 3). *Myrothecium* was most devastating on test plants, followed respectively by *Rhizoctonia* and *Alternaria* (Figure 3).

DISCUSSION

An important aspect of this work involves the safety in transport and handling of foreign pathogens in the United States. Starting with the transport of these cultures into this country from their foreign sources, every care was taken to prevent accidental escape of any of these microorganisms. The quarantine glasshouse at the Plant Pathology Department of the University of Florida in which these organisms are studied ensures maximum possible protection against such accidental escapes.

There are no known reports of diseases of hydrilla; indeed, little is known about diseases of submersed aquatic plants in general (9). Thus, the results in this paper are the first describing conditions that could be called diseases on hydrilla. The damage to hydrilla due to isolates of *Pythium* and *Sclerotium* led to a general decline of the plant, reduced vigor in growth of side shoots, and

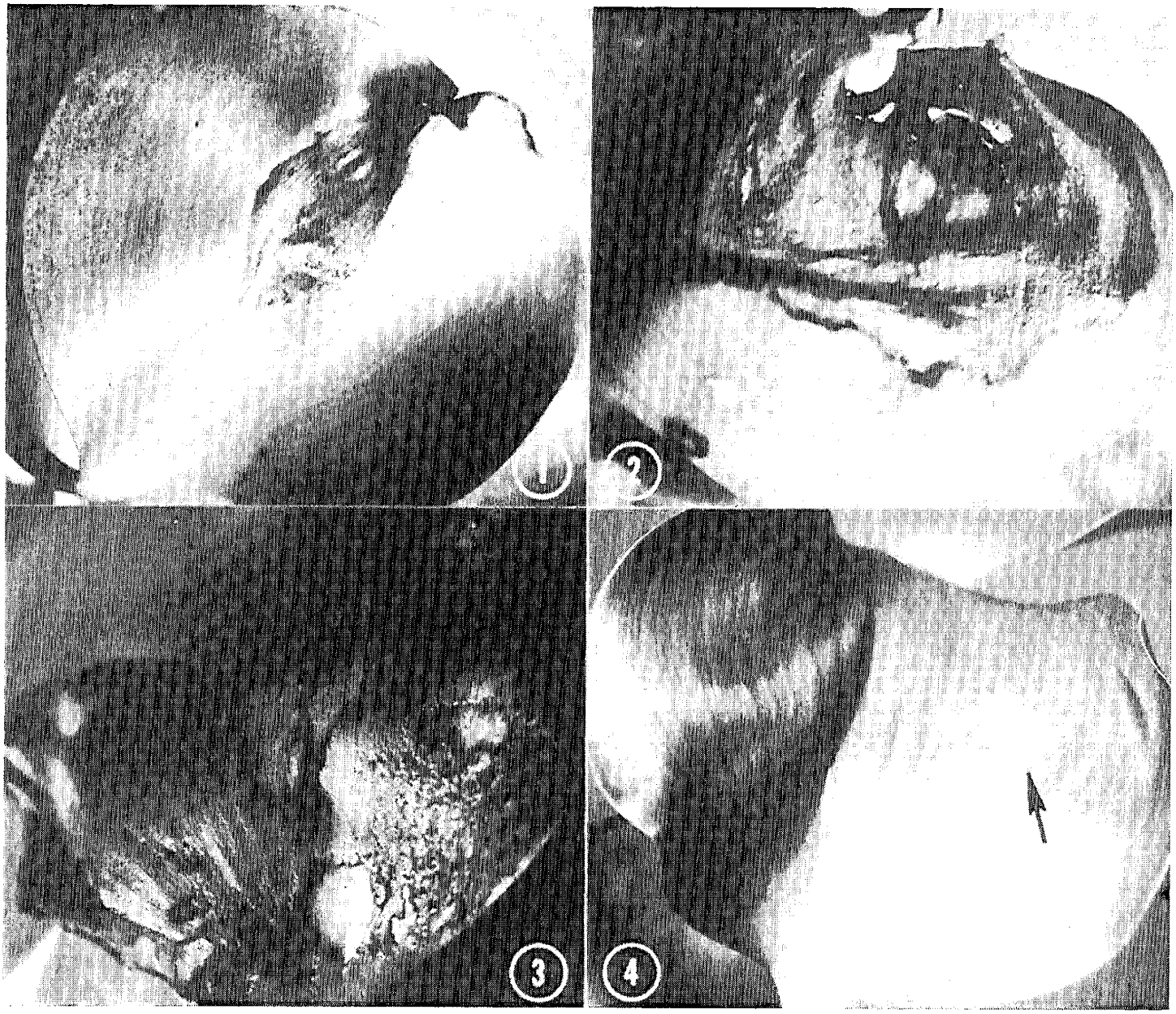

Figure 3. Nature of damage to waterhyacinth leaves caused by *A. eichhorniae* (1) *M. roridum* (2) and *R. solani* (3). Control leaf (4) with only mechanical damage resulting from the inoculation procedure (arrow).

yellowing. Under extreme conditions, infected plants became discolored and decayed.

Hydrilla has the capacity to regenerate itself from any bud along its entire shoot. In these tests, it was found that plants could produce healthy side shoots even from chlorotic regions. However, such axillary shoots were slower in growth, compared to similar ones on control plants. Decaying portions of plants did not produce such side shoots.

A general yellowing of the entire plant or its portions, but no decline, was caused by species of *Penicillium, Aspergillus, Trichoderma,* an unidentified fungus, possibly belonging to Mycelia Sterilia, and a gram negative bacterium. In all instances, the fungi could be reisolated from surface-sterilized symptomatic parts of plants. The apparent pathogenicity of *Aspergillus, Penicillium* and *Trichoderma* to hydrilla seems confusing, since these are generally regarded as non-pathogens of plants. It is possible that they produced toxic metabolites that killed or weakened hydrilla tissues which were later colonized by these fungi saprophytically.

Several fungi and bacteria infected waterhyacinth leaves and caused necrosis, but only ten fungal cultures proved highly pathogenic. The latter were identified as isolates of *A. eichhorniae, M. roridum,* and *R. solani.* They caused extensive necrosis on inoculated leaves, which after a three-week incubation spread to noninoculated leaves and petioles of the same plant. These three species of fungi have been previously reported as pathogens of waterhyacinth in India (3). Also in Panama, *Rhizoctonia solani* was found on *E. azurea* (1). So far, none of these fungi have been reported as pathogens of waterhyacinth in this country. *Myrothecium roridum* and *R. solani* are known to occur on other hosts in this country, while *A. eichhorniae,* being a recently described species (3), has not been found here. The wide host ranges of *R. solani* (4) and *M. roridum* (5) presumably would preclude their use as biocontrol agents. Nag Raj and Ponnappa (3) consider that *A. eichhorniae* and *Marasmiellus inoderma* (Berk.) Sing., the casual agent of the thread blight of waterhyacinth (2) might have some potential for biocontrol of waterhyacinth. Our continuing studies in Florida would include the evaluation of biocontrol potentials of these two fungi and others on waterhyacinth.

SUMMARY

A search was made recently in India for pathogens of hydrilla and waterhyacinth for biological control purposes. Several fungi and bacteria were isolated from surface-sterilized shoots and leaves of these plants that were diseased or suspected to be so. These organisms were brought to the University of Florida and studied in a quarantine glasshouse. Pathogenicity tests with 40 fungi and 15 bacteria revealed that a *Pythium* and a *Sclerotium* species were damaging to hydrilla. Among 32 fungi and 6 bacteria from waterhyacinth, *Alternaria eichhorniae, Myrothecium roridum* and *Rhizoctonia solani* were virulent on this host. These three fungi have been previously reported as pathogens of waterhyacinth in India. The pathogens of hydrilla found in this study as well as known pathogens of waterhyacinth will be assessed as biocontrol agents of these plants in Florida.

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