

Biological Control of Water Hyacinth by a Mycoherbicide in Egypt

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Abstract

An *Alternaria eichhorniae* isolate (#5) pathogenic to water hyacinth was discovered in Egypt in 1984. This fungus appeared to be host specific and capable of severely damaging and thus suppressing the weed. The major constraint to its use as a biocontrol agent—the need for a long period of dew—has been overcome through formulations using oil emulsions. This article summarises the results of 16 years of research culminating in the confirmation of the feasibility of using #5 as a biological control agent against water hyacinth in Egypt.

WATER hyacinth creates serious problems in Egypt: the weed not only affects irrigation, water flow, water use, and navigation, it also poses health risks by enabling the breeding of mosquitoes and schistosomes (bilharzia) and other human parasites. Water quality is affected as well, because of the increasing accumulation of water hyacinth detritus. Fishing may be affected because of the competitive advantage to “trash” fish species in weed-infested water. In many instances, fish are killed when oxygen levels are depleted through plant respiration and decomposition of senescent vegetation. The water hyacinth problem is particularly severe in the Nile Delta and the irrigation systems.

In view of the problems encountered in water hyacinth management, the attention has recently centred on biological control. Biological control could provide a lasting, cost-effective, environmentally safe solution, and is theoretically the best method for solving the water hyacinth problem. Recent conferences and

panels have stressed the need for greater and renewed emphasis on biologically based alternatives (Delfosse and Spencer 1997; Charudattan et al. 1996).

Isolation, Identification, Pathogenicity Testing and Host Range Determination

Between 1984 and 1986, surveys were made in the Nile Delta (Dakahlia and Damietta governorates) where accumulations of floating mats of water hyacinth were located (Shabana 1987). Two hundred fungal isolates belonging to several genera were isolated from diseased water hyacinth plants. These included three isolates of *Alternaria* spp. (numbered 3, 5, and 6) from the Dakahlia Governorate. The isolates were associated with a severe leaf blight that spread rapidly from disease foci (Shabana 1987). Initially, the isolates were identified as belonging to *Alternaria alternata* (Fr.) Keissler and were reported to have potential as biological control agents for water hyacinth (Elwakil et al. 1989). However, the variability in colour of colonies and conidial dimensions among the isolates suggested that more than one species was involved. Taxonomic identification to species level

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was made. Isolates 3 and 5 proved to be *A. eichhorniae* while isolate no. 6 belonged to *A. alternata* (Shabana et al. 1995a). This was the first report of these two species on water hyacinth in Egypt (Shabana et al. 1995b). These three isolates were compared for their bioherbicidal efficacy against water hyacinth. Isolate #5 was the most virulent and, therefore, was selected for further studies of host specificity and safety. Ninety-five plant species and cultivars of economically important crops, representing 21 families, were screened in two consecutive years and only water hyacinth was found to be susceptible to the fungus (Shabana et al. 1995a).

Further Studies for Development of a Mycoherbicide

In an attempt to improve the efficacy of #5, aspects of its sporulation, phytotoxin production, and formulation were studied. Determination of the optimum conditions for production of highly virulent inoculum, and of the epidemiological requirements for disease incidence and disease severity were also investigated. The physiological and ultrastructural host responses were explored as well, to furnish background information for understanding the host–pathogen system.

Sporulation

Among five methods (modified Walker's, Shahin's, modified Cotty's, and sodium alginate method) and factors (temperature, light, pH, aeration, and culture media) tested for inducing spore production by #5, Walker's method (Walker 1980) was the best with respect to time, materials, and the abundance of spores produced (Shabana 1992; Shabana et al. 2001).

In Walker's method, the #5 mycelial mat was blended with equal amount of distilled water and a supplement of antibiotic solution. This mix was plated in a tray lined with aluminium foil in a 4 mm thick layer and incubated under diurnal light, 80–90% relative humidity and 25–30°C for 24–48 h. Then the light was turned off for 24 h. The fungal layer was allowed to air-dry and spores were harvested with a cyclone spore collector.

Phytotoxin Production

An important diagnostic characteristic of this fungus (#5) is the production of crimson-red pigmentation in culture under certain conditions. Studies by Charudattan and Rao (1982) had shown the pigmentation to

be caused by a two compounds (bostrycin and deoxybostrycin) that are phytotoxic to water hyacinth. To determine the optimum cultural conditions for the production of these phytotoxins, six nutrition and environmental factors (culture media, dextrose level, temperature, light regime, aeration, and pH) were tested. Production of pigments as well as nonchromatic, UV-absorbing metabolites was determined. The maximum production of pigments was obtained when cultures were grown on potato dextrose agar (PDA) containing 20% dextrose, with an initial pH of 4.5 at 25–30°C under continuous darkness or diurnal light and without wrapping the culture plates (Shabana et al. 2000b). The maximum yields of the nonchromatic UV-absorbing compounds were obtained when cultures were grown on PDA containing 20–50% dextrose, with the initial pH ranging from 3.8 to 6.2, a temperature range of 20–30°C under continuous darkness in unwrapped plates or in plates sealed with one layer of Parafilm®. The reduction in aeration, presumably proportional to the number of layers of Parafilm® wrappings, led to lower levels of the red pigment(s) and the nonpigmented UV-absorbing compounds, to reduced mycelial growth, and a suppression of sporulation (Shabana 1992). There was a strong inverse relationship between linear growth of #5 and its pigmentation as a function of light regime.

Different fractions of culture filtrates of #5 were tested for their bioherbicidal activity against water hyacinth in a bioassay. Results of the phytotoxin bioassay suggest that a high concentration (10%) of the partially purified culture filtrate was required for symptom expression on water hyacinth. Butanol fractions at 10% (w/v) concentration were the most effective in deteriorating the water hyacinth leaf-segments, followed by the aqueous fractions (10% w/v). The area of necrosis increased with time; the damaged leaf area at 60 hours after applying the partially purified culture filtrate fractions was significantly larger than it was at 30 hours after application (Shabana et al. 2000b).

Culture Conditions and Inoculum Efficacy

Results showed that the efficacy of spore inoculum was equal to that of mycelial inoculum of #5. The disease was initiated with either conidial or mycelial inoculum to yield a similar level of disease severity (Shabana et al. 1995a). The ability to use mycelial inoculum instead of conidia is important to the development and large-scale field use of #5 because the

mycelial inoculum is easier to produce than conidia. Different broth media and culture conditions were evaluated for the ability to produce highly pathogenic mycelial inoculum. Fresh potato dextrose broth, shake-culturing, and incubating cultures under diurnal light for 1 week followed by continuous darkness for an additional week were the most effective (Shabana et al. 1995a). The optimal time of mechanical shear in a blender which produced highly infective mycelial inoculum was 6 seconds. Disease severity increased with increasing inoculum density up to 5% mycelial wet weight; a higher inoculum density did not provide greater disease severity. Exposure of inoculated leaves to at least 10 hours of dew was conducive to a high level of disease, and the use of a hydrophilic mucilloid as a humectant with the mycelial inoculum enhanced the disease level (Shabana et al. 1995a).

Host Responses

Physiological and ultrastructural studies provided interesting details of host-pathogen relationship between #5 and water hyacinth. Infection of water hyacinth with #5 led to a significant decrease in pigments, carbohydrates, and relative water content and to a significant increase in total phenols of water hyacinth leaves. Penetration of water hyacinth leaves by the fungus occurred only through the stomata, and the invading hyphae were located in the intercellular spaces of leaf tissues. Cytological changes noted in infected cells included changes in chloroplast, nucleus, and mitochondria. Invagination of the plasma membrane, particularly at plasmodesmata, was also noticed in infected cells. The associations between the infection process, the physiological disorder, and the ultrastructure of infected leaves have been discussed by Shabana et al. (1997a).

Formulation

A major obstacle to the use of #5 as a foliar pathogen for water hyacinth, is the need for at least 10 hours of dew on the leaf surface to enable the fungal propagules to germinate, grow, infect, and colonise the weed (Shabana et al. 1995a). Longer exposure to dew (e.g. of 26 or 28 hours) might assure disease development, but such uninterrupted, extended exposure to dew periods is not likely to occur under field conditions (Shabana et al. 1995a). For this reason, several ideas have been explored and examined to overcome the lack of dew in the field by formulating the inoculum in hydrophilic polymers (Shabana et al. 1995c, 1997b), in

invert emulsions (Shabana 1997a), or in vegetable oil suspension emulsions (Shabana 1997b). Several structurally different invert or oil emulsions have been tested under field conditions to obtain a good, reliable, and cost-effective formula to help in solving the water hyacinth problem in Egypt and possibly in other countries. Formulation also can be used to increase the efficacy of the biological control agents. In this regard, formulating #5 along with its phytotoxic fractions in oil emulsions produced 100% weed control 7 weeks after application in miniplot trials under field conditions. The oil prevents dehydration of the fungal inoculum and keeps it moistened for long time, facilitating the germination of its propagules, while the phytotoxic fractions serve as disease-promoting factors.

Gene Manipulation

Some very preliminary studies have been initiated to artificially increase the virulence of #5 against water hyacinth by genetic manipulation via inserting gene(s) encoded for phytotoxin production. As a first step, methods have been developed to produce and regenerate protoplasts of this fungus (Shabana and Charudattan 1997).

Conclusions

Alternaria eichhorniae #5 is a safe and effective bioherbicide for water hyacinth. High levels of bioherbicide efficacy and weed stress were seen when an alginate formulation of mycelium and culture filtrate of #5 was used along with a polyacrylamide. Nonetheless, this formulation was deemed insufficient to overcome the weed's growth rate conditions without the benefit of prolonged dew periods. Formulating the fungal inoculum in an invert emulsion helped to overcome this obstacle. The latter formulation caused high disease severity levels on water hyacinth plants even without exposure to dew. However, much more research is needed to provide better stability of the emulsions, easier mixing and spraying, and longer water retention. Practically, the formulation in invert emulsions has a number of disadvantages: the amount of oil required would add greatly to the cost of the product, the material is very viscous and requires special equipment for application, and nontarget contamination by the petroleum oil and other components of the formulation is likely. Therefore, the potential of emulsions with low oil content and emulsions made from vegetable oils was investigated. The rapeseed (Shabana 1997b) and cottonseed (Y. Shabana, unpub-

lished data) oil suspension emulsions are promising in dew-free conditions. Evaluations of these products in large-scale field trials is necessary to confirm the results from miniplot trials.

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