IDENTIFICATION OF SUITABLE POTENTIAL PATHOGENS FOR BIOCONTROL OF WATERHYACINTH [*Eichhornia crassipes* Mart. Solms]

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Abstract

Water hyacinth is one of the most noxious invasive aquatic weed in Sri Lanka.A survey of plant pathogenic fungi associated with naturally infected water hyacinth (Eichhornia crassipes) was conducted in different waterways in the Eastern part of Sri Lanka. Four fungi Alternaria alternata, Cercospora rodmanii, Aspergillus sp.and Trichoderma sp were isolated and confirmedtheir pathogenicity at laboratory. The leaf area affected by the fungal pathogen changes with the intensity (days). The changes in leaf area affected by the pathogen across intensity levels depend upon inoculation method (p=0.003). But there was no statistically significant effect in the inoculation methods on the leaf area affected by the fungal type (p=0.06). All fungal types had different levels of dead lesions formed in water hyacinth. After seven days of inoculation, Alternaria sp. revealed to have the highest affected area, followed by Aspergillus sp. and Cercospora sp but, the Bonferroni mean separation revealed that all four fungal species had similar level of effects in term of dead lesion formed in leaves after 22 days of inoculation. The study suggests that, all the isolated pathogens had potential to control water hyacinth, but the Alternaria alternate was found to be better virulence candidate under laboratory conditions. It may be a potential biocontrol agent against water hyacinth and further studies on performance evaluation under natural environmental conditions and their host specificity test are needed.

Keywords: Water hyacinth, Pathogenic fungi and Biocontrol.

Introduction

Aquatic ecosystems throughout the world are threatened by the presence of invasive aquatic plants, both in the form of floating and submerged. Sri Lanka is an agricultural country with a population of 21 million people. Majority of the farming community relies on irrigation water to raise successful crops as the country experiences periodic droughts during cropping season (FAO's Plant Production and Protection Division-AGP, 2016).The productivity of inland water bodies in the country is affected by the excessive growth of aquatic weeds, such as floating, submerged and rooting emerged species. A recent conducted survey of plant production and protection division of Sri Lanka showed that 45% water bodies are infested with water hyacinth. In Sri Lanka, it has been intentionally introduced for ornamental purpose in 1904 and later it was widely distributed despite its declaration as a prohibited weed under Water Hyacinth Act in 1909 and subsequently under Plant Protection Act in 1924.

Water hyacinth poses serious socioeconomic and environmental problems on millions of people in the world. The weed obstructs electricity generation, irrigation, navigation, and fishing increases evapo-transpiration resulting in water loss increase cost of crop production provides habitat for vectors of malaria and bilharzias, harbors poisonous

snakes, causes skin rashes and can host agents of amoebic dysentery and typhoid (Horwad and Matindi et al.2003; Aneja KR and Srinivas B, 1992).

The main approach for the control of aquatic weeds in Sri Lanka has been traditional biological control, which has been practiced from early fifties. Initial attempts made on controlling aquatic weeds followed mechanical approach supplemented with chemical control (Harry, 1999). However, all these methods failed to provide long lasting effects due to rapid multiplication of the weeds and the relatively low efficacy of control methods. Subsequently, control attempts were diverted towards biological control with insects. However, the insects failed to provide effective control at elevations above 1000 m from sea level due to low (FAO's Plant Production and Protection Division-AGP, 2016).

The management of water hyacinth is possible through physical, chemical, mechanical removal and application of herbicides. All these methods have been found to be either inadequate or too expensive especially in large zones or associated with environmental pollution. Therefore, the present study focuses on identifying potential natural pathogens of water hyacinth with the long term objective of controlling the weed biologically.

Materials and Methods

Surveying diseased water hyacinth

A survey was conducted to document various fungal pathogens of water hyacinth in the water bodies of Batticaloa and Ampara districts. In each district, five locations were surveyed and plants that developed symptoms like leaf spots, lesions, blights, chlorosis, rots and browning were collected.

Sampling procedure, isolation, and fungal identification

Samples were transported to the laboratory in clean plastic bags and stored at 4° C until examined. Stored plant parts were scrubbed under running water to remove surface debris, dissected into small segments (approximately 1 × 1 cm), and surface-sterilized by sequential immersion in 96% ethanol for 30 S, 14% hypochlorite for 30 S, and then rinsed with sterile water for 1 min.

Surface sterilized segments (4 segments per plate) were placed on potato dextrose agar (PDA). Twenty plates were used for petioles and leaves and the plates were incubated for 5 to 7 days. Fungi that were developed on the plant pieces were isolated, and pure cultures were obtained by the single-spore or hyphal-tip technique, depending on the type of fungal isolate. Identification of the different fungal genera was based on morphological characteristics of each growing microbial colony.

Pathogenicity tests

Healthy water hyacinth plants were collected from natural water bodies and they were grown with hydroponics method in lab with suitable environmental conditions. Pathogenicity test for fungal species was performed by preparing the spore suspension from each isolate by adding 15 ml water. The suspensions were centrifuged at 3000ppm for 5mins and the supernatant was removed. Finally, 250ml of fungal suspensions were prepared with sterile water. The number of fungal spores was adjusted under microscope using a dilution series.

Young leaves of some water hyacinth plants were rubbed with carborundum powder for facilitating penetration of spores, and then the leaves were washed-off with sterile distilled water (Firehun et al., 2006). Fungal suspensions were sprayed to the healthy plants using low-pressure hand sprayer followed by the plants were covered with polythene bags to avoid unnecessary spore drift to the next treatment and to maintain the RH.

After inoculation, the four treatments along with the non-inoculated control plants were arranged in randomized complete block design (RCBD) with four replications each. The treated plants were observed for the appearance of disease symptoms and the infected leaf area was measured from 2 to 22 days after inoculation.

Data analysis

Data were subjected to repeated measures ANOVA followed by Bonferroni mean separation at 5% probability level and all data were analyzed using SAS (2004) software.

Mauchly's sphericity test revealed that the Chi-square approximation has an associated p-value less than 0.05. Therefore, the sphericity assumptions have been violated and the data do not meet the sphericity assumptions. Therefore, the multivariate output of the Repeated Measures ANOVA has been used for the interpretation of the results.

Results and Discussion

Alternaria alternata, Cercospora rodmanii, Aspergillus sp. and *Trichoderma sp.* were isolated on PDA from field collected, diseased water hyacinth. Similarly, surveys for natural enemies of water hyacinth that may be used as biological control agents began in 1962 and continued up until recently (Center et al., 2001). Several pathogens gave promising results as biological control agents of water hyacinth in different countries. Among them are *Uredo eichhorniae*, suitable as a classical biocontrol agent and *Acremonium zonatum, Alternaria eichhorniae, A. alternata, Cercospora piaropi, Myrothecium roridum, and Rhizoctonia solani,* which are widely distributed in different continents, as bioherbicides. Other less widely distributed pathogens include notably species of *Bipolaris, Drechslera,* and *Fusarium,* which may hold promise, but further studies are needed to confirm their usefulness (Charudattan et al., 1985 and Shabana et al., 1995).

The changes in leaf area affected by the pathogen across intensity levels depend upon inoculation method ($p=0.003$). Further, the leaf area affected depends on the intensity in conjunction with the fungal type ($p<0.0001$). However, the three-way interaction between the intensity, inoculation method and the fungal type was found to be insignificant at $p=0.05$ (Table 1).

Between subjects effects of repeated measures ANOVA revealed that there was no statistically significant effect of the inoculation methods on the leaf area affected by the fungal type ($p=0.06$). However, different fungal types had different levels of dead lesions formed in the leaf surfaces in water hyacinth. Moreover, inoculation method and fungal type do not combine to influence the overall area affected by the fungus.

Table 1. The leaf area affected by the fungal pathogen changes with the intensity

Effect category	Wilks' Lambda	
	F value	p
Intensity	151.50	<0.0001
Intensity*Inoculation method	5.00	0.0031
Intensity*Fungal type	6.34	<0.0001
Intensity*Inoculation method*Fungal type	1.32	0.2156

Intensity- Days, Inoculation method- Wounded and Unwounded

After seven days of inoculation, *Alternaria sp.* revealed to have the highest affected area, followed by *Aspergillus sp.* and *Cercospora s*). However, bonferroni mean separation revealed that all four fungal species had similar level of effects in term of dead lesion formed in leaves after 22 days of inoculation (Fig.1).

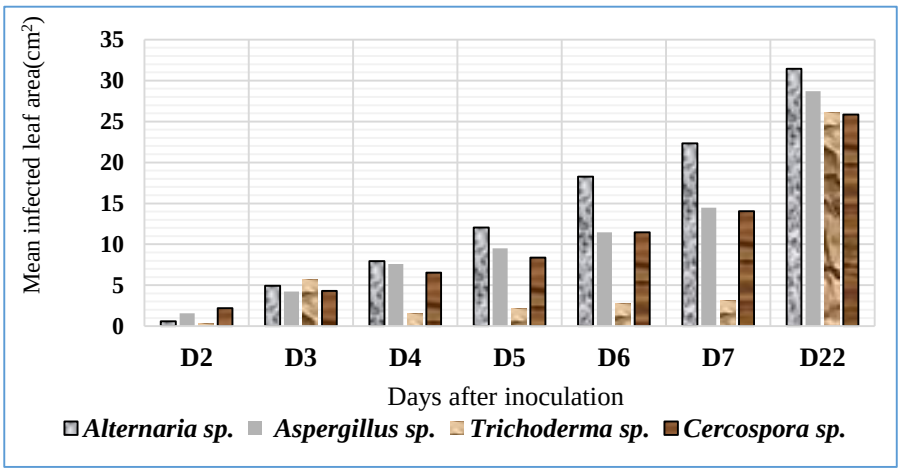

Figure 1. Mean infected leaf area(cm^2) after varying days of inoculation

Similarly, pathogenicity tests of indigenous fungal pathogens on water hyacinth were conducted in different countries. Among the identified pathogens *A.alternata* and *C.rodmanii* have previously been reported (Nag Raj and Ponnappa, 1970; Conway, 1976; Abdel Rahim and Tawfig, 1985; Elwakil et al., 1988; Morris, 1990) and they are considered as potential mycoherbicide agents for integration in biological control of water hyacinth.

Disease started as small necrotic spots and developed into a leaf blight that tended to spread over the leaf. Eventually, these spots enlarge with the rounded side facing the petiole and tapering to a narrow point in the direction of the laminar tip. Additionally, on the upper surface of the leaves, distinct concentric zonation appears giving a target board appearance. The fruiting bodies of the fungus are also noticed on the upper surface along these concentric rings (Fig.2).Similarly, Charudattan (2001) summarized different fungal disease symptoms found in association with water hyacinth as zonate leaf spot, discrete necrotic foliar spots, necrotic spots and blighting on the leaves.

(a) (b) (c) (d)

Figure 2. Leaves showing diseased *Eichhornia crassipes* plants pattern due to different fungal species (a)blighting symptoms(b) zonate leaf spot (c)necrotic leaf spot(d) leaf blight symptoms with conidial mass

Conclusions and Recommendations

The study suggests that, all isolated pathogens *Alternaria alternata*, *Cercospora rodmanii*, *Aspergillus sp.* and *Trichoderma sp*. have potential for control of water hyacinth, but the *Alternaria alternata*was found to be better virulence candidate under laboratory conditions. It may be a potential bio-control agent against water hyacinth and further studies on performance evaluation under natural environmental conditions and their host specificity test are needed.

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