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Survey and selection of potential pathogens for biological control of waterhyacinth

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ABSTRACT

Waterhyacinth is the world worst aquatic weed causing severe ecological and economical losses. Plant pathogens are playing an increasing role in classical biological control of weeds worldwide. The present study attempts to explore some endemic phytopathogens of waterhyacinth. During surveys of waterhyacinth infested water bodies in Jabalpur, 31 isolates of endemic pathogens were recovered from diseased plant parts of the weed. The emergent fungi were purified and screened for their pathogenicity to waterhyacinth in two ways *viz*., spraying of the spore/mycelial suspension and secondary metabolites produced in broth, under growth chamber conditions. Of these *Alternaria alternata* followed by *Curvularia lunata*, *Fusarium pallidoroseum*, *Alternaria eichhorniae* and *Rhizoctonia solani* were found to be highly pathogenic.

Key words : Biological control, Waterhyacinth, Pathogens.

Almost all living organisms including weeds have atleast one enemy. There are several microorganisms including fungi, bacteria, viruses, *etc* that incite disease in plants. Amongst these fungi rank first in pathogenicity with immense mycoherbicidal potential. Plant pathogens are potentially valuable additions to the arsenal of weapons for use against weeds (Babu *et al*. 2003). Selection of a potential and effective agent is one of the most essential steps in any biocontrol programmes.

The aquatic macrophyte, *Eichhornia crassipes* (waterhyacinth) is one of the most severe weeds in several tropical and subtropical regions of the world creating large number of problems particularly related to the use and management of water resources (Jayanth 1987, Schmitz *et al*. 1993, Center 1994). Therefore, need for more and more natural enemies is must inspite of availability of number of biocontrol agents including insect and fungi, The present investigation was carried out to select suitable pathogen for the management of waterhyacinth.

MATERIALS AND METHODS

Collection and identification of pathogens from waterhyacinth

Field survey

Waterhyacinth plant parts with disease symptoms were collected by periodical survey for one year, from various pools, ditches, ponds *etc*. in the vicinity of Jabalpur. The main water bodies surveyed were Gangasagar, Gullauatal, Ranital, Supatal, Junmani pond, ditches and pools near MGM school, Shobhapur railway crossing, Panagar *etc*. The plant specimens were collected and stored in polythene bags and brought to the laboratory where isolation and purification of the fungi was attempted.

Recovery of pathogens

Pieces of 2 mm^2 were segmented from the margins of necrotic or chlorotic lesions on the surface of the lamina and petiole from the waterhyacinth leaf. They were then surface sterilized with 1% sodium hypochlorite (NaOCl) solution for three minutes, followed by rinsing with sterile water to remove any possible contamination. These pieces were then placed on earlier prepared Petridishes containing potato dextrose agar medium amended with 75 mg/l streptomycin and pinhead amount of rosebengal. This was incubated for 3-4 days at 27^0C in BOD incubator. All the emergent fungi were isolated and pure cultures were obtained.

Purification and maintenance of culture

The fungal species isolated earlier were purified by streak-plate and sub culturing techniques (Agarwal and Hasija 1986). About 55 fungi were isolated from waterhyacinth. Cultures that appeared contaminated with other fungus were subcultured and purified. 24 cultures were eliminated from further consideration because they were either contaminated or failed to grow. The purified cultures of the 31 isolates were multiplied on PDA plates. The stock cultures of the microorganisms were maintained on PDA slants supplemented with 10% waterhyacinth

extract and malt extract media and stored at 7^0C in refrigerator. The other slants were kept in the BOD incubator at 25 ± 1^0C and routinely transferred into fresh slants for experimental purposes. The phytopathogens of waterhyacinth were identified at Mycological Research Laboratory, Department of Biological Science, R.D. University, Jabalpur, as per various references (Booth 1977, Ellis 1971, 1976, Gilman 1959, Holliday 1993, Raper and Thom 1984, Sutton, 1980). The fungi found potential against waterhyacinth were sent for further identification to Indian Type Culture Collection (ITTC), Division of Plant Pathology, IARI, New Delhi.

Pathogenicity and screening of the most virulent pathogen

All the pathogens isolated from diseased waterhyacinth were grown on PDA plates and incubated in BOD incubator for 21 days. The spores of the fungi were harvested by flooding the petridishes containing the fungi with 10 ml sterile distilled water. The spores were concentrated by centrifugation and desired inoculum concentration (spores/ml) was adjusted using haemocytometer. Pathogenicity of sclerotial fungi was tested by making mycelial suspension from 21-day-old culture. To this surfactant, Tween-80 (oxysorbic polyxyethylene sorbitan monoleate) was added at the rate of 0.05 ml/ 50 ml of spore suspension.

Virulence of the secondary metabolites secreted by different fungi was also studied for all the fungi isolated. For this 50 ml of Richard's broth was poured in each 100 ml Erlenmeyer flask and sterilized. Using sterile cork borer, 5 mm discs of the fungus were aseptically cut from 7-day old stock culture and inoculated in the broth in three replications. They were incubated for 21 days. At the end of 21 days, the culture filtrates of different fungi were first filtered through eight layers of cheese cloth then through Whatman filter paper no. 1. To these, the surfactant Tween 80 was added at the rate of 0.05 ml per 50 ml of culture filtrate.

Small sized waterhyacinth were brought from local pond and were treated with culture filtrate prepared as described above. For inoculation procedure, waterhyacinth plants were kept in plastic cups (7.5 cm diameter and 9 cm height) filled with water and fertilized as before. They were sprayed with the spore/mycelial suspension with an automizer until wetness. Control plants were

Table 1. Fungal pathogens isolated from waterhyacinth

Fungus Isolated From Waterhyacinth	Associated Plant part	Symptom
Alternaria alternata	Lamina, petiole	Leaf spot/ blight
Alternaria eichhorniae	Lamina, petiole	Leaf spot
Aspergillus flavus	Lamina, petiole, root, dead plant parts	No symptoms
Aspergillus niger	Lamina, petiole, root, dead plant parts	No symptoms
Aspergillus. clavatus	Lamina, petiole, root, dead plant parts	No symptoms
Cephalosporium	Leaf	No symptoms
Cladosporium sp.	Petiole	No symptoms
Coletotrichum dematium	Lamina	Leaf spot
Curvularia clavata	Lamina, petiole	Lesion
Curvularia lunata	Lamina	Slight lesion
Drechslera indica	Lamina, Petiole	Leaf spot
Epicoccum nigrum	Lamina, petiole	No symptoms
Fusarium chlamydosporum	Petiole	Leaf blight
Fusarium equiseti	Lamina, petiole, root	Leaf spot
Fusarium moiliforme	Lamina, petiole	No symptoms
Fusarium oxysporum	Lamina, petiole	No symptoms
Fusarium pallidoroseum	Lamina, Petiole	Leaf spot
Fusarium solani	Lamina, petiole	Lesion
Helminthosporium bicolor	Lamina, petiole	Leaf spot
Helminthosporium sp.	Lamina	Leaf spot
Macrophoma sp.	Petiole, stolen	Leaf spot
Macrophomina sp.	Lamina, Petiole	No symptoms
Penicillum sp.	Lamina	None
Phoma herbarum	Lamina	Leaf spot
Phoma sorghina	Lamina	Leaf spot
Phoma sp.	Petiole	Leaf spot
Pythium sp.	Root	No symptoms
Rhizoctonia solani	Lamina, petiole	Leaf spot, blight
Rhizoctonia sp.	Lamina, petiole, stem	Leaf blight
Sclerotium rolfsii	Petiole	Necrosis
Trichoderma sp.	Lamina	No symptom

sprayed with sterile distilled water containing Tween 80. All the fungi were sprayed in 3 replications. These plants were then kept in environment growth chamber at 28^0C and 70% relative humidity. The plants were individually enclosed in plastic bags. This was done to create a dew effect that is conductive for fungal growth. The disease severity was rated by visual observation at an interval of 24 hours till 7 days.

RESULTS AND DISCUSSION

During a periodical survey of various water bodies of Jabalpur, it was observed that several diseases including leaf spot, leaf blight, die back, petiole rot, root rot, etc were associated with waterhyacinth. A total of 31 fungal pathogens were isolated and purified from various diseased samples of waterhyacinth. The associated plant parts and symptoms are given in Table 1.

The spore/mycelial suspension of pathogens *viz. Alternaria alternata, Rhizoctonia solani, Curvularia lunata, Fusarium pallidoroseum, Alternaria eichhorniae,* incited severe infection and caused drastic damage to the weed while few others *viz. Fusarium chlamydosporum, Drechslera indica, Phoma sorghina, Sclerotium* sp, *Fusarium solani, F. equiseti, Rhizoctonia* sp, *Phoma* sp, and *Curvularia clavata* caused mild disease to waterhyacinth. Several other fungi *viz. F. moiliforme, F. oxysporum, Drechslera* sp, *Pythium* sp,. *Phoma* sp, *Epicoccum nigrum, Colletotrichum dematium, Macrophoma* sp, *Aspergillus flavus, A. niger, A. clavatus, Cladosporium* sp, *Helminthosporium bicolor, Penicillum* sp. *Trichoderma* sp, *Macrophomina* sp, *Cephalosporium* sp. totally failed to incite any disease to the weed.

Similarly the spray of culture filtrate caused diverse effect on different fungi. The culture filtrates of pathogens *viz., A. alternata, A. flavus, C. lunata, F. pallidoroseum, F. solani, D. indica* and *F. equiseti* caused drastic damage to waterhyacinth. Certain fungus like *Helminthosporium bicolor, P. sorghina, A. eichhorniae, F. chlamydosporum, Helminthosporium* sp, *Sclerotium* sp., *C. clavata, Phoma* sp, *Rhizoctonia* sp, *C. dematium, Drechslera* sp. caused mild disease to the weed. Culture filtrate of several other fungi *viz., F. moiliforme, F. oxysporum, Pythium* sp, *Phoma* sp, *E. nigrum, Cephalosporium* sp. *Macrophomina* sp. *Macrophoma* sp, *Aspergillus niger, Trichoderma* sp. *Penicilium* sp. failed to cause any effect (Table 2).

In the first stage of screening, out of 33 isolates, 5 of the most virulent ones were selected for further screening. They were *A. alternata, C. lunata, F. pallidoroseum, A. eichhorniae* and *R. solani*. These isolates were again screened as earlier and *A. alternata* was found to be the most pathogenic. It was followed by *C. lunata, F. pallidoroseum, R. solani* and *A. eichhorniae*. The *A. eichhorniae* culture isolated in the present investigation proved to be mildly pathogenic in the growth chamber test contradictory to many reports by various other

Table 2. Impact evaluation of various fungi on waterhyacinth

Fungus isolated from waterhyacinth	% Damage*	
	Spore suspension	Phytotoxin
Alternaria alternata	85.0 (67.4)	75 (60.1)
Alternaria eichhorniae	65.0 (53.8)	10.0 (18.4)
Aspergillus clavatus	0.0 (4.1)	0.0 (4.1)
Aspergillus flavus	0.0 (4.1)	0.0 (4.1)
Aspergillus niger	0.0 (4.1)	0.0 (4.1)
Cephalosporium	0.0 (4.1)	0.0 (4.1)
Cladosporium sp.	0.0 (4.1)	0.0 (4.1)
Coletotrichum dematium	10.0 (18.4)	0.0 (4.1)
Curvularia clavata	20.0 (26.5)	8.0 (16.4)
Curvularia lunata	70.0 (57.0)	70.0 (57.0)
Drechslera indica	5.0 (12.9)	0.0 (4.1)
Epicoccum nigrum	0.0 (4.1)	0.0 (4.1)
Fusarium chlamydosporum	4.0 (39.2)	35.0 (36.2)
Fusarium equiseti	60.0 (50.8)	10.0 (18.4)
Fusarium moiliforme	0.0 (4.1)	0.0 (4.1)
Fusarium oxysporum	0.0 (4.1)	0.0 (4.1)
Fusarium pallidoroseum	70.0 (57.0)	68.0 (55.6)
Fusarium solani	40.0 (39.2)	50.0 (45)
Helminthosporium bicolor	45.0 (42.2)	0.0 (4.1)
Helminthosporium sp.	25.0 (23.9)	10.0 (18.4)
Macrophoma sp.	0.0 (4.1)	0.0 (4.1)
Macrophomina sp.	0.0 (4.1)	0.0 (4.1)
Penicillum sp.	0.0 (4.1)	0.0 (4.1)
Phoma herbarum	10.0 (18.1)	8.0 (16.4)
Phoma sorghina	40.0 (39.2)	20.0 (26.5)
Phoma sp.	0.0 (4.1)	0.0 (4.1)
Pythium sp.	0.0 (4.1)	0.0 (4.1)
Rhizoctonia solani	60.0 (50.9)	70.0 (57.0)
Rhizoctonia sp.	10.0 (18.1)	10.0 (18.1)
Sclerotium rolfsii	25.0 (29.9)	20.0 (26.5)
Trichoderma sp.	0.0 (4.1)	0.0 (4.1)
LSD (P=0.05)	(NS)	(NS)

*Values in the parenthesis are the arc sin transformed values of the original mean values.

workers (Nag Raj and Ponappa 1967, Charudattan and Rao 1982, Shabana *et al*. 1995, Shabana *et al*. 2000). *Rhizoctonia solani* has been reported as pathogenic to a number of crops, therefore, it was excluded from further consideration.

The present investigation can form a basis for selection of potential biocontrol agents after further studies relating to host range and pathogenic ability of the fungal flora towards waterhyacinth and crop plants.

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