Plant Fungi as Potential Biocontrol agents for Water Hyacinth, *Eichhornia crassipes* Mart. Solms

M.I.S Safeena* and A. Cheanieha Queene
Department of Biological Science, Faculty of Applied Sciences,
South Eastern University of Sri Lanka, Sammanthurai, Sri Lanka
*Corresponding author

ABSTRACT

Water hyacinth (*Eichhornia crassipes*), an invasive alien species in Sri Lanka, has been rated as the world's worst weed. A study was performed to isolate pathogenic fungi of water hyacinth and to evaluate their pathogenicity under field condition. Plant pathogenic fungi associated with naturally-infected water hyacinth were surveyed in selected waterways in the eastern part of Sri Lanka and four potential isolates (*Alternaria alternata, Cercospora rodmanii, Aspergillus* sp. and *Trichoderma* sp.) were identified based on morphological features and koch confirmatory test was carried out. Water hyacinth plots were laid in a Randomized Complete Block Design to spray the fungal spores of pathogens (treatments) in triplicates with an untreated plot as the control.

The affected leaf area was measured per each treatment until 50 days from inoculation. Data were analyzed by means of ANOVA in SPSS version 16, using four isolates as the explanatory variable. The field trial indicated that all four fungal types had different levels of dead lesions formed in water hyacinth ($p<0.01$). Disease started as small necrotic spots and developed into a leaf blight that tended to spread over the leaf. Bonferroni mean separation revealed that the end of 50 days of inoculation, *Alternaria alternata* had the highest affected leaf area (1138 cm2) followed by *Cercospora rodmanii* (1047.13 cm^2) and *Aspergillus* sp. (844.90 cm^2). However, *Trichoderma* sp. revealed the least affected leaf area under field condition. The study suggested that all the isolated pathogens except *Trichoderma* sp. had the potential to control water hyacinth. However, *Alternaria alternata* and *Cercospora rodmanii* were found to be better virulent candidates under field conditions and could be the best candidates to be used in a bio control programme against water hyacinth. Though these fungal species are present in nature, they have failed to control water hyacinth completely perhaps due to limited amount of inoculums present in the natural environment, competition between the concerned fungi and other species and/or the concerned fungi may be antagonized by other microbes. Therefore, further studies on performance evaluation under natural environmental conditions and their host specificity tests are needed.

Keywords: Water hyacinth, Pathogenic fungi, Bio control

INTRODUCTION

Water hyacinth is a perennial, herbaceous, aquatic plant and widely recognized as the world's worst aquatic weed as it poses serious socio-economic and environmental problems (Howard and Matindi, 2003). It has been intentionally introduced to Sri Lanka for ornamental purposes in 1904 and is now widely distributed despite its declaration as a prohibited weed under Water Hyacinth Act in 1909 and subsequently under Plant Protection Act in 1924. It is a common invasive plant in water bodies such as tanks, canals, ponds, paddy fields etc. in almost all the parts of the island. A recently conducted survey showed that 45% of the water bodies are infested with water hyacinth.

It also poses serious socioeconomic and environmental problems on millions of people in the world as it obstructs electricity generation, irrigation, navigation, and fishing, increases evapo-transpiration resulting in water loss, increases 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 (Howard and Matindi, 2003).

The main approach for control of aquatic weeds in Sri Lanka has been traditional ones such as crop rotation, pesticide application and biological control, which have been practiced from early 1950s. Initial attempts were made to use chemicals to control aquatic weeds followed by mechanical approaches (Harry, 1999).Gebregiorgis et al. (2013) explained an opportunity for the joint use of fungal pathogens and the water hyacinth weevil (a biological control method) to manage water hyacinth. In addition, Sutton et al. (2016) indicated that naturally occurring phytopathogens enhance biological control of water hyacinth in eutrophic waters. However, all these methods failed to provide long-lasting effects of control due to rapid multiplication of the weeds and the relatively low efficacy of the methods.

Although, the management of water hyacinth is possible through physical, chemical, mechanical removal and application of herbicides, these all methods have been found to be either inadequate or too expensive especially in large areas or are associated with environmental pollution. Vincent (2001) emphasizes fungal pathogens as bio control agents of water hyacinth. This study aimed to identify and evaluate potential fungal pathogens associated with water hyacinth in Sri Lanka and to use them as effective bio control agents. This may can lead to using natural disease causing fungi as cost-effective and environmentally safe solutions.

MATERIALS AND METHODS

a. Survey of diseased water hyacinth

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

b. Sampling procedure, isolation, and fungal identification

Samples were transported to the laboratory in clean plastic bags and stored at 4^0C until examined. Stored plant parts were scrubbed under running water to remove surface debris, dissected into small segments (approximately 1 × 1 cm^2), and surface-sterilized by sequential immersion in 96% ethanol for 30 seconds, 14% hypochlorite for 30 seconds, and then rinsed with sterile water for 1 minute. Surface sterilized segments (4 segments per plate) were placed on Potato Dextrose Agar (PDA).

Twenty plates each 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 fungal genera was based on morphological characteristics of each growing colony.

c. preparation of fungal suspension

Pathogenicity test for fungal species was performed by preparing the spore suspension from each isolate, obtained by adding 15 ml water onto the spores of each fungal isolate and the suspensions centrifuged at 3000ppm for 5mins. and the supernatant removed. Then 250ml of fungal suspensions were prepared with sterile water. Number of fungal spores was adjusted under microscope (10^7/ l) to maintain the same concentration in each suspension.

d. pathogenicity test in field

A suitable field area with healthy water hyacinth plants (devoid of any disease) was selected and test plots ($2m^2$) for each fungal treatment were made. A field trial was carried out using fungal spore suspensions where three replicates each were carried out for the four isolates. The pathogenicity test was conducted for each isolate at the same concentration for each plot. Treatments were applied to the water hyacinth plots laid in a Randomized Complete Block Design. The complete infected area per each plot was measured up to 50 days from day of inoculation.

e. Host specificity test

Host-specificity tests for the isolated fungi were carried out on economically and ecologically important plants like Chili, Brinjal, Okra and Tomato in an neighboring are (30-50m) away from the testfield. The selected plants were sprayed with equal concentration of fungal spores at seedling and flowering stages using low-pressure hand sprayers and observations of diseased symptoms were recorded.

f. Data Analysis

The affected leaf area was measured per each treatment for 50 days from start of inoculation. Data were analyzed by means of Analysis of Variance (ANOVA) in SPSS version 16, using four isolates as the explanatory variable. Means were separated using Bonferroni mean separation method at 5% probability level.

RESULTS AND DISCUSSION

Surveys for natural enemies of water hyacinth that may be used as biological control agents began in 1962 (Center et al., 2001). Many 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 used in different countries as bioherbicides. Other less widely used pathogens include 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).

In this study based on field trials, after or within three days of inoculation, *Aleternaria alternata* and *Cercospora rodmanii* showed pathogenic effects (Figure1 and Figure 2). First, symptoms appeared on the upper surface of water hyacinth leaves as chlorotic flecks, which developed into lesions with varying degrees of necrosis after 8 days. Brown colouration and coalescence of lesions led to blotches and leaf blights after 13 days. After 25 days, symptoms extended to petioles which were partially killed. After 40 days, partial defoliation became prominent and entire plants dried within 50 days (Table 1).

The disease symptoms were attributed to the severe stress caused by the pathogens to the water hyacinth plants, which affected the ability of the mature plants to produce fresh leaves and daughter plants. There was a positive correlation between leaf susceptibility and age. The first leaf was more susceptible than the second, third or fourth leaves. Overall result of Bonferroni mean separation revealed that, at the end of 50 days of inoculation by *Alternaria* sp. revealed to have the highest affected area followed by *Cercospora* sp. *Aspergillus* sp. and *Trichoderma* sp. (Table 2).

Table 1. Different stages of disease development on water hyacinth inoculated with *Alternaria alternata* and *Cercospora rodmanii*

Days after inoculation	Description of symptoms
1	No appreciable symptoms
3	Chlorotic flecks
6	Lesions with some necrosis on upper surface of leaves
8	Chlorotic lesions with varying degrees of necrosis
13	Enlarged lesions of deep yellow color
19	Excessive lesions with brown color
30	Lesions coalesce and form blight in many areas of leaves
40	Petioles and stolons infected
50	Plants parts dried and died

Table 2. Mean infected leaf area after 50 days of inoculation in the field

Fungus sp.	Mean infected leaf area (cm^2)
Alternaria alternate	1138.24[a]
Cercospora rodmanii	1047.13[b]
Aspergillus sp.	844.90[c]
Trichoderma sp.	72.03[d]
Control	27.73[e]



Figure 1-Disease development with *Alternaria alternata* on water hyacinth plants

After 50 days of inoculation, death of the outer and many inner leaves was evident throughout the treated area in the field except for *Trichoderma* sp. Shabana (1997) reported that, the degree of disease infection increased with increasing incubation period and gave maximum severity after 30 days of incubation. This occurred along with a decrease in chlorophyll content. Similarly other field studies also confirmed that the use of potential fungal pathogens would result in reduced water hyacinth biomass (Martyn and Freeman, 1978; Charudattan et al., 1985; Shabana et al., 1995). Firehun Yirefu et al. (2017) have found that among the fungal pathogens, *A. alternata, A. tenuissima, F. oxysporum*, *F. equiseti*, and *Neofisicoccum parvum* were highly pathogenic to water hyacinth.



1st day **3rd day** **19thday** **30th day** **50th day**

Figure 2-Disease development with *Cercospora rodmanii* on water hyacinth plants

In these studies disease started as small necrotic spots and developed into a leaf blight that spread over the leaf. Finally, these spots enlarged 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 appeared giving a target board appearance. The fruiting bodies of the fungus were also apparent on the upper surface along these concentric rings. Similarly, different fungal disease symptoms were found in association with water hyacinth as zonate leaf spots, discrete necrotic foliar spots, necrotic spots and blighting of leaves.

Shabana (1997) reported significant physiological changes in water hyacinth infected with *Alternaria* sp., such as decrease in pigments, carbohydrates, and relative water content which negatively affect the growth and development of the weed.Julian et al. (2001) also indicated that potential fungal pathogens could reduce plant growth rate and production of new leaves and new stolons. Moreover, increased severity results in rotting of the lower petioles, water logging of the crown and gradual sinking of the plant. Abbas et al. (1995) and Mailu et al. (1998) also reported that biological control agents (either insects or pathogens) caused disintegration of original water hyacinth mats into smaller ones; stunted growth; decline in water hyacinth biomass, reduced flowering potential, reduced daughter plant production and finally rotting of the petioles followed by sinking. Disease development was also noticed on uninoculated water hyacinth grown in neighboring areas of the test field.

However, disease symptoms were not observed on any of tested economically important plants at seedling and flowering stage. Therefore, the three fungi including A. alternata, *Aspergillus* sp. and *C. rodmanii* appear to be specific to water hyacinth. Similar results are reported by Firehun Yirefu et al. (2017) where *Alternaria alternata* and A. *tenuissima* did not cause disease symptoms on ecologically important plant species (e.g. Noug, Tef, and Coffee) in the Rift Valley of Ethiopia.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that, all isolated pathogens had the potential to control water hyacinth, but *Alternaria alternata* and *Cercospora rodmanii* were found to be better virulent candidates under the field conditions. Further studies on performance evaluation under natural environmental conditions and their host specificity tests are needed.

REFERENCES

Abbas HK, Duke SO, Paul RN, Riley RT, Tanaka T (1995). AAL-toxin, a potent natural herbicide which disrupts sphingolipid metabolism of plants. *Pestic. Sci., 43(3):* 181-187.

Center TD, Julien MH, Hill MP, Jianqing D (2001). Biological and Integrated Control of Water Hyacinth, *Eichhornia crassipes. In: Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth, Beijing, China, 9–12 October 2001,* 102: 152.

Charudattan R (2001). Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. *Bio Control*, 46(2) 229-260.

Charudattan R, Kluepfel BM, Linda S, Osman YA (1985). Biocontrol efficacy of *Cercospora rodmanii*on water hyacinth. *Phytopathology*, (75) 1263-1269.

Firehun Yirefu C. Struik EA. Lantinga, Taye Tessema (2017). Occurrence and diversity of fungal pathogens associated with water hyacinth and their potential as biocontrol agents in the Rift Valley of Ethiopia. *International Journal of Pest Management*, 63(4): 355-363.

Gebregiorgis FY, Struik PC, Lantinga EA, Taye T. (2013). Joint use of insects and fungal pathogens in the management of waterhyacinth (*Eichhornia crassipes*): Perspectives for Ethiopia. *Journal of Aquatic Plant Management*, 51: 109 – 121.

Julien MH, Griffiths MW, Stanley JN (2001). Biological control of water hyacinth: The moths *Niphograpta albiguttalis* and *Xubidain fusellus*: biologies, host ranges, rearing, releasing and monitoring techniques. *ACIAR Monograph*, pp. 79-91.

Harry C. Evans (1999). Biological control of weed and insect pests using fungal pathogens with particular reference to Sri Lanka. *Biocontrol news and Information*, (20) 63N-68N.

Holm, LG, Plucknett DL, Pancho J V and Herberger JP (1977). The World's *Worst Weeds: Distribution and Biology.* University Press, Hawaii, Honolulu, H. I. 609pp.

Howard GW, Matindi SW (2003). Alien Invasive Species in Africa's Wetlands. Some threats and solutions. IUCN Eastern African Regional Program, Nairobi, Kenya, February, 2003.

Mailu AM, Ochiel GRS, Gitonga W, Njoka SW (1998). Water hyacinth: an environmental disaster in the Winam Gulf of Lake Victoria and its control. In: Proc.Ist IOBC Water Hyacinth Working Group 101, Kenya Agricultural Research Institute (KARI), Nairobi, Kenya.

Martyn RD, Freeman TE (1978). Evaluation of *Acremonium zonatum* as a potential biocontrol agent of water hyacinth. *Plant Dis. Rep.*, 62: 604-608.

Shabana YM (1997). Vegetable oil suspension emulsions for formulating the weed pathogen (*Alternaria eichhorniae*) to bypass *dew. ZeitschriftfürPflanzenkrankheiten und Pflanzenschutz,* (1040) 239-245.

Shabana YM, Charudattan R, Elwakil MA (1995). Identification of pathogenicity and safety of *Alternaria eichhorniae* from Egypt as a bioherbicide agent for water hyacinth. *Biol. Control*, (5) 123-135.

Sutton GF, Compten SG, Coetzee JA (2016). Naturally occurring phytopathogens enhance biological control of water hyacinth (*Eichhornia crassipes*) by Megamelus scutellaris (Hemiptera: Delphacidae), even in eutrophic water. *Biological Control*, (103): 261-268.

Vincent AC (2001) *Cercospora piaropi and Myrothecium roridum as potential bioherbicides to control water hyacinth, Eichhornia crassipes*. Thesis, University of Florida, USA.