EFFECT OF MYCOHERBICIDES ON WATERHYACINTH [*EICHHORNIA crassipes* MART. SOLMS]

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ABSTRACT: One of the major problem accompanying water resource development in Sri Lanka is the explosive proliferation of water hyacinths (*Eichhornia crassipes*). A survey of plant pathogenic fungi associated with naturally infected water hyacinth was conducted in different waterways in the Eastern part of Sri Lanka and potential isolates including *Alternaria alternata, Cercospora rodmanii*, *Aspergillus sp*.and *Trichoderma sp.* were identified. The four identified fungi were evaluated for their pathogenicity on water hyacinth at laboratory conditions. The first pathogenicity trial indicates, 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, all four fungal species had similar level of effects in term of dead lesion formed in leaves after 22 days of inoculation. During the confirmation test, observed diseased symptoms also similar to first laboratory trial which confirmed that the disease syndromes of first lab trial were caused by the inoculated pathogenic fungi, not from the other factors and also indicate that, after one week of inoculation *A.alternata* revealed to have the higher affected area followed by *C rodmanii* and *Aspergillus sp*. and the *Trichoderma sp.* did not give a significant effect on confirmation test. The selected three pathogenic fungi including *Alternaria sp.*, *Aspergillus sp.*, and *Cercospora sp.* can easily culture and disease can cause by conidia, mycelial fragment or mycoherbicidal preparation. Different carriers produced different levels of affected leaf area upon infection by the fungal type ($p<0.0001$). However, fungal types alone did not show differences in the formation of dead lesions in the leaf surfaces of water hyacinth ($p=0.31$). Moreover, the carrier type and fungi do not combine to influence the overall area affected by the fungus ($p=0.49$). A better and quick infection caused by the mycoherbicidal preparation with oven ash. The extent of infection depends on the concentration of mycoherbicide and the 60% of oven ash concentration showed better result on water hyacinth control. We concluded that, *A. alternata* and *Cercospora rodmanii* could be used as effective biocontrol agents against water hyacinth following performance evaluation under natural environmental conditions and their host specificity test.

Keywords: Water hyacinth, Pathogenic fungi, Bio control, mycoherbicide

1.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

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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.

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 C Evans, 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.

In recent years, attention has centered on biological control, which could provide a cost-effective environmentally safe, solution to the water hyacinth problem (Charudattan, R. and Dinoor, A. et al., 2000) and most emphasis has been given to fungal pathogens as biocontrol agents (Vincent, 2001).

So, this study's objective was to identify and evaluate potential pathogens associated with water hyacinth to use as biocontrol agents in laboratory conditions.

2.METHODOLOGY

The laboratory trials were carried out during 2016 at Department of biological science, Faculty of Applied Sciences, South Eastern University of Sri Lanka to evaluate the pathogenicity of selected fungi to control the water hyacinth.

2.1 Surveying diseased water hyacinth

A survey was conducted to document various fungal pathogens of waterhyacinth in the water bodies of Batticaloa and Ampara districts 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.

2.2 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.

2.3 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

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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 then 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.

2.4 Confirmation tests of pathogenicity

The diseased leaves of pathogenicity tests were collected for each treatment. Associated fungi on the collected leaves were isolated and pure cultures were prepared. The isolated fungi from the confirmation test were inoculated to young healthy plants which are grown hydroponically and the inoculated plants were incubated for 5 days. After inoculation, the diseased symptoms that were appeared in the pathogenicity test were confirmed and infected area of leaves was measured from 2 to 7 days.

2.5 Formulation of the pathogenic fungi for application as mycoherbicides

Isolates of 3 selected pathogenic fungi were grown in 250ml Erlenmeyer flasks, each containing 100ml of potato dextrose broth. The PH was adjusted to 8 after treatment with sterile 1N NaOH. After incubating the cultures at 25° C for 3 weeks, mycelial mats were filtered for each isolates. The fungal preparations were obtained by air – dried (48 hrs.) and ground by motor and pestle to get fine powder. Corn flour and oven ash were added to each treatment (30% and 60% w/w concentrations). Two replicates were conducted of each treatment and the control treatment was maintained with no additives and fungi. Treatments were water hyacinth controls, and plants were sprayed with; a fresh suspension of fungal spores and mycelium fragments; corn flour suspension; oven ash suspension; a mixture of corn flour and oven ash. One –half of each formulation was added to 20 ml of sterile water and sprayed on healthy water hyacinth plants at the 3-4 leaf stage. Treated plants were covered with polythene bags for 5-10 days after inoculation. Finally, the infected areas of the diseased leaves were determined from 2 to 14 days.

2.6 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.

3 RESULTS AND DISCUSSION

3.1 Results of Pathogenicity tests

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Alternaria alternata, Cercospora rodmanii, Aspergillus sp. and *Trichoderma sp.* were isolated on PDA from field collected, diseased waterhyacinth. 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). 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 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.

After seven days of inoculation, *Alternaria sp.* revealed to have the highest affected area, followed by *Aspergillus sp*. and *Cercospora sp.* (Table 2). 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 (Table 2).

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

Table 2. Mean leaf area affected by fungi after varying days of inoculation.

Fungal type	Mean leaf area affected (mm^2)						
	D2	D3	D4	D5	D6	D7	D22
Alternaria sp.	0.61^{bc}	4.95^{a}	7.94^{a}	12.06^{a}	18.28^{a}	22.34^{a}	31.43^{a}
Aspergillus sp.	1.58^{ab}	4.24^{a}	7.60^{a}	9.49^{ab}	11.45^{b}	14.47^{b}	28.70^{a}
Trichoderma sp.	0.31^{c}	0.57^{b}	1.57^{b}	2.12^{c}	2.75^{c}	3.13^{c}	26.12^{a}
Cercospora sp.	2.18^{a}	4.30^{a}	6.53^{a}	8.38^{b}	11.46^{b}	14.04^{b}	25.85^{a}

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Mean values with same superscript letters in each column are not significantly different from each other at α=0.05).

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. 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.

3.2 Results of Confirmation test

The same four fungi including *A. alternata, Aspergillus sp., Trichoderma sp.* and *C. rodmanii* were isolated on PDA from the leaf samples collected from first trial and observed diseased symptoms also similar to first pathogenicity tests (Figure 1 and Figure 2) which confirmed that the disease syndromes were caused by the inoculated pathogenic fungi, not from the other factors.

Repeated measures ANOVA revealed that intensity effect was significant. Therefore, there is an effect of time. The results further demonstrated that there is a significant effect of fungal types on the leaf area affected by each fungus upon inoculation (F=0.002). Moreover, within-subject main effect for intensity was also statistically significant (p<0.0001). Interaction between fungal type and intensity was also found to be significant at p value of 0.05.

Table 3. Mean leaf area affected by fungi after varying days of inoculation.

Fungal type	Mean leaf area affected(mm^2)					
	D2	D3	D4	D5	D6	D7
Alternaria sp.	8.09^{a}	14.97^{a}	19.36^{a}	39.67^{a}	59.77^{a}	72.39^{a}
Aspergillus sp.	6.91^{a}	11.79^{a}	13.83^{a}	21.81^{ab}	27.81^{bc}	35.53^{bc}
Trichoderma sp.	0.00^{b}	0.00^{b}	2.73^{b}	5.03^{b}	6.84^{c}	9.46^{c}
Cercospora sp.	5.33^{a}	11.94^{a}	14.02^{a}	28.13^{a}	37.66^{ab}	48.54^{ab}

Mean values with same superscript letters in each column are not significantly different from each other at α=0.05).

The disease symptoms started to appear within 2 days on waterhyacinth leaves inoculated with the *A. alternata*, *Aspergillus sp.* and *C. rodmanii*. The plants which were inoculated with the *Trichoderma sp.* started to appear disease symptoms after 4 days of inoculation (Table 3).

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Within 4days the fungi including *A.alternaria*, *Aspergillus sp.* and *C.rodmanii* did not showed significant different on pathogenicity but after 4 days *A.alternata* and *C.rodmanii* started to appear significant effect than other 2 fungi. After one week of inoculation *A. alternata* revealed to have the higher affected area followed by *C. rodmanii* and *Aspergillus sp.* (Table 3).

The plants inoculated with the *Alternaria alternata* dried and undergo defoliation within one week and the *Trichoderma sp.* did not give a significant effect on confirmation test.

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

Figure 1. Disease symptoms of first trial on waterhyacinth after one week of inoculation. (a) Control plant (b) Infected plant with Alternaria alternata (c) Infected plant with Cercospora rodmanii (d) Infected plant with Aspergillus sp. (e) Infected plant with Trichoderma sp.

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

Figure 2. Disease symptoms of confirmation test on waterhyacinth after one week of inoculation. (a) Control plant (b) Infected plant with Alternaria alternata (c) Infected plant with Cercospora rodmanii (d) Infected plant with Aspergillus sp. (e) Infected plant with Trichoderma sp.

Charudattan (2001) reported that *Alternaria sp*. was highly virulent and severely damaged the inoculated water hyacinth leaves. Moreover, the potential of *A. alternata* as biocontrol agent was reported by Abbas et al., (1995).

Some studies confirmed that, studies use of potential fungal pathogens would result in reduced water hyacinth biomass (Martyn and Freeman, 1978; Charudattan et al., 1985; Shabana et al., 1995). On the other hand, Shabana (1997) reported significant physiological changes in water hyacinth infected

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with *Alternaria sp*., such as decrease in pigments, carbohydrates, and relative water content which negatively affect the growth and development of the weed. Abbas et al. (1995) and Mailu et al. (1998) reported that biological control agents (either insects or pathogens) caused disintegration of original water hyacinth mats into smaller mats; stunted growth; decline in water hyacinth biomass, reduced flowering potential, reduced daughter plants production and finally rotting of the petioles followed by sinking.

3.3 Results of mycoherbicide formulation test

The leaf area affected by the fungal pathogen changes with the intensity (days after inoculation). Further, the changes in leaf area affected by the pathogen across intensity levels also depend upon the effects of the carrier type used as it has an associated p value less than 0.05 (Table 4). However, the leaf area affected does not depend on the intensity in conjunction with the fungal type (p=0.06). The results showed that there was a significant interaction between the intensity, carrier type and fungal type (Table 4).

Table 4. Within-subjects main effect test for micro-herbicide test.

Effect category	Wilks' Lambda	
	F value	P
Intensity	58.20	<0.0001
Intensity*Inoculation method	2.07	0.01
Intensity*Fungal type	2.04	0.06
Intensity*Inoculation method*Fungal type	1.76	0.01

Between subject effects of repeated measures ANOVA revealed that there was a statistically significant effect of the type of carrier used and therefore different carriers produced different levels of affected leaf area upon infection by the fungal type (p<0.0001). However, fungal types alone did not show differences in the formation of dead lesions in the leaf surfaces of water hyacinth (p=0.31). Moreover, the carrier type and fungi did not combine to influence the overall area affected by the fungus (p=0.49).

Table 5. Mean leaf area affected by different carrier type for each fungus after varying days of inoculation (as revealed by Bonferroni t-tests).

Carrier type	Mean leaf area affected(mm^2)						
	D2	D3	D4	D5	D6	D7	D14
Corn flour 30%	0.00^{c}	0.00^{c}	2.30^{c}	5.61^{c}	8.40^{b}	11.36^{b}	17.87^{b}
Oven ash 30%	5.18^{b}	8.97^{b}	13.17^{b}	17.49^{b}	21.33^{b}	27.25^{b}	43.94^{b}
Cornflour+Ash30%	0.00^{c}	3.08^{bc}	5.85^{bc}	9.44^{bc}	12.99^{b}	18.34^{b}	35.50^{b}
Corn flour 60%	0.00^{c}	2.20b^{c}	6.68^{bc}	10.63^{bc}	14.25^{b}	18.70^{b}	35.90^{b}
Oven ash 60%	12.70^{a}	22.64^{a}	34.46^{a}	43.63^{a}	57.00^{a}	71.07^{a}	111.94^{a}
Cornflour+Ash60%	3.10b^{c}	6.08b^{c}	7.98^{bc}	11.29^{bc}	14.92^{b}	18.46^{b}	41.63^{b}

Mean values with same superscript letters in each column are not significantly different from each other at α=0.05).

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The results indicate that oven ash 60% prominently stays significantly different from other carrier types contributing appreciably to the lesion formation by fungi. This pattern of lesion formation or dead leaves by oven ash 60% found to be repeating over the intensity (days after inoculation) (Table 5). Six days after inoculation, oven ash 60% produced 57.00 mm^2 dead lesion formations, which is significantly different from the rest. The other carrier types produced lesions in the range of 8.40-21.33 mm^2, all of which did not differ from each other. Similar pattern was observed after seven and 14 days after inoculation though lesion formation was increasing over time (Table 5). The oven ash 60% produced 111.94 mm^2 of dead lesion formation after 14 days of inoculation and this stays significantly different from other carrier types after the same days of inoculation (Table 5). 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.

CONCLUSIONS

The attack of the pathogens resulted in killed plants as time progressed and higher level of pathogenicity exhibited by *A. alternata* followed by *C. rodmanii* and *Aspergillus sp.* In the formulation test, *A. alternata* and *C. rodmanii* have the best potential to kill water hyacinth. A better and quick infection caused by the mycoherbicidal preparation with oven ash and the extent of infection depends on the concentration of mycoherbicide and the 60% of oven ash concentration showed better result than other mycoherbicide preparations.

The study suggests that, among the four pathogenic fungi *Altrnaria alternata* and *Cercospora rodmanii* could be used as effective biocontrol agents against water hyacinth following performance evaluation under natural environmental conditions and their host specificity test before large scale biological control programmes.

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