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EFFECT OF PATHOGENIC FUNGI ON WATER HYACINTH (*EICHHORNIA CRASSIPES* MART. SOLMS)

Aruna Dhakad*, Pokhar Rawal and Brijesh
[1]Department of Plant Pathology, Rajasthan College of Agriculture, M.P.U.A.T., Udaipur – 313001, Rajasthan, India
*Corresponding author E-mail: dhakadaruna3@gmail.com
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ABSTRACT

A comprehensive survey was conducted to isolate, purify, and identify fungal pathogens associated with water hyacinth (*Eichhornia crassipes*) across various regions. Diseased plant samples were collected and subjected to tissue isolation techniques on Potato Dextrose Agar (PDA) to obtain pure fungal cultures. Morphological and molecular analyses revealed several pathogenic fungi, including *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium* spp., *Rhizoctonia solani*, and *Alternaria alternata*. Pathogenicity tests confirmed the virulence of these pathogens on water hyacinth, with *Alternaria eichhorniae* causing up to 74.73 per cent infection intensity. Host range studies were conducted on cultivated crops such as brinjal, chilli, cowpea, maize, sorghum etc. to assess non-target effects. Results indicated that while some pathogens like *Fusarium* spp. exhibited limited host specificity, others like *Alternaria eichhorniae* and *Cercospora rodmanii* demonstrated high specificity to water hyacinth, infecting only a few weed species and sparing cultivated crops. These findings suggest the potential of certain fungal pathogens as biocontrol agents against water hyacinth, emphasizing the importance of host specificity to prevent unintended impacts on agriculture. Further research into formulation and field application is warranted to develop effective mycoherbicides.
Keywords **:** Mycoherbicide, formulation, pathogenicity, PDA, inoculum, BOD.

Introduction

Water hyacinth (*Eichhornia crassipes*), an invasive aquatic plant native to the Amazon basin, was introduced to India in 1896 for ornamental purposes. Since then, it has proliferated across the country's water bodies, including Rajasthan's Pichhola Lake, Ayad River, and Swaroop Sagar in Udaipur. By 2022, infestations had expanded to over 4, 55,000 hectares, affecting more than 350 districts nationwide. Traditional control methods like manual removal are labour-intensive and costly, and chemical herbicides pose environmental and health risks. Biological control has emerged as a promising alternative. Fungal pathogens such as *Cercospora rodmanii*, *Curvularia lunata*, *Fusarium oxysporum*, *Fusarium pallidoroseum*, *Fusarium equiseti*, *Colletotrichum gloeosporioides*, and *Alternaria eichhorniae* have demonstrated efficacy in suppressing water hyacinth growth. Regarding the host range of these fungal pathogens, studies have shown varying degrees of specificity. These fungi can be formulated into bioherbicides that are environmentally friendly and cost-effective. Integrating these biological agents with physical and chemical methods offers a sustainable approach to managing water hyacinth infestations and mitigating their impact on water resources and ecosystems. Which necessitates careful evaluation before its application as a biocontrol agent. Therefore, while certain fungal pathogens offer promising avenues for biological control, their host specificity must be thoroughly assessed to prevent unintended impacts on agriculture.

Material and Methods

Isolation, purification and identification

The water hyacinth diseased leaves were collected from different sites around Udaipur and brought to the laboratory for isolation. The leaves with spots were cut into small pieces, surface sterilized with 0.1% $HgCl_2$ or 1% sodium hypochlorite, for 30 to 60 seconds, rinsed thoroughly in three changes of sterile distilled water

and dried with sterile filter paper. The leaf pieces were plated on potato dextrose agar (PDA) supplemented with 250 ppm streptomycin for the fungi and incubated at 28±2°C in biological oxygen demand (BOD) incubator. Sub-cultured from uncontaminated margin by hyphal tip culture method was performed on PDA slants. Similarly, all the other pathogens were isolated from infected leaves. Identification of fungus was done by comparing the morphological characters and reproductive structures under compound microscope using preparatory slide with lactophenol cotton blue stain**.**

Fungi were isolated and pure cultures obtained using single spore or hyphal tip methods on PDA. Sporulating cultures were diluted to prepare a spore suspension, which was mixed with warm water agar and plated. Under a microscope, single spores were identified and transferred to PDA using a cork borer. Cultures were incubated at 28±2°C and maintained on PDA slants, with monthly revival and storage at 5°C for further study (Choi *et al*., 1999).

The pathogens were identified on the basis of their morphological and colony characters using standard literature (Holliday, 1980). These cultures were maintained on PDA slants at 4ºC for further studies. The pure culture of fungal pathogen was submitted for confirmation of identification to ITCC, IARI, New Delhi.

Pathogenicity test

Pathogen's pure culture was tested to establish their pathogenicity on water hyacinth leaves following Koch's postulates under *in vitro* condition. Water hyacinth plants were transplanted and maintained in plastic pots filled with mud and tap water. Healthy plants with five leaves were selected; two leaves other than the youngest and the oldest served as test leaves and the third leaf served as a control. The leaves blade & petiole base and root zone were pinpricked before inoculation. Seven to ten days old culture bits of the pathogens grown on PDA were placed on the injured portion and covered with small bits of moist cotton wool. The entire plants were covered with polythene sheet to maintain sufficient humidity. Control plants were maintained by applying cotton wool soaked with sterile water alone on the injured leaves and observations were recorded for symptoms development, pathogen was re-isolated and were matched with original symptoms and pathogen to established Koch's postulates.

Symptomatology

In general symptoms produced by *Alternaria eichhorniae* were, initially small brown spots with yellow halo developed on the leaf lamina, which coalesced to form large spots, later resulted in blighting. *Fusarium oxysporum* and *Fusarium pallidoroseum* produced, initially water-soaked dark brown spots and later drying or wilting of entire plants. *Curvularia lunata* produced small brown spots which remained without further spread. Small, brown flecks with reddish borders that develop into circular spots, necrotic spots on leaves, such symptoms produced by *Cercospora rodmanii*. *Colletotrichum gloeosporioides* produced dark brown spots on the leaves later enlarged and coalesced to from large patches.

Based on the time taken for the symptom's development, the pathogens were grouped into three categories (Naseema, 2003). Group I fungi are those which took less than five days to develop symptoms. Group II which took five to seven days to develop symptoms. Those fungi which are classified under group III took more than seven days for symptoms development.

Effect of pathogenic fungi on water hyacinth

Water hyacinth plants were transplanted and maintained in plastic pots filled with mud and tap water. Healthy plants with five leaves were selected. The leaves blade, petiole base was pinpricked and were spray inoculated separately with spore suspension of $1x\ 10^6$ conidia ml^{-1} prepared in sterilized distilled water from 12-15 days old culture grown on PDA. The plants were covered with polythene sheet to maintain sufficient humidity. Control plants were maintained by spray of sterile water. Observations were recorded at 15 days after inoculation using the disease rating scale (0-9) and disease severity was calculated.

Disease rating scale (0-9) of water hyacinth

Grade/Score	Description
0	No symptoms
1	1-10 per cent symptoms development around the pinpricked area only
2	11- 20 per cent leaf area showing yellowing / browning symptoms
3	21-30 leaf area showing yellowing / browning symptoms
4	31-40 per cent of leaf area including petiole showing symptoms
5	41-50 per cent of leaf area including petiole showing symptoms

6	51-60 per cent of leaf area including petiole showing symptoms
7	61-75 per cent of leaf area including petiole showing symptoms
8	More than 75 per cent of leaf area
9	Complete drying of plant

No symptoms

1-10 per cent symptoms

11- 20 per cent of leaf area showing symptoms

21-30 per cent of leaf area showing symptoms

31-40 per cent of leaf area showing symptoms

41-50 per cent of leaf area showing symptoms

51-60 per cent of leaf area showing symptoms

61-75 per cent of leaf area showing symptoms

More than 75 per cent of leaf area

Complete drying of leaf

Disease rating scale (0-9) for water hyacinth

* Fractional values of per cent was round up

Per cent disease index(PDI)=

$$\frac{\text{Sum of all individual disease numerical rating}}{\text{Total number of plant assessed} \times \text{Maximum disease rating scale}} \times 100$$

Effect of fungal culture filtrate on water hyacinth.

(a) Preparation of culture filtrate

The pathogenic fungi of water hyacinth were grown in 250 ml conical flasks containing 100 ml PDA broth, sterilized in autoclave at 121°C for 20 minutes (1.1 kg cm^{-2} pressure). Each of the flasks were inoculated with 5 mm culture disc cut from actively growing seven to ten-days culture of each fungi and incubated for 15 days at room temperature (28 ± 2°C). The broth culture was stirred for 1 to 2 minutes and sieved through double layer of muslin cloth.

(b) Effect of culture filtrate on water hyacinth

Water hyacinth plants transplanted in pots were sprayed with culture filtrate of each fungi @ 10 ml/ plant separately using an atomizer. Three replications were maintained for each fungi. Water hyacinth plants sprayed with sterile water and 15 day old uninoculated PDA broth sprayed were served as control. Intensity of damage caused by culture filtrate was recorded using the rating scale (0-5) given by Praveena (2003) and per cent disease index (PDI) was calculated.

Disease rating scale (Praveena, 2003)

Grade /Score	Description
0	No symptoms
1	1-10 per cent symptoms development around the pinpricked area only
2	11- 25 per cent leaf area showing yellowing / browning symptoms
3	26-50 per cent leaf area including bulbous portion showing blighting
4	51-75 per cent of leaf area with shrinkage of bulbous portion
5	>75 per cent of leaf area affected or complete decaying of the plant

Result

Alternaria eichhorniae

The fungus produced velvetty mycelium growth with brownish white mycelium. In culture, fungus produced intense red pigment which darkened with age. Conidiophores were simple, at times branched bearing conidia which were round to ovate, consist with 3-6 longitudinal septa and 1-4 transverse septa and long cylindrical beak which tapered gradually. The conidia were 32-64.5 × 9.84-13.4 µm in size.

Colletotrichum gloeosporioides

It produced abundant aerial mycelium which was white at first and later changed to greyish white. Hyphae were branched and septate. Fungus produced large number of acervuli in culture, which were globose, dark brown to black coloured. Conidiophores were non septate and hyaline. Conidia were single celled, hyaline, straight with blunt ends, oil globule in the centre and 12.2-16.9 × 3-8 µm in size.

Cercospora rodmanii

The fungus produced greyish white or olive white with a velvetty mycelium growth with white to creamy texture mycelium. In culture, fungus produced pale pink colonies with a light pink reverse side. Hyphae were elongated and multiseptated. Conidiophores were branched often monopodial, long and slender, pale to greyish or olive coloured. The apex of conidiophore is often geniculate. Conidia were hyaline and truncate.

Curvularia lunata

Mycelium produced by fungus were black velvetty, initially appearing grey but turning greyish black with age, with a black reverse and moderate growth. Conidia were three celled, with the middle cell slightly curved and 18.72-29.1 × 9.1-13.9 µm in size.

Fusarium oxysporum

The fungus produced white mycelium first, which later turn whitish pink on upper side and light pink below. Hyphae and conidiophores were hyaline. Macroconidia seen in large numbers which were hyaline and cylindrical to falcate, 4-6 septate and 18.5-48.2 × 2.5-4.3 µm size. Microconidia were single celled, oval, hyaline and 6.24-13.2 × 2-3.1 µm in size.

Fusarium pallidoroseum

It produced aerial mycelium that was dull white initially but later deep yellow at the centre of the colony below. Conidiophores were branched. Falcate macroconidia which gradually tapered from both the ends, which were 3-4 septate and 15.2-37.53 × 2.3-4.1 µm in size. Microconidia hyaline, single celled, oval and 4.13-14.5 × 1.6-7.2 µm in size.

Pathogenicity test

To prove Koch's postulates of fungal pathogens, plants of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] were maintained in plastic pots and spray inoculated. The variations in symptoms produced by the pathogenic fungi on water hyacinth were studied. It was observed that time taken for symptom development were varied with the pathogen.

In general symptoms produced by *Alternaria eichhorniae* were, initially small brown spots with yellow halo developed on the leaf lamina, which coalesced to form large spots, later resulted in blighting. Small brown spots were seen on petiole also .*Fusarium oxysporum* and *Fusarium pallidoroseum* produced, initially water-soaked dark brown spots and later drying or wilting of entire plants. *Curvularia lunata* produced small brown spots which remained without further spread. Small, brown flecks with reddish borders that develop into circular spots about 4mm wide, necrotic spots on leaves, such symptoms produced by *Cercospora rodmanii* .*Colletotrichum gloeosporioides* produced dark brown spots on the leaves later enlarged and coalesced to from large patches. Brown spots were seen on petiole resulted in complete drying up of the plant.

Based on the time taken for the symptom development, the pathogens were grouped into three categorised according to Naseema (2003). Group I fungi are those which took less than five days to develop symptoms. *Alternaria eichhorniae*, *Fusarium oxysporum* and *Fusarium pallidoroseum* belonged to group II which took five to seven days to develop symptoms. Those fungi which are classified under group III *viz., Colletotrichum gloeosporioides, Cercospora rodmanii* and *Curvularia lunata* took more than seven days to symptoms development. Though *Colletotrichum gloeosporioides*, took more time to develop symptoms (eight to ten days).

Re-isolation of pathogens from diseased leaves were carried out on PDA plates. The recovered cultures of fungi were identical to the original culture. Thus, fulfilled Koch's postulates and the pathogenicity of the test pathogens.

Effect of pathogenic fungi on water hyacinth

Disease severity (PDI) was ranged from 26.89 to 75.55 per cent among the pathogens at 15 days after inoculation. The maximum extent of damage (PDI 75.55) was caused by *Alternaria eichhorniae*, followed by *Cercospora rodmanii* (PDI 66.45), *Fusarium pallidoroseum* (PDI 48.93), *Colletotrichum gloeosporioides* (PDI 44.48), *Fusarium oxysporum* (PDI 36.46) (Table 1). However, least intensity of diseased (26.89%) was caused by *Curvularia lunata*.

Table 1: Disease severity caused by pathogenic fungi on water hyacinth

S.No.	Fungi	Disease index % (*PDI)
1	*Cercospora rodmanii*	66.45 (54.58)
2	*Alternaria eichhorniae*	75.55 (60.34)
3	*Colletotrichum gloeosporioides*	44.48 (42.38)
4	*Fusarium oxysporum*	36.46 (37.13)
5	*Curvularia lunata*	26.89 (31.21)
6	*Fusarium pallidoroseum*	48.93 (42.65)
SEm±		0.67
C.D. at 5%		2.09
C.V. %		2.35

*Average of three replications; Figures in parentheses are arcsine √ per cent angular transformed values.

Effect of fungal culture filtrate on water hyacinth

The pathogenic fungi showed significant difference in extent of damage caused by the culture filtrates (Table 2).

Culture filtrates symptoms were scorching on leaf lamina coupled with yellowing and finally drying up of the leaves and petiole. It was observed that maximum intensity of damage *i.e*., 73.77 per cent was produced by the culture filtrate of *Alternaria eichhorniae* followed by 65.48, 48.09, 44.64, 35.37 and 25.62 per cent respectively by *C. rodmanii, F. pallidoroseum, C. gloeosporioides, F. oxysporum* and *C. lunata*. However, least damage (25.62%) was caused by culture filtrate of *C. lunata* (Table 2).

Table 2: Effect caused by culture filtrate of pathogenic fungi on water hyacinth

S.No.	Fungi	Disease index % (*PDI)
1	*Cercospora rodmanii*	65.48 (53.99)
2	*Alternaria eichhorniae*	73.77 (59.17)
3	*Colletotrichum gloeosporioides*	44.64 (41.91)
4	*Fusarium oxysporum*	35.37 (36.47)
5	*Curvularia lunata*	25.62 (30.39)
6	*Fusarium pallidoroseum*	48.09 (42.47)
SEm±		0.68
C.D. at 5%		2.13
C.V. %		2.44

*Average of three replications; Figures in parentheses are arcsine √ per cent angular transformed values.

Discussion

The present study was focused on isolation and screening of most virulent pathogens with aim to develop bioformulations for effective biological management of water hyacinth. During the research studies, 6 pathogenic fungi were isolated and found pathogenic to water hyacinth were *Alternaria eichhorniae, Cercospora rodmanii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium pallidoroseum* & *Fusarium oxysporum* {Holliday (1980); ITCC I.D. No. 12131.25 (IARI, 2025)}.

Pathogenicity test was conducted to prove Koch's postulates. The symptoms and pathogen culture were found similar with original field symptoms and initial pure culture of the pathogen. Thus, fulfilled Koch's postulates and the pathogenicity of the test fungus. Pathogenicity tests on water hyacinth using

Cercospora rodmanii produced symptoms similar to those reported by Conway, (1976). Symptoms produced by *Alternaria eichhorniae* on water hyacinth were small brown spots, which coalesced to form large spots, later resulted in blighting. Small brown spots were seen on petiole also. Similar to those reported by Nagraj and Ponnappa (1970). In the case of *Colletotrichum gloeosporioides*, symptoms were limited to older leaves as small dark brown spots with yellow halo on the leaf lamina only. Later these spots enlarged and adjacent spots coalesced to form large patches, similar symptoms were observed by Santhi and Naseema, 1995. *Curvularia lunata* produced brownish black coloured pin head sized spots on leaf lamina and petioles. Similar observation was made by Santhi (1994). *Fusarium* spp. produced brown spots with yellow halo both on leaf lamina as well as on petiole which resulted in yellowing of the lamina. Infection was more pronounced on the older leaves. Praveena (2003) also reported similar observations.

Based on the time taken for the symptom's development, the pathogens were grouped into three categories. Group I fungi are those which took less than five days to develop symptoms. *Alternaria eichhorniae*, *Fusarium oxysporum* and *Fusarium pallidoroseum* belonged to group II which took five to seven days to develop symptoms. Those fungi which are classified under group III *viz., Colletotrichum gloeosporioides, Cercospora rodmanii* and *Curvularia lunata* took more than seven days for symptoms development. Though *Colletotrichum gloeosporioides*, took more time for develop symptoms (eight to ten days). All these fungi developed symptoms on leaves blade as well as on petioles and petiole base with a prominent yellow halo on the leaf lamina. In the later stages, the infection led to blighting and drying up of the plants.

A fungus can be considered as an effective biocontrol agent of water hyacinth only if it can infect both the foliage and the petioles. Once the petioles are infected, the plants will sink to the bottom of water thereby suppressing its regeneration. Water hyacinth multiplies at a very fast rate and can double its biomass in ten days (Singh, 1999). Therefore, fungus, which is effective in killing the weed in less than ten days, can be selected as an effective biocontrol agent. The fungi belonging to group III with retarded symptom development is less appealing as biocontrol agent. On the contrary, fungi coming under groups I and II seem more potent with quick symptom production on both leaves and basal swollen parts (petioles).

The disease severity was varied among the pathogens of water hyacinth. The extent of infection (PDI) ranged from 26.89 to 75.55 per cent. The maximum intensity of infection 75.55 per cent was caused by *Alternaria eichhorniae*, followed by *Cercospora rodmanii* being 66.45 per cent. *Alternaria eichhorniae* was most virulent and significantly better than other fungi. Least intensity of infection was caused by *Curvularia lunata* (25.89 per cent).

Variation in the extent of damage by the fungal pathogens has been reported by several workers. Shabana *et al.* (1995) isolated most virulent isolate of *Alternaria eichhorniae*, causing leaf blight of water hyacinth, produced intensity of infection of 79.33 per cent. Santhi (1994) reported 51.10 per cent intensity of infection of *F. pallidoroseum* on water hyacinth plants. Conway (1976) isolated *Cercospora rodmanii* from declining water hyacinth in reservoir and was evaluated for its biocontrol potential, produced disease intensity of 70.00 per cent.

Alternaria eichhorniae produced symptoms within a very short period and also high intensity of damage, it is probable that it may contain a potent toxin. So, there is high possibility of exploiting this toxin for the management of water hyacinth. Followed by *C. rodmanii, F. pallidoroseum, C. gloeosporioides, F. oxysporum* and *C. lunata* had ability to produce symptom on leaf as well as on petiole region. The variation in the symptoms produced by these fungi may be due to the difference in the nature and type of toxin & enzymes produced by the them.

The extent of damage caused by the culture filtrates of the pathogenic fungi showed significant difference. Culture filtrates symptoms were scorching on leaf lamina coupled with yellowing and finally drying up of the leaves and petiole. It was observed that maximum intensity of damage *i.e*., 73.77 per cent, was produced by the culture filtrate of *Alternaria eichhorniae*. Followed by *C. rodmanii, F. pallidoroseum, C. gloeosporioides, F. oxysporum* and *C. lunata* being 65.48, 48.09, 44.64, 35.37 and 25.62 per cent respectively. Least damage was in culture filtrate of *C. lunata* 25.62 per cent. Similarly, Abbas *et al.* (1991) also reported that culture filtrate of *F. moniliforme* exhibited phytotoxicity symptoms of mild to severe necrosis on jimson weed. Cell free metabolites of *A. eichhorniae* produced small necrotic spots with prominent yellow halo on the leaves. According to Nagraj and Ponnappa (1970), culture filtrate of *A. eichhorniae* showed necrosis on water hyacinth plants within 24 hours and in the next 24 hours the leaves scorched and dried up. Whereas in the present study, small necrotic spots developed and resulted in scorching and drying. Extent of damage of 73.77 per cent was caused by its culture filtrate. The

pathogen also took almost same time and same extent of damage for developing symptom as the culture filtrate. Regarding the extent of damage, it was comparable with *F. moniliforme*. Brown patches followed by scorching and yellowing was developed by the culture filtrate of *F. moniliforme*

Culture filtrate of *F. pallidoroseum* produced scorching coupled with yellowing of leaves. *F. equiseti* produced minute irregular spots on the leaves. Santhi and Naseema (1994) observed that the culture filtrate of *F. equiseti, F. pallidoroseum* and *F. solani* produced symptoms on water hyacinth as these contained toxin.

Pandey *et al.* (2002) reported that the phytotoxicity of culture filtrate of various fungal isolates varied significantly. Stevens *et al.* (1979) isolated and characterized a phytotoxic substance bostrycin from *A. eichhorniae*, which showed no herbicidal activity towards water hyacinth. Maity and Samaddar (1977) isolated a stable toxic metabolite from fourteen-day old culture filtrate of *A. eichhorniae*.

Conclusion

The development of a plant pathogen into a bioformulation involves using a naturally occurring microorganism (like a fungus, bacterium) that causes disease in specific weed species to control them. However, a critical concern in this process is ensuring the safety of non-target cultivated plants. Plants that are not intended to be affected, such as crops, native flora, or other economically important species. This is crucial for both environmental and regulatory reasons.

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