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THE USE OF PATHOGENS FOR BIOLOGICAL CONTROL OF HYDRILLA, A SUBMERSED AQUATIC WEED

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ABSTRACT

During a one year survey for microorganisms associated with hydrilla (*Hydrilla verticillata*), approximately 2200 microbes were recovered from hydrilla, surrounding water, and sediment collected from 10 man-made ponds and three natural lakes in Florida, USA. A representative selection of fungi (651), bacteria (330), and actinomycetes (38) from this collection was screened against hydrilla in bioassays. Severity of damage (DS) was determined on a scale of 0 to 4, where 0 = healthy, 1 = 1-25% damage, 2 = 26-50% damage, 3 = 51-75% damage, and 4 = 76-100% damage (100% = complete kill). Of the 1019 isolates that were examined in this manner, none of the actinomycetes were pathogenic on hydrilla, however 2 strains of bacteria (0.6% of the bacteria tested), and 42 isolates of fungi (6.5% of the fungi tested) were effective at DS level 4 on hydrilla 3 weeks post inoculation. Fungi and bacteria capable of killing hydrilla are considered to have potential as biocontrol agents or as sources of novel herbicidal metabolites. These included *Acremonium* sp., *Botrytis* sp., *Curvularia* sp., *Cylindrocarpon* sp., *Fusarium culmorum*, and *F. moniliforme*. The biocontrol potential of *F. culmorum* has been established in several tests. For maximum bioherbicidal activity of this fungus against hydrilla, its inoculum should be applied at a rate of 5 x 10^5 conidia/ml at 20 to 25 °C water temperature.

Keywords: *Hydrilla verticillata*, submerged aquatic plant, biological control, bioassay, spore production, temperature, *Fusarium culmorum*, *Acremonium* sp., *Botrytis* sp., *Cylindrocarpon* sp.

INTRODUCTION

Hydrilla [*Hydrilla verticillata* (L. f.) Royle; Hydrocharitaceae] is considered to be one of the worst aquatic weeds in Florida and in other geographic regions of the United States. Native to the warmer regions of the Old World, it is a cosmopolitan species that now occurs in Africa, Europe, Asia, Australia, New Zealand, the Pacific Islands, South America, and North America. Serious economic losses and ecological damage occurs when hydrilla impedes navigation, clogs drainage and irrigation canals, interferes with recreational activities, and disrupts wildlife habitats. This plant grows rapidly, tolerates very low light intensities and produces two types of specialized hibernating organs (turions). It can survive unfavorable conditions for growth and outcompete other species. Its potential to invade new areas is alarming, as has been shown in the United States, where it was introduced

around 1960 and now has expanded from Florida to Conneticutt on the east coast, across southern US, and extended from California to Washington on the west coast.

Hydrilla is controlled largely through the use of chemical herbicides or mechanical removal. The high cost of these control measures, as well as concern for the environment, has increased interest in biological control of this noxious weed. Diseases of submerged weeds are poorly known and very few plant pathogens have been found on hydrilla (Charudattan, 1973; Shearer, 1995). The fungus *Mycoleptodiscus terrestris* was considered as a potential mycoherbicide for hydrilla control (Shearer, 1996; Shearer and Jackson, 2001). Therefore, attempt to discover additional biological control agents for hydrilla was the specific objective of the present investigation.

MATERIALS AND METHODS

Sampling strategy

Water, hydrosoil, and hydrilla were sampled every 3 to 4 weeks for one year from ten man-made ponds located at the Center for Aquatic Plants, University of Florida, Gainesville, USA and three freshwater lakes around Gainesville, Orange Lake, Rowell Lake and Rodman Reservoir. Three sampling points within each pond and 25 points in each lake were sampled each sampling time. Water samples were taken by submerging sterile 5-dram glass vials and opening them 10 cm under the water surface. Sediment samples were collected from the ponds by pushing a 1.8-m long, 1.8-cm-diam PVC syringe into the sediment and retrieving the top 5-cm. The PVC syringe was sterilized by dipping it in 95% ethyl alcohol for 5 min prior to use in the next pond. Sediment was collected from lakes by pushing a 3-m long, 2.25-cm-diam PVC tube into the sediment, closing the upper end of the tube with a rubber stopper, and retrieving the top 10-15 cm. At each sampling point, 10 to 15 apical shoots of hydrilla (20- to 30-cm-long) were collected from the top 30 cm of the water column. All samples were kept cool and processed within 3 to 4 h of collection. Each plant sample was divided into 2 subsamples. One was surface sterilized in 3% H_2O_2 for 3 min and the other was repeatedly washed in running tap water for 3 min to remove epiphytes. Both subsamples were ground with a sterile pestle and mortar prior to making dilutions.

Recovery of microbes

Aqueous dilutions of water, surface sterilized plants, repeatedly washed plants, and soil were plated on 9-cm agar plates of six selective or general purpose culture media as described by Shabana and Charudattan (1996). Culture media used for isolation of microbes were Sneh & Stack selective medium (SS) (Sneh and Stack, 1990), Komada's medium (K) (Komada, 1975), PART selective medium (PART) (Shabana and Charudattan, 1996), chitin agar selective medium for actinomycetes (CA) (Lingappa and Lockwood, 1962), nutrient agar (NA) (DIFCO laboratories, Detroit, Michigan, USA), and hydrilla extract plus half strength potato dextrose agar (HPDA) (Shabana and Charudattan, 1996). Dilution plating, colony

counting, and culture storage were conducted as described by Shabana and Charudattan (1996).

Identification of microbes

Pure cultures of highly effective isolates against hydrilla were identified according to Ellis (1971 & 1976), Barnett and Hunter (1972), and Booth (1971). Bacterial strains were tested for Gram reaction.

Screening microbes against hydrilla in a test tube bioassay

A random selection of fungi (651), bacteria (330), and actinomycetes (38) from the collected microbes were screened against hydrilla in a bioassay. Two-wk-old agar-plate fungal cultures, 3-wk-old agar-plate actinomycete cultures, and 3- to 4-day-old streaked culture plates (6-cm-diam) were flooded with 4 ml of sterile distilled water and the cultural growth dislodged by thoroughly rubbing the agar surface with a rubber knife. One milliliter of the resulting suspension was dispensed into 22-mm-diam x 150-mm-long glass tubes, each containing 49 ml of sterile tap water and a 9-cm-long healthy, terminal shoot of hydrilla. The tubes were covered with sterile plastic caps and placed under diurnal light (12 h light, 137 $\mu E/m^2.s$) at 25 $\pm$ 2^0C for 3 weeks. Each treatment was replicated 3 times. Three to 9 tubes containing hydrilla shoots were left without microbial inoculation to serve as controls in each test. Three weeks post inoculation, the hydrilla was rated for severity of damage (DS). DS was determined for each shoot on a scale of 0 to 4, where 0 = healthy, 1 = 1-25% damage, 2 = 26-50% damage, 3 = 51-75% damage, and 4 = 76-100% damage (100% = complete kill) (Fig. 1). Kock's postulates were fulfilled for fungal and bacterial isolates that had highly pathogenic capabilities on hydrilla. The bioassay was repeated three times for microbes that caused DS level 4.

Pathogenicity of selected isolates in two bioassays

a) Large test tube bioassay

Four isolates of fungi, *Acremonium* sp. (F71PJ), *Cylindrocarpon* sp. (F531), *Botrytis* sp. (F542), and *Fusarium culmorum* (F964) that were highly effective against hydrilla in the aforementioned bioassay were chosen for further testing. Conidial suspensions were prepared for each fungus and were quantified with a hemacytometer. Spore concentrations ranging from 2 x 10^6 to 1 x 10^8 conidia / ml were added in 1 ml aliquots to 22mm x 150 mm test tubes filled with 49 ml of sterile tap water and containing one 6-9 cm long terminal shoot of hydrilla. Following dilution, the final concentrations in the tubes ranged from 4 x 10^4 and 2 x 10^6 conidia per ml of water. Inoculated and uninoculated tubes were covered with sterile plastic caps and placed under diurnal light (12 h light, 137 $\mu E/m^2.s$) at 25 $\pm$ 2^0C for 3 weeks. Treated and untreated control tubes were replicated 6 times. Three weeks after inoculation, hydrilla was rated for DS.

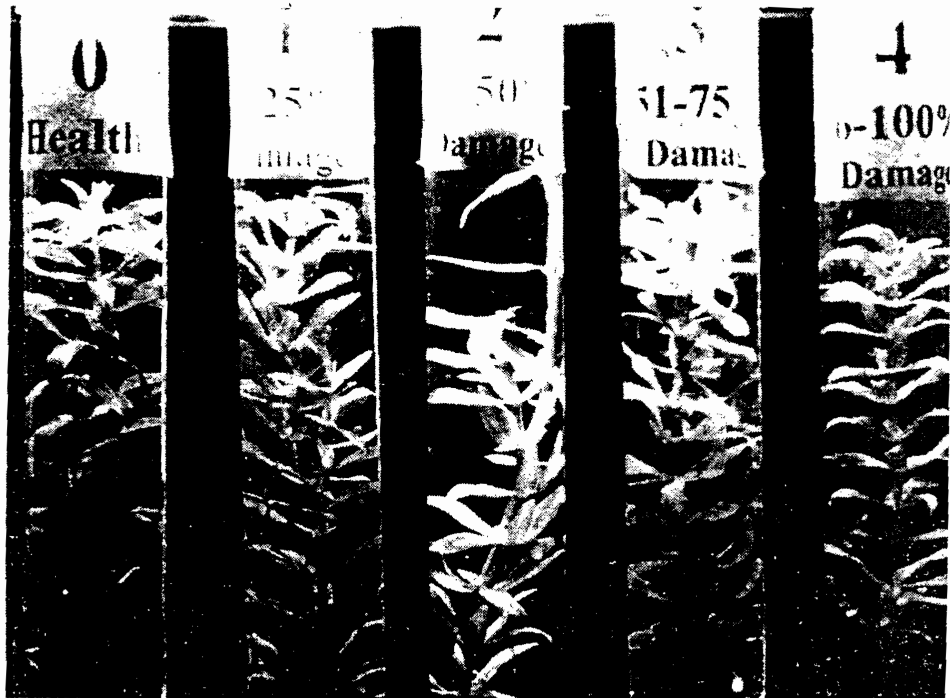

Fig.(1): **A 0-4 disease severity rating system for hydrilla, where 0 = healthy, 1 = 1-25% damage, 2 = 26-50% damage, 3 = 51-75% damage, and 4 = 76-100% damage (100% = complete kill).**

b) Aquarium bioassay

Fusarium culmorum (F964) was selected for aquarium testing because it sporulated more profusely than the other biocontrol candidates. Ten healthy, vigorously growing shoots of hydrilla (10 to 12 cm long) were planted singly in sterile 1-gallon aquaria containing 5 cm height of sterilized washed sand. The aquaria were carefully filled with sterilized 5% Hoagland's solution (Hoagland and Arnon, 1950) containing 0.1% $KHCO_3$. The pH was adjusted to 6.5 with 1 *N* HCl or 1 *N* NaOH. After two days, treated aquaria were inoculated with a conidial suspension of *F. culmorum* to produce a final concentration of 1 x 10^6 conidia per ml of solution. Treated and untreated reference aquaria were replicated 4 times. The aquaria were maintained in a completely randomized design under diurnal light (190 $\mu E/m^2.s$) at 25 $\pm$ 2^0C in a plant growth room. After 4 weeks, the hydrilla in each aquarium was rated for DS.

Large-scale spore production of selected biocontrol candidates

The four isolates of fungi that were highly effective against hydrilla in the test tube bioassay were used in this study. Two 500-ml Erlenmeyer flasks containing 100 ml of V-8 juice broth (200 ml V-8® + 3 g $CaCO_3$ + 800 ml tap

water) were inoculated with 3 ml of a mycelial suspension of each isolate and incubated at 27^{0}C $\pm$ 2^{0}C on a shaker at 120 strokes / min for 7 days. Contents of each flask were comminuted in a blender to form a very smooth fungal suspension. Plastic trays (20 x 40 cm^2) lined with aluminum foil and containing 500 ml of V-8 agar medium supplemented with streptomycin sulphate (0.3 g/l) were inoculated with 30 ml of fungal suspension. The trays were covered with plastic wrap and placed under diurnal light (137 μE/m^2.s) at 27 $\pm$ 2 ^{o}C for 3 days. Spores of isolates F964, F531 and F542 were harvested at 3 and 12 days post inoculation. Spores of F71PJ were harvested at 7 and 12 days post inoculation. In addition, some trays containing isolates F542 and F964 were harvested only once at 12 days post inoculation. Harvest times varied depending on spore production. Spores were harvested by adding 50 ml of sterile distilled water to each tray and thoroughly rubbing the agar surface with a sterile rubber knife to dislodge the conidia. The spores were collected in sterile 500 ml screw-capped glass bottles and stored in a cold room at 2^{0}C. Each treatment was replicated 5 times. Spore production in each tray was determined using a hemacytometer.

Effect of inoculum concentration of *F. culmorum* on hydrilla

Conidia produced as described above were used to set up 8 concentrations of inocula for *F. culmorum* (F964) (0, 2 x 10^3, 2.25 x 10^4, 1.25 x 10^5, 2.5 x 10^5, 5 x 10^5, 1 x 10^6 and 24 x 10^6 spores / ml) following dilution in a test tube bioassay as previously described. Each treatment was replicated 6 times. Three weeks post inoculation, hydrilla in each tube was rated for DS.

Determination of the optimum incubation temperature conducive for disease promotion by *F. culmorum* on hydrilla

To determine which temperature induced the highest level of *F. culmorum* DS, apical shoots of hydrilla were placed in test tubes as described previously and inoculated with a conidial suspension of *F. culmorum* (F964) to produce a final concentration of 1 x 10^6 conidia/ml. The tubes were placed in 5 growth chambers set to 15, 20, 25, 30, and 35^{0}C under diurnal light (210 μE/m^2.s). Fifteen days post inoculation, hydrilla was rated for DS. Treated and untreated reference tubes were replicated 6 times.

Statistical analysis

The experiments were repeated at least twice and the data were analyzed with the Statistical Analysis System (SAS Institute, 2000). All multiple comparisons were first subjected to analysis of variance (ANOVA). Significant differences among treatments were determined with Fisher's least significant difference (LSD) separation test. Square-root transformation was applied when appropriate to obtain homogeneity of variances among treatment.

RESULTS

Recovery of microbes

A total of 2193 microorganisms were isolated from water, sediment and plant samples collected in ponds and lakes during one year of sampling (Table 1). The ponds yielded the highest number of microbes followed respectively by RL, OL and Rodman Reservoir.

Nearly equal numbers of fungal and bacterial isolates were recovered from ponds and lakes. It appears that the actinomycetes were restricted to ponds with one exception from OL (Table 1). The majority of actinomycetes were recovered from pond soils with a few from pond hydrilla (data not shown).

Of the total number of microbes recovered (2193), fungi represented 50.6%, bacteria represented 46.6% and actinomycetes only 2.8%.

Screening microbes against hydrilla in bioassay tests

Of the 651 fungal isolates and the 330 bacterial strains tested, only 6.5% of fungi and 0.6% of bacteria were effective at DS 4 three weeks post-inoculation (Table 2). Diversity of symptoms included chlorosis, necrosis, blight, reddening, browning, and wilt. About 10% of fungi versus 1.5% of bacteria tested were effective at DS 3. None of the actinomycetes tested was effective at DS 3 or 4.

Table 3 summarizes selected isolates that caused more than 75% damage on hydrilla (DR 4), their identification, and source.

Table 1. Number of microbial isolates recovered from ponds and lakes during one year of sampling

Sample site	Bacteria	Fungi	Actinomycetes	Total
Ponds	557	636	61	1254
Orange Lake	144	144	1	289
Rowel Lake	215	205	0	420
Rodman Reservoir	105	125	0	230
Total	1021	1110	62	2193

Pathogenicity of selected fungi in large tube test bioassay

Symptoms appeared on hydrilla shoots 5 to 7 days post inoculation with isolates F71PJ, F531, and F964 and after 7 to 9 days with isolate F542. Disease symptoms of *Acremonium* sp. (F71PJ) first appeared as reddening of leaf margins followed by a chlorosis (Fig. 2). Isolate F531 bleached chlorophyll from leaves and stems and softened plant tissues causing defoliation of the lower leaves (Fig. 3). Hydrilla inoculated with *F. culmorum* developed severe chlorosis and discoloration of inoculated shoots. Death and lyses of tissues were observed 3 weeks post inoculation however if hydrilla tissues were only partially damaged, regrowth was observed. Typical symptoms as browning of leaves and stems were observed when hydrilla was inoculated with *Botrytis* sp.

There was a significant effect of treatment ($P > 0.0001$) in the test tube bioassay. Isolates F964, F71PJ, and F531 were more efficacious on hydrilla than isolate F542 (Table 4).

Fig. (2): Disease symptoms by *Acremonium* sp. (Isolate F71PJ) on hydrilla 8 days post inoculation. Notice the reddening of leaf margins and the decline of the chlorophyll.

Pathogenicity of *F. culmorum* (F964) in an aquarium bioassay

Two weeks post inoculation with *F. culmorum*, hydrilla shoots started to discolor and develop signs of rotting. By 4 weeks, most of the shoots (93%) had disintegrated. *F. culmorum* was reisolated from infected hydrilla shoots in treated aquaria but was absent from the controls.

Fig (3): Disease symptoms by *Cylindrocarpon* sp. (isolate F531) on hydrilla 10 days post inoculation. Notice the severe yellowing of leaves and stem of hydrilla (right) in comparison with the control (left).

Table (2): Disease severity[a] (DS) for fungi (651 isolates), bacteria (330 strains), and actinomycetes (38 isolates) tested against hydrilla in a test tube bioassay

Microbe	% of microbes tested exhibiting				
	DS 0	DS 1	DS 2	DS 3	DS 4
Fungi	25	35	23	10.4	6.5
Bacteria	56.1	34.8	7	1.5	0.6
Actinomycetes	60.5	36.8	2.6	0	0

[a] Damage caused by microorganisms tested on hydrilla shoots was rated on a scale of 0 to 4, where 0 = healthy, 1 = 1-25% damage, 2 = 26-50% damage, 3 = 51-75% damage, and 4 = 76-100% damage (100% = complete kill).

Table (3): **Selection of effective biocontrol agents for hydrilla and their sources**

Isolate code	Taxon	Isolated from	
		Sample site	Sample type[a]
F71PJ	*Acremonium* sp.	Ponds	H2
F531	*Cylindrocarpon* sp.	Ponds	W, S, H1, H2
F542	*Botrytis* sp.	Ponds	W, S, H1, H2
F964	*Fusarium culmorum*	Rowell Lake	W
F966	*F. culmorum*	Rowell Lake	H1, H2
F1786	*F. culmorum*	Rowell Lake	W, S, H1, H2
F2774	*F. culmorum*	Rowell Lake	W, S, H1, H2
F3775	*F. culmorum*	Rowell Lake	W, H1
F4768	*F. culmorum*	Rowell Lake	W, H1, H2
F2060	*F. moniliforme*	Ponds	W
F1083	*Acremonium* sp.	Ponds	H1
F2035	*Curvularia* sp.	Ponds	W, S, H1, H2
B3535	Unidentified (G⁻, short rod)	Orange Lake	H1
B115	Unidentified (G⁻, short rod)	Ponds	W, S

[a] W = water, S = sediment, H1 = nonsurface sterilized hydrilla, and H2 = surface sterilized hydrilla.

Table (4): **Disease severity of selected biocontrol candidates on hydrilla three weeks post inoculation in large tubes**

Treatment	Fungus	Disease severity (%)
Control	Fungus free	0.0 c[a]
F71PJ	*Acremonium* sp.	92.5 a
F531	*Cylindrocarpon* sp.	89.0 a
F542	*Botrytis* sp.	74.5 b
F964	*Fusarium culmorum*	100 a

[a] Values represent the means of six replicates. Values followed by the same letter are not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Large-scale spore production of selected biocontrol candidates

Isolates differed in spore production over time. Isolates F964 (*F. culmorum*) and F542 (*Botrytis sp.*) yielded the largest number of spores (Table 5). The second harvest of the isolates F71PJ and F531 yielded more spores than the first harvest. This observation was reversed in the case of isolates F542 and F964, where fewer spores were present in the second harvest than in the first harvest (Table 5). A single harvest after 12 days lead to a notable reduction in spore yield of isolate F542 compared to a harvest at day 3 (Table 5).

Effect of inoculum concentration of *F. culmorum* on hydrilla

Inoculum concentration had a significant effect (P = 0.0001) on efficacy. C concentrations of 5 x 10^5 conidia per ml and above in the water column were effective in killing hydrilla (Table 6). Concentrations lower than 5 x 10^5 conidia per ml in the water column were significantly less effective.

Table 5. Numbers of spores produced by a tray method for selected biocontrol candidates

Isolate code	Fungus	Yield of spores (spores/ml)		
		Two harvests		One harvest (after 12 d)
		First harvest (after 3 or 7 d)	Second harvest (after 12 d)	
F71PJ	*Acremonium* sp.[a]	4×10^5	1.3×10^6	NC[b]
F531	*Cylindrocarpon* sp.[c]	2×10^6	1.3×10^8	NC
F542	*Botrytis* sp.[c]	1.2×10^8	1.5×10^6	3.7×10^6
F964	*Fusarium culmorum*[c]	1.3×10^8	3.2×10^7	1.1×10^8

[a] First harvest of F71PJ was done after 7 days since there were only sparse spores after 3 days.
[b] Not counted.
[c] First harvest of F531, F542, and F964 was done after 3 days.

Table 6. E ffect of inoculum concentrations of *Fusarium culmorum* on hydrilla three weeks post inoculation

Inoculum concentration (conidia/ml)[a]	% Infection
0 (Control)	3 d[b]
2×10^3	45.50 c
2.25×10^4	48.83 c
1.25×10^5	55.17 c
2.50×10^5	70 b
5×10^5	96.83 a
1×10^6	100 a
24×10^6	100 a

[a] As final concentrations in hydrilla tubes containing 49 ml of sterile water.
[b] Values represent the means of six replicates. Values followed by the same letter are not significantly different according to Fisher's LSD test ($P \leq 0.01$).

Optimum incubation temperature conducive for disease promotion by *F. culmorum*

Temperature had a significant effect on DS (P = 0.0001). Incubation temperatures of 20 and 25^{0}C resulted in the highest DS (Table 7). Incubation at 35^{0}C induced algal blooms. The least effective level of temperature was 15^{0}C (Table 7). Disease was not noted on the controls at any temperature.

Table 7. Effect of incubation temperature on the efficacy of *Fusarium culmorum* against hydrilla

Temperature (^{0}C)	% Infection (two weeks after inoculation)	
	Non-inoculated control	Inoculated
15	0	20.50 c[a]
20	0	92.33 a
25	0	90.67 a
30	0	53.17 b
35	0	47.83 b

[a] Values represent the means of six replicates. Values followed by the same letter are not significantly different according to Fisher's LSD test ($P \leq 0.01$).

DISCUSSION

This is one of only a few studies reporting pathogenicity of several microorganisms on hydrilla. The microorganisms isolated in the study are endemic and native to the USA and were shown to induce leaf necrosis, chlorosis, and lyses of hydrilla. In nature, these potential biocontrol agents do not impact hydrilla, either due to high host growth rates or they occur at concentrations below the threshold necessary for disease initiation. But if applied in an inundative approach, a high level of disease severity and rapid disease progress may be induced. In addition, the biocontrol activity of these pathogens may be enhanced by bioherbicidal formulation applied with proper timing.

Using six selective or general-purpose culture media, a considerable diversity of microorganisms associated with hydrilla were isolated.

The number of microbes recovered had an inverse relation with the size of the water body. More microbes were recovered from small ponds than either of the lakes or Rodman Reservoir. Higher water flows may reduce the microbial community.

Only 6.5% of the fungi tested and 0.6% of bacteria caused significant DS on hydrilla. Those isolates are considered to have potential biocontrol activity and/or potential capability of producing herbicidal metabolites.

The most effective isolates against hydrilla were *F. culmorum* (F964), *Acremonium* sp. (F71PJ), *Cylindrocarpon* sp. (F531), and *Botrytis* sp. (F542). Of these fungi, only *F. culmorum* isolated in the Netherlands from a related plant (*Stratiotes aloides*, Hydrocharitaceae) had previously been reported to be a pathogen of hydrilla (Charudattan and McKinney 1978). Joye (1990) and Shearer (1996 & 1998) have also reported an endemic fungus, *Mycoleptodiscus terrestris* (Gerd.) Ostazeski that has bioherbicidal activity against hydrilla.

The tray method allowed multiple harvestings of fungal spores which would be advantageous for large-scale spore production by the potential biocontrol agents. It was far more efficient with respect to time and spore yield than conventional methods based on Petri dish cultures (data not shown). However, incubation conditions would need to be optimized for each agent to yield maximum spore production. Modifications to the single harvest method of Walker (1980), Shabana (1992), and Shabana *et al.* (2000) allowed multiple harvestings of spores. This method may be applicable to other spore producing fungi when aseptic conditions are not required.

A high inoculum concentration provides better distribution of inoculum on plant surfaces and therefore induces a higher initial level of disease from which successive cycles of infection and invasion can develop (Charudattan, 1988 & Van der Plank, 1975). However, it would be uneconomical and unrealistic to apply inoculum at levels above those required for field efficacy. Thus, a rate of 5×10^5 spores/ml of *F. culmorum* is considered as sufficient to promote a significant level of DS. This rate may be reduced by formulations that provide excellent dispersal characteristics and good coverage over plant tissues.

Post-inoculum temperature may have significant effects on penetration and subsequent mycelial growth (Ghorbani *et al.* 2000). Optimum temperature for disease promotion by *F. culmorum* on hydrilla was 20 to 25 °C. Lower and higher temperatures probably had negative effects on pathogen survival and growth rate resulting in reduced disease development. These results are similar to those reported by Walker (1981) and Ghorbani *et al.* (2000) for other plant pathogenic fungi. Walker (1981) found that the maximum penetration and the amount of infection of *Anoda cristata* by *Alternaria macrospore* occurred at 25 °C rather than 15 and 20 °C. Ghorbani *et al.* (2000) reported that the optimum temperature for disease development and dry weight reduction by *Alternaria alternata* on *Amaranthus retroflexus* was 20 to 30 C.

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استخدام المسببات المرضية فى المقاومة البيولوجية للهيدريللا (حشيشة مائية مغمورة)

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يعتبر نبات الهيدريللا أحد أخطر الحشائش المائية المغمورة فى كثير من دول العالم فى المناطق المعتدلة والشبه استوائية والاستوائية. ويعتبر الموطن الأصلى لهذه الحشيشة هو المناطق الاستوائية من العالم القديم. تنتشر هذه الحشيشة حاليا فى أفريقيا وآسيا وأوروبا واستراليا ونيوزيلاندا والأمريكتين الشمالية والجنوبية وجزر المحيط الهادى مسببة خسائر اقتصادية فادحة، علاوة على تأثيرها السلبى الكبير على البيئة المائية. لذلك فقد كان الهدف الرئيسى لهذا البحث هو محاولة اكتشاف بعض المسببات المرضية من البيئة المحلية بولاية فلوريدا بالولايات المتحدة الأمريكية والتى لها قدرة عالية فى إصابة هذه الحشيشة بغرض استخدامها فى المكافحة البيولوجية لهذه الحشيشة المائية.

وقد أجرى حصر للكائنات الدقيقة المصاحبة لهذه الحشيشة استمر لمدة عام كامل كان نتيجته عزل حوالى ٢٢٠٠ كائن دقيق من النبات نفسه وكذلك من الماء والتربة المحيطة به والتى جمعت من ١٠ برك صناعية وثلاثة بحيرات طبيعية فى ولاية فلوريدا بالولايات المتحدة الأمريكية. وقد تم دراسة القدرة المرضية لعدد منتخب من هذه الكائنات المعزولة على تلك الحشيشة كان عبارة عن ٦٥١ عزلة فطرية و٣٣٠ عزلة بكتيرية و٣٨ عزلة أكتينوميسيتات. وبناء على درجة شدة المرض التى سببتها هذه الكائنات على الحشيشة، فقد قسمت هذه الكائنات إلى خمسة مجاميع كالآتى: المجموعة الأولى: وهى الكائنات الغير ممرضة والتى لم تسبب أى أعراض مرضية على الهيدريللا ، المجموعة الثانية: وهى تلك الكائنات الى أظهرت ١-٢٥% تلف على الحشيشة ، المجموعة الثالثة: هى الكائنات التى أظهرت ٢٦-٥٠% تلف للحشيشة ، المجموعة الرابعة: هى التى سببت ٥١-٧٥% إصابة ، المجموعة الخامسة: هى التى سببت ٧٦-١٠٠% إصابة للحشيشة. أظهرت هذه الدراسة أنه من بين العزلات المختبرة (١٠١٩عزلة) لم يكن للأكتينوميسيتات أى قدرة مرضية على الهيدريللا فى حين أظهرت سلالتين فقط من البكتريا (٦و % من البكتريات المختبرة) و ٤٢ عزلة فطرية (٥ و٦% من الفطريات المختبرة) قدرة عالية فى مقاومة تلك الحشيشة (٧٦-١٠٠% إصابة) وذلك بعد ثلاثة أسابيع من المعاملة. ولذلك فقد أعتبرت هذه العزلات (التى سجلت قدرة على قتل الحشيشة) عوامل مكافحة حيوية للهيدريللا، وقد اشتملت هذه المجموعة على فطريات الأكريمونيوم والبوترايتس والكيرفيولاريا والسيلندروكاربون والفيوزاريوم كالمورم والفيوزاريوم مونيليفورمى. وقد تم اختيار الفطر فيوزاريوم كالمورم لعمل دراسات أكثر تقدما وذلك بسبب سرعة نموه وانتاجه أعدادا كبيرة من الجراثيم بالإضافة إلى قدرتة العالية على قتل الحشيشة حيث تم دراسة أنسب تركيز لقاح يمكن استخدامه من هذا الفطر ووجد أن التركيز الأمثل والذى يعطى أقصى درجة من الإصابة على الحشيشة هو ٥ ع ١٠ ٩ جرثومة/مل ، كما درست أنسب درجة حرارة للماء والتى عندها يعطى الفطر أقصى درجة من الإصابة على الحشيشة فكانت الحرارة المثلى لذلك من ٢٠-٢٥ م.