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FUNGAL PATHOGENS ASSOCIATED WITH WATER HYACINTH (Eichhornia crassipes Mart.) AND THEIR POTENTIAL AS BIOLOGICAL CONTROL AGENTS

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By

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Water hyacinth, (*Eichhornia crassipes* Mart.), is an aquatic weed in South America, Europe and Asia. Water hyacinth has been identified as an aggressive invasive aquatic weed that could double up its population in two-weeks-time, making it detrimental once established. Water hyacinths form dense and thick mat across the water surface making it limited for sunlight to penetrate which made aquatic organisms deprived of sunlight. Water hyacinth also disrupts human activities such as in rice cultivation area, irrigation, water drainage, fishing for living and also recreational activities. A number of control methods for water hyacinth are currently implemented such as legislative, chemical and mechanical methods, but these methods are expensive, labor intensive and still give unsatisfactory result. Biological control method is a promising and eco-friendly method that is used to manage a number of pests, including invasive weeds. Several studies have been evaluating potential fungal pathogen and the effectiveness of fungal pathogens as mycoherbicides in suppressing water hyacinth populations, but the mycoherbicides developed are still in research stage. One of the aims of this study is to evaluate pathogenicity of fungal isolates obtained from diseased water hyacinth in Malaysia. 82 fungal isolates were obtained from five sampling locations, Sg. Limau (Kedah), Tasik Seri Serdang (Selangor), Kolam Seri Melor (Selangor), Tanjung Karang (Selangor) and Taman Tasik Cempaka Bangi (Selangor). Twenty-one fungal isolates grew full plate in 5 days of incubation and categorized as fast-growing fungi. Among the 21 fungal isolates, 12 isolates were found to be pathogenic to water hyacinth in detached leaf assay. Isolates derived from Sg. Limau and Tanjung Karang (D1 and D3) were found to be severely pathogenic (showing >80% incidence) to water hyacinth. Eight isolates that showed ≥ 50% disease incidence in detached leaf assay were subjected to whole plant bioassay for preliminary screening. All pathogenic isolates tested in whole plant bioassay showed significant differences from untreated water hyacinth except for isolate B2, *Diaporthe drenthii* sampled from Tasik Sri Serdang. Isolate D3, derived from Tanjung Karang, showed the highest percentage of disease incidence with means of 51.58% during the evaluation period. Both morphological and molecular analyses showed that the fungal isolates belonged to seven genera including *Curvularia lunata*, *Epicoccum sorghinum*,

Diaporthe drenthii., *Colletotrichum siamense*, *Xylaria* sp., *Myrothecium roridum* and *Mycoleptodiscus terrestris*. This study provides an updated information on fungal isolates and identification of potential fungal pathogens associated with water hyacinth in Malaysia. Fungal isolates that were effective in suppressing water hyacinth, not pathogenic to paddy plants and not pathogenic to economically important crops were selected for assessment of disease severity. The selected isolates were *Myrothecium roridum, Mycoleptodiscus terrestris* and *Xylaria* sp. *Myrothecium roridum* was the most virulent and induced disease symptoms as lesions on leaves three days after inoculation. It also showed the highest disease severity (10.2%) and disease incidence (36.3%). This study developed *Myrothecium roridum* wettable powder formulation as mycoherbicide and evaluated the efficacy of the wettable powder formulation for controlling water hyacinth in glasshouse condition. *Myrothecium roridum* was selected as the active ingredient in wettable powder mycoherbicide formulation. The components of the wettable powder formulation in this study were active ingredient (40%), surfactants (10%), UV protectant (3.64%) and carrier (46.36%). Surfactants used were sodium lignosulfonate (Lig), sodium polyacrylate (PAAS), ELTESOL®SC (SC) and sodium naphthalene sulfonate (SNS). Surfactants used in the formulation did not inhibit the growth of *M. roridum*. Lig which took up the most percentage in the surfactant system had no inhibition towards *M. roridum*. Two different surfactant combination were incorporated in the formulations, Lig:PAAS:SC and Lig:PAAS:SNS with a ratio of 52:33:15. SC formulation had higher performance enhancement for individual physical tests but both formulations were graded as B. *In vitro* test showed viability of *M. roridum* growth for both SNS and SC formulation. SNS formulation on potato dextrose agar PDA media developed higher colony forming unit (cfu/mL), 8.1 $\times 10^3$ cfu/mL and fast sporulation as compared to SC formulation with 3.6 $\times 10^3$ cfu/mL. Disease severity in detached leaf assay when treated with SNS formulation was higher than non-inoculated treatment while SC formulation showed no difference with non-inoculated and SNS formulation. In glasshouse experiment, SNS formulation showed less control efficacy in controlling water hyacinth compared to non-formulated *M. roridum* for disease incidence (DI) and disease severity (DS) after 10 days of inoculation.

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KULAT PATOGENIK KELADI BUNTING (*Eichhornia crassipes* Mart.) DAN POTENSINYA SEBAGAI AGEN KAWALAN BIOLOGI

Oleh

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Keladi bunting, (*Eichhornia crassipes* Mart.), adalah rumpai akuatik di Amerika Selatan, Eropah dan Asia. Keladi bunting merupakan sejenis rumpai akuatik yang agresif dan invasif yang mampu melipatgandakan populasinya dalam tempoh dua minggu. Pembentukan jaringan stolon keladi bunting yang tebal diatas permukaan air menyebabkan jumlah cahaya matahari kurang menembusi permukaan air dan menyebabkan organisma akuatik kurang menerima cahaya matahari. Keladi bunting juga mengganggu aktiviti manusia sebagai contoh di kawasan penanaman padi, pengairan, perparitan, aktiviti menangkap ikan dan aktiviti rekreasi. Beberapa usaha pembasmian keladi bunting termasuk secara kawalan legislatif, kimia dan mekanisasi telah dilakukan namun tidak dapat membasmi keladi bunting secara sepenuhnya. Kawalan secara biologi merupakan kaedah kawalan yang kos efektif dan mesra alam dan mampu untuk mengawal sebilangan perosak termasuk rumpai invasif. Kajian telah banyak dijalankan bagi menilai keberkesanan kulat pathogen sebagai racun rumpai berasaskan kulat bagi mengawal populasi keladi bunting. Kajian ini mempunyai objektif untuk menjalankan ujian patogenisiti terhadap kulat yang dipencilkan dari keladi bunting. Sebanyak 82 pencilan kulat telah diperolehi dari lima lokasi pensampelan iaitu dari Sg. Limau (Kedah), Tasik Seri Serdang (Selangor), Kolam Seri Melor (Selangor), Tanjung Karang (Selangor) dan Taman Tasik Cempaka Bangi (Selangor). Dua puluh satu pencilan kulat telah dapat memenuhi piring petri dalam masa lima hari inkubasi. Dua belas pencilan daripada 21 pencilan kulat mempunyai kepatogenan terhadap keladi bunting dalam ujian helaian daun. Pencilan dari Sg. Limau dan Tanjung Karang (D1 dan D3) merupakan pencilan yang mempunyai kepatogenan paling tinggi (lebih dari 80% kejadian penyakit) terhadap keladi bunting. Lapan pencilan kulat yang menunjukkan ≥50% kejadian penyakit dalam ujian helaian daun telah dipilih bagi ujian inokulasi pokok keladi bunting bagi penyaringan awal. Semua pencilan kulat menunjukkan kepatogenan kecuali satu pencilan, B2 *Diaporthe drenthii* dari Tasik Sri Serdang. Pencilan D3 dari Tanjung Karang menunjukkan peratus kejadian penyakit paling tinggi (51.58%). Identifikasi melalui sifat morfologi dan pencirian secara molekul menunjukkan bahawa pencilan kulat merupakan *Curvularia lunata, Epicoccum sorghinum, Diaporthe drenthii., Colletotrichum siamense, Xylaria* sp., *Myrothecium roridum* dan *Mycoleptodiscus*

terrestris. Kajian ini memberikan maklumat terkini tentang isolasi dan identifikasi kulat patogen yang dipencilkan dari keladi bunting di Makaysia. Pencilan kulat yang berkesan dalam mengawal keladi bunting, tidak patogenik terhadap padi dan tanaman penting ekonomi telah dipilih bagi penilaian tahap keseriusan penyakit. Pencilan kulat yang dipilih adalah *Xylaria* sp., *Myrothecium roridum* dan *Mycoleptodiscus terrestris. Myrothecium roridum* merupakan kulat paling virulen dan mengakibatkan simptom dalam bentuk lesi pada daun dalam tempoh tiga hari selepas inokulasi. Tahap keseriusan (10.2%) kejadian penyakit (36.3%) oleh *Myrothecium roridum* juga adalah paling tinggi. Kajian ini telah membangunkan formulasi serbuk boleh basah menggunakan kulat *Myrothecium roridum* sebagai mikoherbisid dan menilai keberkesanan serbuk boleh basah tersebut dalam mengawal keladi bunting di rumah kaca. *Myrothecium roridum* dipilih bagi menjadi bahan aktif dalam formulasi serbuk boleh basah. Komponen serbuk boleh basah dalam kajian ini terdiri dari bahan aktif (40%), surfaktan (10%), perintang sinaran UV (3.64%) dan bahan lengai (46.36%). Surfaktan yang digunakan adalah sodium lignosulfonate (Lig), sodium polyacrylate (PAAS), ELTESOL®SC (SC) dan sodium naphthalene sulfonate (SNS). Surfaktan yang digunakan tidak menghalang pertumbuhan miselium *M. roridum*. Dua kombinasi surfaktan berbeza telah digunakan dalam formulasi tersebut iaitu, Lig:PAAS:SC and Lig:PAAS:SNS dengan nisbah 52:33:15. Formulasi tersebut dinamakan sebagai SC dan SNS mengikut kombinasi surfaktan. Formulasi SC menunjukkan peningkatan prestasi yang lebih tinggi dalam ujian fizikal secara individu namun kedua dua formulasi diberikan grade B. Ujian secara *in vitro* menunjukkan kemandirian *M. roridum* dalam formulasi SC dan SNS. *M. roridum* mempunyai formasi koloni yang lebih banyak diatas kultur PDA dari semburan formulasi SNS 8.1×10^3 cfu/mL berbanding dari semburan formulasi SC 3.6×10^3 cfu/mL. Tahap keseriusan atas helaian daun oleh semburan formulasi SNS lebih tinggi dari daun kawalan yang tidak mempunyai inokulat manakala semburan formulasi SC tiada perbezaan dengan kawalan dan formulasi SNS. Dalam ujian keadaan rumah kaca, formulasi SNS tidak dapat mengawal keladi bunting dibandingkan dengan *M. roridum* yang tidak diformulasi dari segi kejadian penyakit dan tahap keseriusan.

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LIST OF ABBREVIATIONS

°C	degree celcius
µ	micro
µm	micrometer
µL	microlitre
ANOVA	analysis of variance
BLAST	basic local alignment system tool
CAL	Calmodulin
CFU	colony forming unit
CRD	completely randomized design
ddH2O	double distilled water
DI	disease incidence
DNA	deoxyribonucleic acid
DS	disease severity
EDTA	Ethylenediamine tetraacetic acid
g	gram
ITS	internal transcribed spacer
kb	kilo base pair
L	litre
Lig	sodium lignosulfonate
LSD	least significant difference
mg	miligram
mL	milliliter
NCBI	National Center for Biotechnology Information
PAAS	sodium polyacrylate
PCR	polymerase chain reaction

PDA	potato dextrose agar
PDB	potato dextrose broth
SC	ELTESOL®SC
SE	standard error
SNS	sodium naphthalene sulfonate
TBE	tris base ethylenediaminetetraacetic acid
UV	Ultra violet

CHAPTER 1

INTRODUCTION

Eichhornia crassipes Mart., also known as water hyacinth of family Pontederiaceae is a major freshwater weed in most part of the world that is frost free (Dagno et al. 2012). This weed originates from tropical South America. In Malaysia, water hyacinth has been identified as one of the most aggressive weed species. Water hyacinth thrives in water systems such as ponds, lakes and rivers. The rate of growth is vigorous where the water hyacinth population could cover the entire water system bodies in a short time, therefore, causing it to be a hard to control type of weed (Téllez et al., 2008).

Water hyacinth in Malaysia was introduced in the early 1900s. Water hyacinth was brought in as, but later on being discarded improperly causing the problem to arise (Mohd. Shariff and Abu Bakar, 2006).

To date, there is no official hectarage of water hyacinth infestation in Malaysia. Information regarding areas of infestation can only be obtained through published studies, personal reports and news reports across Malaysia. Even then, latest published study on water hyacinth distribution across Malaysia can only be found up to 1996 (Bakar, 2004). Areas that were found to be infested with water hyacinth from this study were:

1. Selangor: Taman Tasik Seri Serdang, Taman Tasik Cempaka Bangi, Kolam Sri Melor Kajang and Tanjung karang
2. Kedah: Sungai Limau

Other published studies, personal communication and news reports include areas such as:

Area of water hyacinth infestation	Reference
Perlis: Sungai Perlis, Sungai Aran,	Mansor, 1996
Kedah: Sg Padang Terap, Sungai Kedah, Sungai Muda, Sungai Jarak	Mansor, 1996
Penang:,Chenderoh, Sungai Pinang	Mansor, 1996
Perak: Bukit Merah, Sungai Kerian, Sungai Kuran, Sungai Beruas, Sungai Perak, Sungai Bernam (in between Perak and Selangor), Taman Tasik Taiping, Tanjung Tualang, Teluk Intan	Mansor, 1996; Che Lah, 2019; Salleh 2020; Tanzizi 2021
Selangor: Lake Aman, Pandamaran, Sungai Tinggi, Sungai Selangor, Sungai Kelang, Sungai Langat, Sungai Sepang	Mansor, 1996; Chan, 2018; MyBIS, August 2, 2021
Negeri Sembilan: Sungai Linggi	Mansor, 1996
Melaka: Sungai Melaka, Taman Semabok Perdana	Mansor, 1996; Murali, 2020

Johor: Sungai Muar, Sungai Benut, Sungai Pulai, Sungai Johor	Mansor, 1996
Pahang: Beserah, Lake Ringlet, Sungai Pahang	Mansor, 1996; M. A. Masran (personal communication, April 30, 2017)
Terengganu: Sungai Besut	Mansor, 1996
Kelantan: Sungai Kelantan, Tumpat	Mansor, 1996; Idris, 2020
Sarawak: Sungai Sarawak	Mansor, 1996
Sabah: Sungai Papar	Mansor, 1996; MyBIS, August 2, 2021

A few methods had been taken to control infestation of water hyacinth such as legislative, chemical and mechanical but still give unsatisfactory results. Mechanical control of water hyacinth such as physical removal is not practical in large scale weed control programs (Department of Ecology State of Washington, 2016). Mechanical control has been the most commonly used method in Malaysia to remove water hyacinth from water bodies. However, this method could be temporary acting as seeds of water hyacinth that has already been dispersed are still in the area and will be able to cause another infestation. Chemical controls are not only temporary acting, but also expensive way to control weeds in long term. Biological control can be done by release of insects or using fungal pathogens. Fungal pathogens have shown positive result outlook in studies done in other countries such as Egypt and Pakistan. The pathogen found in the studies were found to have the possibility of being developed into mycoherbicide. Since there are limitations and possibilities of legislative problem on importing fungal pathogen from other countries, it is a good measure to find and identify indigenous potential pathogen and formulate it into effective mycoherbicide. Commercialized mycoherbicide targeting water hyacinth are still in development. One of the two is still in development but has been registered which is mycoherbicide formulation containing *Cercospora rodmanii* specifically for controlling water hyacinth by Abbot Laboratories and another one already developed but not yet commercialized because of its wide host range is called Hyakill™. Hyakill™ contains *Sclerotinia sclerotiorum* (Awasthi and Mishra, 2020).

Many studies have been conducted to identify potential of fungal pathogens as biological control agent of water hyacinth and it has a future outlook thus further study needs to be conducted (Charudattan, 2001). Most of the studies conducted focuses on potential of the water hyacinth pathogen as the pathogen itself where pathogenicity, infectivity and identity of the pathogens have been researched upon. However, formulation and evaluation of formulation efficacy of said pathogens have a long way to be made available. Data on fungal pathogens that can be found in Malaysia has also been old and not renewed. Effective mycoherbicide on controlling water hyacinth have yet to be extensively available, making it an area that needs exploration. Thus, the objective of this research would be:

1) to isolate and identify potential fungal pathogens associated with water hyacinth;
2) to evaluate pathogenicity of fungal isolates obtained from diseased water hyacinth;

3) to develop *Myrothecium roridum* wettable powder formulation as mycoherbicide;
4) to evaluate efficacy of the *Myrothecium roridum* wettable powder formulation for controlling water hyacinth in *in vitro, in vivo* and glasshouse condition.

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