

Screening of Fungal Pathogens for Biological Management of Noxious Weed *Eichhornia crassipes*.

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Abstract:

Waterhyacinth (*Eichhornia crassipes*) which have been described as the most troublesome weed in the world. 15 fungal isolates belonging to different genera were isolated. *Alternaria alternata* (FCWH#46) conway which was isolated from declining waterhyacinths in Lamina, Petiole in Florida. Maximum infection was observed in the Lamina of the leaves along with the expression minimum symptoms. *Alternaria alternata*, (FCWH#46) was found in maximum frequency and hence, it would be the best fungal candidate for biological management of Water hyacinth.

Key Words: *Eichhornia crassipes*, *Alternaria alternata*, Leaf spot, Lamina.

Introduction:

The aquatic weeds are those unwanted aquatic plants which grow in water and complete at least a part of their life cycle in water. Aquatic ecosystems all around the globe are threatened by the presence of various aquatic weed. Some of the most common dangerous aquatic weed are (*Eichhornia crassipes* [Mart.] Solms), alligator weed, *Alternanthera philoxeroides* (Mart.), giant salvinia, *Salvinia molesta* and water lettuce (*Pistia stratiotes* L.) (Ray and Hill, 2013). Aquatic weeds are generally classified into three groups: - Floating Aquatic weeds, submerged Aquatic weed and Emergent Aquatic weed.

Waterhyacinth is a free-floating perennial aquatic plant (or hydrophyte) native to tropical and subtropical South America. With broad, thick, glossy, ovate leaves, waterhyacinth may rise above the surface of the water as much as 1 meter in height. The leaves are 10–20 cm across, and float above the water surface. One of the fastest growing plants known, waterhyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. Each plant can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8-15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. When not in bloom, waterhyacinth may be mistaken for frog's-bit (*Limnobium spongia*).

The most ignored aspect of the harm done by waterhyacinth is its direct impact on fresh water itself. The plant cover provides obnoxious smell, suspended particulate matter in water. It also causes oxygen depletion load on the water body. It also lowers temperature, pH and bicarbonate alkalinity and increases the carbon dioxide concentration. The decomposition of dead plants increases the B.O.D. and chlorine demand and also leads to eutrophication and rapid siltation of the water bodies. We have isolated, identified and screened the most widely occurring fungal pathogens associated with water hyacinth in Jabalpur and determined their frequency on the water bodies.

Material and Methods

Survey and collection

Infected *Eichhornia crassipes* plant parts were collected by various ponds, Ganga Sagar, Gullauatal, Ranital, Adhartal, Bajanamath, Supatal, Mahandda pond, ditches and pools near MGM school, Shobhapur railway crossing, Panagar etc from the Jabalpur. The plant specimens were collected and stored in polythene bags and brought to the laboratory where isolation and purification of the fungi was attempted.

The developed symptoms like spots, lesions, roots were collected then surface sterilized with 1% sodium hypochlorite solution for about three minutes, followed by rinsing with sterile water to remove any possible contamination. (Hawker, L. E. 1950 and Chopada *et al.*, 2014) then treated pieces were placed on potato dextrose agar medium amended with 75 mg/ 1 streptomycin. This was incubated for 5-7 days at 28°C in BOD. (Agrawal and Hasija 1986). All the fungal pathogen was obtained. (Marak *et al.*, 2014).

Pathogenicity Test

The pathogenicity was determined Koch Postulates method. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884. Fungi isolated from a diseased water hyacinth and grown in pure culture. The cultured strain should cause disease when introduced into a healthy water hyacinth.

- 1) 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.
- 2) The suspension was then strained through muslin cloth and the concentration of 3 the spore suspension was maintained at about 25×10^3 cfu/ml. Healthy, uniform and small sized water hyacinth plants of about 10 cm length were collected and surface sterilized by using 20% ethanol.
- 3) 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.
- 4) Pathogenicity of the pathogens were tested. (Karuna and Kolte 2005). Broths containing fungal spores (25×10^3 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 0 chamber at 28 ± 2 C temperature and $75 \pm 5\%$ relative humidity for a period of 15 days. Spots appeared on the leaf surface of plants (Saleem *et al.*, 2012).

Purification and maintenance of fungal culture

Fungi isolated were aseptically transferred to PDA slant and by repeated sub culturing pure cultures of different isolates were obtained. The stock culture of the micro-organisms were maintained on PDA media and stored at a low temperature in the refrigerator (Agrawal Hasija, 1986). The other slants were kept in the BOD incubator at $25 \pm 1^\circ\text{C}$ routinely transferred into fresh slants for experimental purposes.

Identification of the Fungi

The Identification was done after studying the morphological cultural characteristic with the help of manuals monographs papers of various workers. (Subramaniam 1971; Ellis 1971, Barnet and Hunter 1972, Sutton 1980).

Frequency of Fungi

Study the distribution of fungi isolated earlier from the various samples the percentage frequency of various species were detected. Frequency as introduced Raunkier (1934) indicates the number of sampling units in which a given species occurred thus expresses the distribution or dispersion of various species in a community. From this, percentage frequency is calculated as follows.

$$\text{Percentage frequency} = \frac{\text{Number of sampling units in which the species occurred frequency}}{\text{Total number of units studied}} \times 100$$

Results and Discussion

During a periodical survey of various water bodies of Jabalpur viz the main water bodies surveyed were Ganga Sagar, Gullauatal, Ranital, Bajnamath, Supatal, Mahandda pond, ditches and pools near MGM school, Shobhapur railway crossing, Panagar, etc. it was observed that leaf spot, leaf blight, die back, petiole rot, root rot, etc, diseases were associated with waterhyacinth. A total of 15 fungal pathogens were isolated and purified from various diseased samples of waterhyacinth (Table - 2)

The spore mycelial suspension of pathogens viz. *Alternaria alternata* (FCWH#46), *Fusarium oxysporium* (FCWH#2), *Fusarium solani* (FCWH#06) and *Rhizoctonia solani* (FCWH#20), incited severe infection and caused drastic damage to the weed while few others viz. *Phoma sorghina* (FCWH#31), *Sclerotium spp* (FCWH#16), *Curvularia lunata* (FCWH#34) and *Colletotrichum dematium* (FCWH#18) caused mild disease to water hyacinth. Several other fungi viz. *Fusarium moiliforme* (FCWH#1), *Fusarium pallidroseum* (FCWH#12), *Rhizoctonia spp* (FCWH#4), *Aspergillus flavus* (FCWH#42), *Aspergillus niger* (FCWH#23), *Penicillium spp* (FCWH#13), *Trichoderma spp.* (FCWH#5), totally failed to incite any disease to the weed.

Similarly the spray of culture filtrate caused diverse effect on different fungus. The culture filtrates of pathogens viz., *Alternaria alternata* (FCWH#46), *Curvularia lunata* (FCWH#34), *Aspergillus flavus* (FCWH#42), *Fusarium pallidroseum* (FCWH#12) and *Fusarium solani* (FCWH#06), caused drastic damage to waterhyacinth. Certain fungus like *Phoma sorghina* (FCWH#31), *Sclerotium spp.* (FCWH#16), *Rhizoctonia spp.* (FCWH#4), and *Colletotrichum dematium* (FCWH #18) caused mild disease to the weed. Culture filtrate of several other fungi viz., *Fusarium moiliforme* (FCWH#1), *Fusarium oxysporium* (FCWH#2), *Aspergillus niger* (FCWH#42), *Trichoderma spp.* (FCWH#5) failed to cause any effect.

In the first stage of screening of 15 isolates 4 of the most virulent ones were selected for further screening. They were *Alternaria alternata* (FCWH#46), *Fusarium oxysporium* (FCWH#2), *Fusarium solani* (FCWH#06), and *Rhizoctonia solani* (FCWH #20). These were again screened as earlier and *Alternaria alternata* (FCWH#46) was found to be the most pathogenic. It was followed by *Fusarium oxysporium* (FCWH#2), *Fusarium solani* (FCWH#06) and *Rhizoctonia solani* (FCWH#20) is reported as pathogenic to a number of crops thus it was excluded from further consideration. Thus finally *Alternaria alternata* (FCWH#46) was selected for further studies.

Maximum damage to the test plants was caused by *Alternaria alternata* FCWH#46. Contradictory to many reports by various other workers, (Nagraj and Ponappa, 1967; Charudattan and Rao, 1982; Shabana *et al.*, 1995a; Shabana *et al.*, 1995b; Shabana *et al.*, 2000a; Shabana *et al.*, 2000b; Molo *et al.*, 2001; Hubballi *et al.*, 2010; Pathak and Kannan, 2011;). The pathogens of the genus *Alternaria*, *Fusarium* and *Aspergillus* are facultative parasites and do not have much specialized mechanisms for their entry into the host (Aneja *et al.*,

1993). The pathogens of genus, *Fusarium*, *Alternaria*, *Curvularia* are facultative parasites do not have much specialized mechanisms for their entry into the host (Aneja et al., 1993).

Table 1 : Pathogenicity of some fungi on water hyacinth.

S. No.	Fungi	Pathogenicity levels
1	<i>Fusarium pallidoroseum</i>	+
2	<i>Fusarium solani</i> (Martin)	+
3	<i>Penicillium</i> sp.	-
4	<i>Phoma sorghina</i>	+
5	<i>Rhizoctonia solani</i>	++
6	<i>Aspergillus niger</i>	-
7	<i>Sclerotium</i> sp.	+
8	<i>Trichoderma</i> sp.	-
9	<i>Fusarium moiliforme</i>	-
10	<i>Fusarium oxysporium</i>	++
11	<i>Rhizoctonia</i> sp.	-
12	<i>Coletotrichum dematium</i>	-
13	<i>Curvularia lunata</i>	-
14	<i>Alternaria alternata</i>	+++
15	<i>Aspergillus flavus</i>	-

Pathogenicity level :

- +++ → Lesion on more than 75% of infected leaves.
 ++ → Above broad than 50 to 75% of infected leaves.
 + → Lesion on less than 50% of infected leaves.
 - → No lesion.

Table 2: Fungi Strains isolated from infected *Eichhornia crassipes*

S. No.	Isolate No.	Fungus Isolated From Waterhyacinth	Associated Plant part	Symptom
1	FCWH#12	<i>Fusarium pallidoroseum</i>	Lamina, Petiole	Leaf spot
2	FCWH#6	<i>Fusarium solani</i> (Martin)	Lamina, petiole	Lesion
3	FCWH#13	<i>Penicillium</i> sp.	Lamina	None
4	FCWH#31	<i>Phoma sorghina</i>	Lamina	Leaf spot
5	FCWH#20	<i>Rhizoctonia solani</i>	Lamina, petiole	Leaf spot, blight
6	FCWH#23	<i>Aspergillus niger</i>	Lamina, petiole, root, dead plant parts	No symptoms
7	FCWH#16	<i>Sclerotium</i> sp.	Petiole	Necrosis
8	FCWH#5	<i>Trichoderma</i> sp.	Lamina	No Symptom
9	FCWH#1	<i>Fusarium moiliforme</i>	Lamina, petiole	No symptoms
10	FCWH#2	<i>Fusarium oxysporium</i>	Lamina, petiole	No symptoms
11	FCWH#4	<i>Rhizoctonia</i> sp.	Lamina, petiole, stem	Leaf blight

12	FCWH#18	<i>Coletotrichum dematium</i>	Lamina	Leaf spot
13	FCWH#34	<i>Curvularia lunata</i>	Lamina	Slight lesion
14	FCWH#46	<i>Alternaria alternata</i>	Lamina, petiole	Leaf spot/blight
15	FCWH#42	<i>Aspergillus flavus</i>	Lamina, petiole, root, dead plantparts	No symptoms

FCWH= (Fungal Culture Waterhyacinth)

Conclusion:

On the basis of above observation it can be concluded that the strain *Alternaria alternata* (FCWH#46) found to be the most virulent strain so, it was selected as a biological agent for the management of the noxious weed Water hyacinth.

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