MICROBIOLOGICAL CONTROL OF WATER HYACINTH
(Eichhornia crassipes)
IN EGYPT

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A potentially serious aquatic weed problem in Egypt is the water hyacinth (*Eichhornia crassipes*). Problems caused by it become increasingly critical as man's use of waterways, natural or artificial impoundments, and irrigation systems increases. Conventional methods of control have not been entirely satisfactory because of cost, overall ineffectiveness, or environmental pollution.

In the present investigation five Dematiaceous Hyphomycetes were tested for their capabilities of attacking the *E. crassipes* plant. *Alternaria grisea* was found to be strong in inciting pathogenesis in the plant, while the other tested fungal species, namely *Alternaria alternata*, *Alternaria humicola*, *Cladosporium cladosporioides* and *Cladosporium herbarum*, appeared to be slight or very weak pathogens inducing small zonate yellowish-brown spots on leaves.

The cell-free culture filtrate of *A. grisea* was capable of inciting the same syndrome as *A. grisea* spore suspension. The active substance was extracted, purified, and obtained as orange plate crystals; m. p. = 112-115°C; [α]$_D^{26}$ = -15.2°C. It has a molecular formula of C$_{16}$H$_{22}$O$_2$N. It is soluble in chloroform, ether, butanol, benzene, ethyl acetate, butyl acetate, acetone, ethanol, methanol, and scarcely soluble in water. This necrogenic substance proved to be related to the vicrorin group of toxins. This substance appears to hold some promise as a possible biocontrol agent for water hyacinth.
The aquatic weed problem is of considerable proportion and appears to be growing rather than diminishing in magnitude or even stabilizing. This is occurring despite the expenditure of considerable sums of money and human energy in the application of conventional methods of mechanical and chemical control. Water hyacinths continue to be among the most serious of aquatic plant pests in the world (Martin and Nailon 1977, Guerra 1976, Kassas 1972, Moursi 1976). Regarding the possibility of aquatic weeds infestation of the man-made lake of Aswan High Dam, Kassas (1972) states "It will take about 10 years to fill the High Dam reservoir of Egypt and the episode of dramatic fluctuations will result and provide habitat features that are not, in general, different from those of a natural lake. Invasion by water weeds will follow sooner or later, subject only to their migration efficiency and local conditions of water depth...."

In Egypt, surprisingly, until our research program for aquatic weeds' control was initiated, plant pathogens rarely had been considered as biocontrols. They have all the prerequisites of a biocontrol agent and, thus, offer an untapped reservoir of potential usefulness either alone or in an integrated program with fungi and perhaps insects.

In the present investigation we have dealt with the biological control of water hyacinth as one of the most noxious aquatic weeds in Egypt, particularly after the construction of High Dam. The purpose of the overall study is to determine: 1) the ability of some Dematiaceous Hyphomycetes to attack the plant, and 2) the potentiality of fungal metabolite to induce suppressing effect on E. crassipes growth.
Water hyacinths (Eichhorniae) are represented in Egypt by two species, namely, Eichhornia azurea and E. crassipes (Takholm and Drar 1950). E. azurea (Sw.) Kunth is occasionally cultivated in the gardens of Cairo. Its leaves are not rosetted and the leaf petioles are not (or hardly) swollen. It flowers in June through August. E. crassipes (Mart.) Solms-Laub., on the other hand, is very common and widely spread in Egypt. Its leaves are rosetted with inflated bladder-like petioles and it flowers in May through September. Sometimes flowering extends to December.

E. crassipes is a free-floating pontederiaceous aquatic weed, native to South America. It was introduced into the United States in 1884 (Sculthorpe 1967). It flourishes in the swampy habitats with warm, slow moving, fresh (or brackish) water. As sea water contains excess salts, it becomes unfavorable for E. crassipes growth. The most favorable temperature for growth is 27.6°C, and at lower temperatures E. crassipes growth tends to decrease (El-Fiky 1974). This may explain its absence in waters of the cold countries.

E. crassipes was introduced into Egypt as early as 1879-1892 during Khedive Tawfiq governorship. Hence, for many years it has been grown to a limited extent in certain public and private gardens of Cairo and Alexandria as an ornamental plant with beautiful flowers (Anonymous 1971).

During the last 15 years and after the establishment of High Dam at Aswan, the growth and distribution of water hyacinths in Egypt are increasing considerably and it is hard to find a canal, stream, or drainage system free of this weed infestation (Zahran 1976). Sculthorpe (1967) states "Once well established in an area, E. crassipes will
successfully suppress competing species." This may show that the problem is so serious that safe and urgent solution is necessary.

MATERIALS AND METHODS

According to Conway et al. (1974) several species of Deuteromycotina were found to possess varying pathogenicity to water hyacinth. In the present investigation, five species of Dematiaceous Hyphomycetes, namely Alternaria alternata, A. grisea, A. humicola, Cladosporium herbarum and C. cladosporioides, were tested for their capabilities of attacking the water hyacinth growing in Egypt.

Pathogenicity Tests

On August 10, 1977, samples of healthy water hyacinths (E. crassipes) were collected from Damietta Branch of the River Nile in the vicinity of Mansoura City. The plants were kept in six glass jars (24 X 40 X 50 cm) filled to 2/3 of their heights with fresh water and maintained at air temperature (maximum 36°, minimum 22° C). Pathogenicity tests were conducted using the above mentioned fungal species to five jars of plants while those of the sixth one were left without infection (control jar). Inocula were prepared by growing the tested fungi on slant cultures of potato dextrose agar (Riker and Riker 1936) at 28° C. The conidia of 7 day old cultures were suspended in sterile water, and the spore suspensions of each individual fungus were then combined and homogenized. Infestation was carried out by spraying water hyacinth with the inoculum of each fungus at the rate of 10 ml spore suspension per plant and keeping at open air conditions for 7 days. Meanwhile, daily observations of the syndrome were noted. This experiment was set up to find out the infective potency of the experimental fungi to E. crassipes plants.

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A subsequent pathogenicity trial was carried out using only one fungus (A. grisea) to qualify the infestation syndrome and/or to ascertain validity of this organism in biocontrol. On September 1, 1977, new samples of water hyacinth, collected from the same location, were dispensed in five jars. One was left as a control, while the other four jars of plants were sprayed with the spore suspension of A. grisea. The jars were maintained at open air conditions (maximum temperature 34°C, minimum 21°C) and the syndrome was noted over a period of 45 days.

Deteriorative Potentiality of Fungal Metabolite

The fungus A. grisea was cultured in 250 ml conical flasks each containing 50 ml of potato dextrose liquid medium. The fungus-free fluid of cultures, incubated for ten days at 28°C, was obtained by centrifugation, then Seitz filtration. This cell-free filtrate was sprayed over the water hyacinth (new healthy samples) and kept in a group of jars for seven days. Meanwhile, the syndrome was noted.

Subsequent extraction of the fungal metabolite of the tested culture filtrate was undertaken using seven different solvents; namely, diethyl ether, chloroform, n-butanol, ethyl acetate, butyl acetate, benzene and petroleum ether at pH 5, 7, and 9. The extract of each individual trial was evaporated under vacuum and suspended in 10 ml of water. The obtained suspensions were sprayed over healthy plants of E. crassipes and the syndrome was noted.

RESULTS

Pathogenicity of the Tested Fungi

As indicated in Table 1, the tested fungi appeared to differ in their infective potency for the water hyacinth. Pathogenesis appeared on the sixth day of inoculation with the two Cladosporium species, while it started on the third day with Alternaria grisea and on the fifth day with A. alternata and A. humicola.
Table 1. Development of symptoms on Eichhornia crassipes due to infection with the tested fungal species during seven days.

<table>
<thead>
<tr>
<th>Time elapsed after infection (Days)</th>
<th>A. grisea</th>
<th>A. alternata</th>
<th>A. humicola</th>
<th>C. cladosporioides</th>
<th>C. herbarum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>yellow spots</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>yellow spots</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>yellow spots</td>
<td>+++</td>
<td>yellow spots</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>yellowing of leaves</td>
<td>++++</td>
<td>yellowing of leaves</td>
<td>+</td>
<td>faint yellow</td>
</tr>
<tr>
<td>7</td>
<td>yellowing of leaves and browning</td>
<td>++++</td>
<td>yellowing of leaves and browning</td>
<td>++</td>
<td>yellowing of leaves and browning</td>
</tr>
</tbody>
</table>

* Severity of disease symptoms
- No disease symptoms
+ Slight syndrome; increasing number of + indicates increasing severity of disease symptoms
It is a well known fact that yellow or brown spottings mean damage in assimilation systems of plants, which sooner or later would be accompanied by deterioration of leaves and/or the whole plant.

From the results herein reported, it is apparent that *A. grisea* is the most active organism in inciting disease for water hyacinth, while *A. humicola* and *A. alternata* and the *Cladosporium* species are respectively weaker as disease inciters. The higher infective potency of *A. grisea* was the cause for using it in the last stages of this investigation so as to qualify its virulence and/or to ascertain validity of its application in biocontrol of *E. crassipes*.

**Efficiency of Alternaria grisea as Water Hyacinth Deteriorative**

The results shown in Table 2 clearly elucidate that after or within five days of spraying the spore suspension of *A. grisea*, the fungus exhibited its pathogenic effects. First, faint yellow spots appeared on the upper surface of *E. crassipes* leaves. These spots enlarged gradually and turned yellowish-brown in color after 10 days. Development of brown color and coalescence of spots led to blotches and blights on leaves on the 20th day. On the 30th day, these symptoms extended to petioles and stolon of the plant which appeared partially or totally killed as partial defoliation became a prominent feature after this period. Disappearance of the plant remains took place after 45 days and the contents of nearly all the jars turned into dark brown or dirty fluid. This may be due to tissue necrosis and/or bacterial decomposition of the deteriorated tissues of the *E. crassipes* plant.

**Necrogenic Capability of Fungal Metabolite**

The cell-free filtrate of a 10 day old culture of the candidate fungus *A. grisea* was found to be equally active as its spore suspension in inciting necrosis of water hyacinth tissue. This indicated that the fungus elaborates a toxic metabolite in the culture broth which usually induces such
Table 2. Development of symptoms on *Eichhornia crassipes* due to infection with *Alternaria grisea* during 45 days.

<table>
<thead>
<tr>
<th>Time elapsed after infection (Days)</th>
<th>Description of Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>No appreciable symptoms.</td>
</tr>
<tr>
<td>5</td>
<td>Small water-soaked yellow spots on the upper surface of leaves.</td>
</tr>
<tr>
<td>7</td>
<td>Enlarged spots of yellow color.</td>
</tr>
<tr>
<td>10</td>
<td>Enlarged spots of deep yellow color with brown colored margins.</td>
</tr>
<tr>
<td>14</td>
<td>Excessive spotting; some leaves manifest brownish spots and others tend to be necrotic; spots coalescence appeared in many areas of leaves.</td>
</tr>
<tr>
<td>20</td>
<td>The affected areas turn necrotic and appearance of blotchy lesions and/or leaf blights.</td>
</tr>
<tr>
<td>30</td>
<td>The aforementioned syndrome extend to petioles (bladders) and/or stolon of the plant, some parts get dried; partial defoliation, while others get dried.</td>
</tr>
<tr>
<td>45</td>
<td>The weed individuals disappeared and the contents of nearly all jars appeared to turn into dirty or dark brown colored fluid.</td>
</tr>
</tbody>
</table>
syndrome resulting in destruction of the cellular contents and collapse of
the cell walls of water hyacinth.

Chloroform, ether, benzene, ethyl acetate, butyl acetate, and n-butanol,
particularly at pH 5, were capable of extracting the active necrogenic
substance. This was indicated by the necrosis induced by the aqueous sus-
pensions of dried extracts of each solvent; however, chloroform proved,
comparatively, the most efficient solvent.

Characterization of the Active Biocontrol Agent

The chloroform extract was evaporated under vacuum to the least volume
and the necrogenic substance was precipitated by addition of petroleum ether.
The crude substance, which was yellowish-brown in color, was purified by
chromatography and obtained from ethanol as orange plate crystals. It has
a melting point of 112-115 C and an optical activity of $[\alpha]^{26}_D = -15.2^\circ C$.
It is soluble in chloroform, ether, butanol, benzene, ethyl acetate, butyl
acetate, acetone, ethanol, methanol, and scarcely soluble in water.

The elemental analysis indicated that this substance contains carbon
(72.62%), hydrogen (8.95%), nitrogen (3.62%), and oxygen (14.81%). The
molecular weight was found to equal 262 as indicated by high resolution mass
analysis. The molecular formula, therefore, is suggested to be $C_{16}H_{22}O_{2}N$.

The ultraviolet spectrum showed absorption maxima at 223, 278, and
352 nm. The infrared spectrum exhibited 5 bands at 2525, 2435, 1740, and
1275 cm$^{-1}$. The bands at 2525 and 2435 cm$^{-1}$ correspond to OH group, while
the bands at 1740 and 1715 cm$^{-1}$ are assigned to C-N. The necrogenic
substance, therefore, is a low molecular weight peptide.

Based on the foregoing results, the toxic agent synthesized by
A. grisea can be identified as belonging to the victorin group of toxins.
The name victorin was coined by Wheeler and Luke(1954) to include toxic
agents produced by some species of Helminthosporium and Alternaria.
DISCUSSION AND CONCLUSION

The biocontrol refers to the use of one or more kinds of organisms to stress a pestiferous population of other organisms whether by physical destruction, direct consumption, parasitism, or pathogenicity. A most important characteristic of the control agent is that it must not pose a threat to other species whose presence in the ecosystem is valued, and, in particular, it must not pose a threat to any economic species in any area where it may be introduced (Martin and Nailon 1977).

Plant pathogens have many characteristics that make them ideal candidates as biocontrols for aquatic weeds (Zettler and Freeman 1972, Rintz 1973, Freeman et al. 1976, Charudattan et al. 1976).

It may be recalled here that Conway and coworkers (1974) referred that most Alternaria species are saprophytic on water hyacinth and their pathogenicity is not confirmed. The results obtained from the present study proved the pathogenicity of Alternaria grisea which killed Eichhornia crassipes plants. Accordingly, this fungus may be used as a promising biological control organism for the floating aquatic weeds. Before suggesting its use on a large scale, the side effect of the candidate pathogen should be considered. First of all is the probability of infecting other cultivated plants near the areas of spore suspension spray. To overcome this problem, the active substance (necrotic agent) through which pathogenesis could be brought by the candidate fungus was isolated. The use of this substance instead of spore suspension will limit the growth rate or destroy E. crassipes only and may diminish the infection of other cultivated plants.
ACKNOWLEDGEMENTS

The authors are grateful to Dr. M. M. Mostafa, Chemistry Department, Faculty of Science, Mansoura University, for his invaluable help in connection with the physico-chemical analysis.


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