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Isolation and pathogenicity of some native fungal pathogens for the biological management of water hyacinth

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ABSTRACT

Water hyacinth (*Eichhornia crassipes*) is one of the most predominant, persistent and troublesome aquatic weeds. Periodical surveys of various water bodies in and around Jabalpur were under taken with the objective to isolate and evaluate the indigenous strains of fungal pathogens as myco-herbicides to manage water hyacinth. Three fungal pathogens, *viz*., *Fusarium oxysporum, Curvularia lunata* and *Alternaria alternata* were isolated. Efficacy studies of the pathogens were done by inoculating water hyacinth plants, either directly or after creating wounds. Artificial injury created in the plants by pin pricks before inoculation of the pathogens aided in the entry of the pathogens there by resulting in the better infection of the plants. Among the three pathogens, *Fusarium oxysporum* was found to be the best resulting in the killing of inoculated water hyacinth in about 15 days.

Key words : Water hyacinth, Biological control, *Fusarium oxysporum, Alternaria alternata*, *Curvularia lunata*

Water hyacinth (*Eichhornia crassipes)* is one of the most successful colonizers of the aquatic plant world. It is a vigorous grower, known to double its population in two weeks. Its rapid rate of proliferation over large area of water causes a variety of problems like impenetrable barriers in lakes, obstructing the navigation, blocking drainage in lower channels thereby causing flooding in upper channels and obstructing water flow in irrigation pipes (Charudattan 1982) besides fish mortality and deteriorating water quality (Seabrook 1962, Gopal and Sharma 1981). Governments all over the world are spending millions of rupees to manage this weed (Praveena and Naseema 2004). The success of the host specific fungi *Cercospora rodmanii* in controlling water hyacinth greatly stimulated interest in the management of this weed using fungal pathogens (Conway and Freeman 1977). Abbot laboratory of USA developed an experimental formulation of *C. rodmanii*, named *ABG-5003* against *E. crassipes* (Te Beest 1991). Charudattan (2005) has given an indepth view of the selection of weeds target for the good biocontrol programme.The current paper discusses the isolation of some common fungi occurring naturally on water hyacinth and testing their pathogenic potential in order to develop an effective biocontrol agent for the management of water hyacinth.

MATERIALS AND METHODS

Survey and sampling

A periodical survey was conducted for isolation of plant pathogens. Water hyacinths showing diseased symptoms were collected from different ponds and ditches in and around Jabalpur. The diseased plants were collected and brought to the laboratory for further studies. The diseased plant parts were cut into small pieces and placed on the potato dextrose agar (PDA) plates which surface was sterilized using sodium hypochlorite solution followed by distilled water and maintained at 28^0C for 24-48 hrs in BOD incubators. The fungi were pure cultured by hyphal tip method and stored in PDA slants. The isolated fungal pathogens were then identified based on their colony, morphology and characters at the genus level (Barnett 1972).

Pathogenicity

The pathogens were grown in wide bottomed flasks in potato dextrose (PD) broth for seven days. Spore suspension was prepared from, freshly developed conidial growth by harvesting the entire fungal mat along with the broth and crushing in a clean mortar. The suspension was then strained through muslin cloth and the concentration of the spore suspension was maintained at about 25 x 10^3 cfu/ml. Healthy, uniform and small sized water hyacinth plants of about 10 cm length were collected from the local ponds and surface sterilized by using 20% ethanol. The plants were kept in plastic pots filled with sterile water. After two days of acclimatization in the new environment, the fungal treatments were applied as spore suspension using a garden sprayer. Pathogenicity of the pathogens were tested in one set by making an artificial wound by pricking the stem from all the four sides with a needle for facilitating the entry of the pathogen (with pin prick) and

other set without the artificial pricking (Karuna and Kolte 2005). Broths containing fungal spores (25 x 10^3 cfu/ml) were sprayed. The experiment was done in three replication and control plants were sprayed with distilled water. The experiment was conducted in the containment chamber at 28±2^{0}C temperature and 75±5% relative humidity for a period of 15 days. The infection percentage of leaf pathogens, *A. alternata* and *C. lunata* were calculated by using following formula given by (Dingra and Sinclair 1995).

$$\% \text{ of infection} = \frac{\text{No. of leaf infected}}{\text{Total no. of leaves}} \times 100$$

A disease index of lesions formed by *Fusarium* was determined by the length and severity of lesions as per the following scale 0 = no visible lesions; 1 = lesions 1 mm long, light brown; 2 = lesions 1 to 5 mm long, brown; 3 = lesions longer than 5 mm, dark brown; 4 = root totally infected and brown; 5 = root destroyed and plant dying or dead (McGee and Kellock 1974).

RESULTS AND DISCUSSION

Ten different fungal species were isolated and identified (Table 1). Based on the frequency of their infection and severity of symptoms, only 4 species were used for further experiment. However among the four tested, only three of them were found to be pathogenic on water hyacinth. Among the different pathogens isolated, *A. alternata* and *F. oxysporum*, were the most common and found in all the ponds surveyed. *C. lunata* was found in three out of five ponds while *Trichothicium* sp., *Trichoderma viride* and *Pythium* sp. were found in only two samples and did not come up in the subsequent surveys. Attempts to re-isolate these pathogens were not successful for some unknown reason. The pathogens isolated exhibited a range of different symptoms depending on their site of infection in water hyacinth. It was observed that all the pathogens showed higher and rapid infection when the stems of water hyacinth were wounded by pin pricking method. This showed that the fungal pathogens needed a point of entry into the host so that they establish in a more rapid manner.

The pathogens of genus, *Fusarium, Alternaria* and *Curvularia* are facultative parasites and do not have much specialized mechanisms for their entry into the host (Aneja *et al*. 1993). Several highly virulent fungal parasites have been reported to cause diseases of water hyacinth. Among them *Acremonium zonatum, A. alternata, A. eichhorniae, Bipolaris* spp*., F. chlamydosporum, Helminthosporium* spp*. Cercospora rodmanii, Myrothecium roridum, Rhizoctonia solani* and *Uredo eichhorniae* were able to cause significant damages in water hyacinth (Charudattan 1982). An isolate of *Colletotrichum* sp. collected in China were found to be highly virulent with a disease index of 65.28% after 30 dyas of inoculation. However, an isolate of *Alternaria* from the same place was found to cause 67% Disease Index (DI) upon 7 days of inoculation (Ding *et al.* 2008).

Pathogens when applied as a mixture caused more infection than applied alone. Maximum infection was caused by the consortia of the three pathogens *viz., F. oxysporum, C. lunata* and *A. alternata in* the pin prick method, causing 76% infection, while it was only 30.2% in plants without pin prick (Table 2). *F. oxysporum* alone caused 68% infection with pin prick and 24.2% infection as foliar spray without pin prick. Whereas *A. alternata* and *C. lunata* caused 48% and 40.1% infection, respectively with pin prick method and 32% and 30% infection, without pin pricking respectively. *A. alternata* has a worldwide distribution and has been isolated from almost all habitats. However *A. alternata* and *C. lunata* are both foliar pathogens, their ability to manage the water hyacinth which produces a new flush of leaves in every week may be less when compared to the vascular pathogen *F. oxysporum* which targets the stem portion of water hyacinth. Thus *F. oxysporum* may offer better advantages in the biological management of water

Table 1. Fungal isolates from water hyacinth at different ponds around Jabalpur

Fungi isolated	Adhartal Pond	Shobhapur Pond	Budhagar Pond	Soopatal Pond	Panagar Pond
Alternaria alternata	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Curvularia lunata	+	-	+	-	+
Fusarium oxysporum.	+	+	+	+	+
Penicillium chrysogenum	+	+	+	+	+
Pythium sp.	-	-	-	+	-
Trichoderma viride	-	-	+	-	+
Trichothecium sp.	-	-	-	+	-

+ Indicates presence of the organisms in that ponds, - Indicates absence of the organisms in that ponds

Table2. Percentage infection by different fungal pathogens on water hyacinth

Treatment	Infection % (with pin prick)	Infection % (without pin prick)
Control	No infection	No infection
Curvularia lunata	40.1	30.0
Alternaria alternata	48.0	32.0
Fusarium oxysporum	68.0	24.2
Consortia	76.0	30.2
LSD (P= 0.05)	8.1	4.6

hyacinth when compared with the other pathogens used in this study. Though *F. oxysporum* has been reported pathogenic on many crops, it has also been observed that *F. oxysporum* has many specialized biotypes/ strains (Farmae specialis) which are highly host specific. Thus in this case attempts are being made to identify it to the strain level and study the host specificity. Further since the target area for use of this fungus as biocontrol agent is water hyacinth, the probability of infecting field crop is very less.

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