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ISOLATION AND IDENTIFICATION OF INDIGENOUS MYCOFLORA OF *EICHHORNIA CRASSIPES* FOR THE BIOLOGICAL CONTROL OF WEED

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ABSTRACT

*Eichhornia crassipes*is one of the most invasive and troublesome aquatic weed and causes serious ecological and financial harm to the aquatic ecosystem. Survey of water body, Taran Taaran taal of Datia (M.P) were conducted with the objective to isolate and evaluate the pathogenicity of native strains of fungal pathogens as biocontrol agent of *Eichhornia crassipes.* The fungal pathogens*Aspergillus ochraceus, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum, Mucor irregularis, Mucor hiemalis and Rhizopus stolonifera* were isolated and identified. After the pathogenicity test it has been found that *Aspergillus niger*and *Mucor irregularis* causes disease symptoms resulting in the death of inoculated *Eichhornia crassipes*plants in about 18 days.

Keywords:*Aspergillus niger, Aspergillus ochraceus, Biological control, Eichhorniacrassipes, Fungal, Isolation, Indigenous Mycoflora, Inoculation, Mucor irregularis, Penicillium chrysogenom.*

INTRODUCTION

Eichhornia crassipes is an invasive aquatic plant that poses a threat to both the environment and the economy worldwide. Originally from the Amazon Basin, this perennial plant has rapidly proliferated various aquatic environments, posing a serious ecological threat. *E. crassipes* is a resilient weed that outcompetes native plants and clogs rivers due to its rapid growth and reproduction capacity. These infestations have a negative influence on irrigation, hydropower production, fisheries, and water chemistry in addition to reducing biodiversity. The aquatic macrophyte *Eichhornia crassipes*, also referred to as the water hyacinth, is a free-floating plant that normally grows to a height of 0.5 meters but can reach nearly 1 meter in some Asian regions. Dense, floating mats of *Eichhornia crassipes* are found on water reservoirs such as lakes, ponds, marshes, rivers, etc. since they float freely. The leaves are glossy, spherical, slightly waxy, and they rise well on the water's surface on the stalks. The leaf stalks are spongy and bulbous. There's a flower at the top named Blue Devil. It has six petals that range in hue from purplish blue to lavender to pinkish, and at the centre of the highest petal is a yellow splotch surrounded by blue. Feathery and Purplish black roots.Its growth is life threatening to aquatic vertebrate and invertebrates because it absorbs oxygen from the water resulting in oxygen depletion in the waterbodies which leads to the loss of habitat for aquatic life, including fish and other aquatic species.,. They can tolerate a variety of challenging environmental conditions, including those related to temperature, salinity, water pH, nutrition availability, and the presence of toxic contaminants. As a result, it has expanded to more than 50 countries across five continents despite being native to the Amazon River basin. The shoot *Eichhornia crassipes* is a branching, stoloniferous rhizome with multiple short internodes that is up to 30 cm long and 6 cm in diameter. The plant is considered a noxious weed in Arizona. It is frequently considered to be the world's most problematic aquatic weed.

Datia is a historic city in Madhya Pradesh since Mahabharata kal. The city is surrounded by lakes and ponds. These waterbodies are a main source of city water supply and possess great historic values. Now a days these waterbodies, Taran Taaran tal is infested by *Eichhornia crassipes*weeds which damages the pond.

Therefore, an attempt has been made to control the growth of this noxious weed through the biological control of native fungal pathogen found on the leaf of *E. crassipes*.

Extensive survey have been carried out to find potential biocontrol agent for water hyacinth. M.A Elwakil *et al*. (1989) isolated Alternaria alternata number 5 for the biological control of *Eichhornia crassipes* in Egypt and found it best suitable agent. H.C Evanc and R.H Reeder (1998) reported *Alternaria alternata* and *Cercospora* as biocontrol agent of weed. Aditi Pathal and C. Kannan (2011) reported Fusarium oxysporum as a promising agent for the biocontrol of *Eichhornia crassipes.*R. Pavithra *et al*. (2020) reported isolates of *Fusarium* WH-5 having potential biocontrol property for the management of water hyacinth. Several researchers have found *Fusarium oxysporum* (WHK-59) had high effectiveness against water hyacinth.

MATERIALS AND METHODS

SAMPLE COLLECTION

Diseased plants of *Eichhornia crassipes* showing symptoms of chlorosis, brown leaf spots and necrosis were collected from Taran Taaran taal of Datia (M.P). collected plants were washed under tap water to remove all debris.

STERILIZATION OF GLASSWARE

Petri plates, flasks, beakers, slides, test tubes, measuring cylinder were autoclaved at 15 psi at 120ºC for 15-20 minutes.

MEDIA PREPARATION: PDA CONCENTRATION

Potato -200 g
Dextrose – 20g
Agar agar -17 g were mixed in 100mL distilled water
Distilled water –1000mL
And were sterilized at 15psi for 30 minutes
3.4 pH – 5.5

POURING AND INOCULATION

After the sterilization of media, melted PDA was poured into sterilized petri plates under aseptic condition. After solidification of media, samples were taken from the diseased part of *E. crassipes* leaves and inoculate the fungi in zig-zag manner making fine streaks.

INCUBATION

Inoculated petriplates with 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 pH were kept in incubator at 28ºC temperature for 72hours at pH 5.5.

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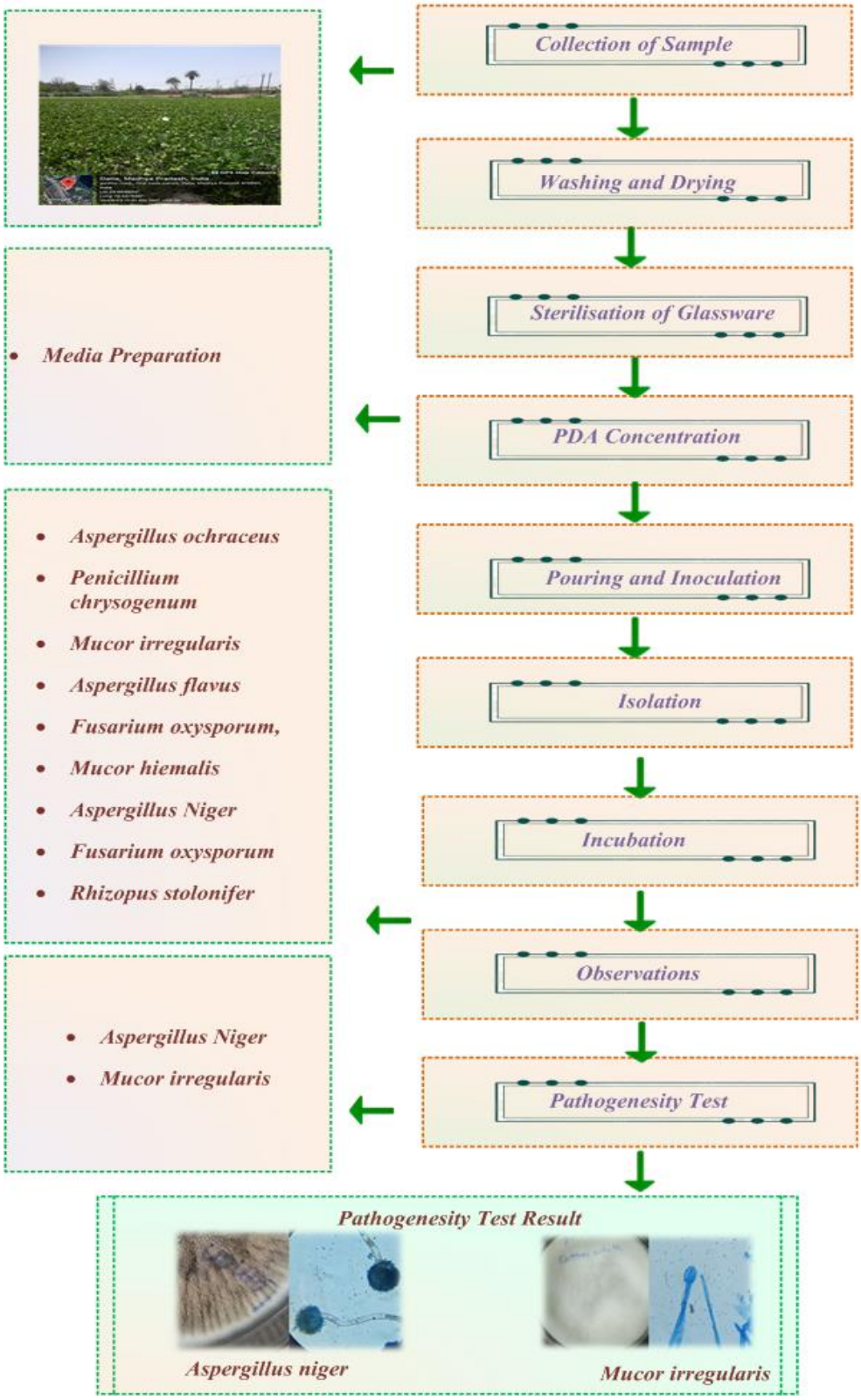

Figure: 1 Proposed Method Block diagram

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OBSERVATION

After incubating for 72 hours fungal species of *Aspergillus ochraceus, Aspergillus flavus,Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum, Mucor irregularis, Mucor hiemalis and Rhizopus stolonifera* have been isolated from *Eichhornia crassipes*and identified using the standard text.

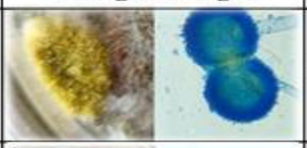
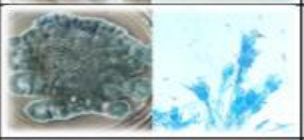
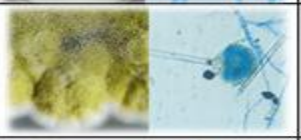
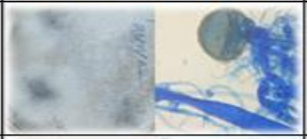
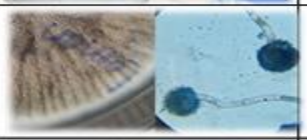
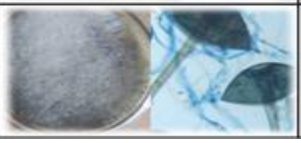
S.No	Fungal Image	Fungal Name
1		*Aspergillus ochraceus*
2		*Penicillium chrysogenom*
3		*Mucor irregularis*
4		*Aspergillus flavus*
5		*Fusarium oxysporum*
6		*Mucor hiernalis*
7		*Aspergillus Niger*
8		*Rhizopus stolonifer*

Figure: 2 Fungal Species

PATHOGENICITY TEST

Fungal isolates were tested for disease development in healthy plants. Two methods have been used i.e. *in vitro* detached leaf assay and whole plant assay (R. Pavitra *et al*, 2020)

a) *In vitro* detached leaf assay:

Healthy leaves were collected from *Eichhornia crassipes* plants and washed thoroughly under running water. These leaves were surface sterilized using 70% ethanol and placed in sterile petri plates containing wet blotting paper. Small open wounds were made using sterile needle and fungal isolates were inoculated on the leaves at

1×10^4/mL and incubated for 7 days. Inoculated leaves were observed for every 24hrs, and symptoms were recorded.

b) WHOLE PLANT ASSAY:

Fresh water hyacinth plants were collected, washed and placed in 500mL glass beaker containing water. Plant leaves were wounded artificially and sprayed with 1×10^6/mL spore suspension of fungal isolates. Inoculated plants were covered with polyethylene bags to maintain humidity. Plants were observed for disease development up to 25 days.

Figure: 3 Pathogenicity Test Process

RESULT AND DISCUSSION

From the diseased plants of *Eichhornia crassipes* 8 fungal isolates were obtained. It has been found that *Aspergillus niger*, *Mucor irregularis* and *Fusarium oxysporum* grow well at 28ºC, pH 5.5 for 72 hrs of incubation. It can be used for pathogenicity test.

PATHOGENICITY TEST RESULT

Aspergillus niger and *Mucor irregularis* fungi were identified again in pathogenesity test.

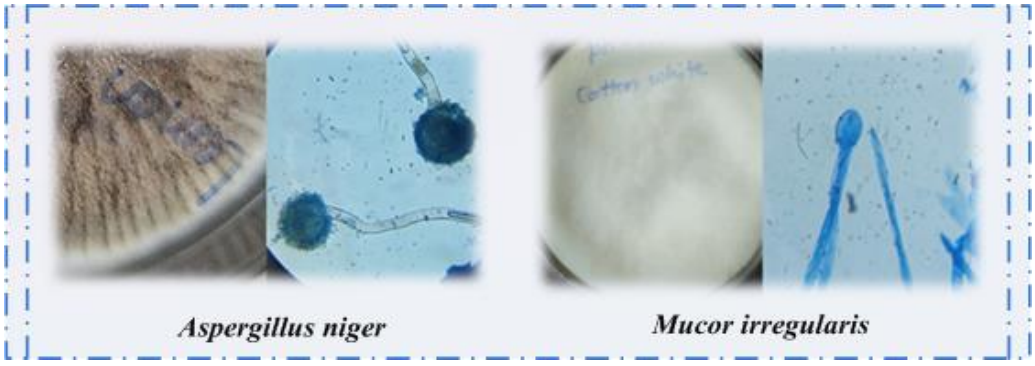


Figure: 1 Pathogenesity Test Result

a) *Invitro* detached Leaf Assay:

In the *in vitro* detached leaf assay, the isolates *Aspergillus niger*and *Mucor irregularis* produced symptoms about 48 hours of incubation whereas *Fusarium oxysporum* produced symptoms after 72 hours of incubation.

b) Whole plant Assay:

In this assay three isolates namely- *Aspergillus niger*, *Fusarium oxysporum* and *Mucor irregularis* were inoculated on the leaf and petiole of the *Eichhornia crassipes* and found that plant infected with *Aspergillus niger* produced symptoms like brown spots after the 17th day of incubation whereas *Mucor irregularis* produced symptoms within 20 days and *Fusarium oxysporum* produced symptoms up to 26th day of incubation.

During the whole plant assay, it has been found that symptoms like brown spots first appeared on leaf and spread up to petiole resulting in rotting of the whole plant.

CONCLUSION

Water hyacinth was shown to be an abundant source of new and useful antibiotics active against some pathogenic strains of bacteria, fungi and algae. It also decreases the emissions of small particles from chemical fuels and green house gases. It decreases GHG emissions by approximately 71% compared to fossil fuels. It also decreases emissions of other pollutants to improve the air quality. It has low toxicity and is easily biodegradable. Studies show the choice of microbial inoculant for the better bioethanol production. Ethanol production is observed using inoculant. It is economically feasible method for high yield production of bioethanol using aquatic plant such as water hyacinth. Compared to landfill water hyacinth Bioethanol production is a best choice of economics, which means it can improve social welfare. Instead of disposing water hyacinth by landfill, it may be contributed to decrease in GHG emission by producing bioethanol. The bioethanol cost, rate of yield is more feasible economically. Water hyacinth can be used for producing bioethanol output targets. The active compounds were complex in structure and so would be difficult and expensive to synthesize chemically. Water hyacinth is also useful for pharmaceutical uses. Extracts and fractions used pharmaceutically could require the harvest of millions of tones/year. To sum up, *Aspergillus niger* and *Mucor irregularis* can be used as a promising biocontrol agent for the biological control of *Eichhornia crassipes.*

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