UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF ALLELOPATHIC NOXIOUS WEED SPECIES AS POTENTIAL BIOHERBICIDE FOR WEED CONTROL IN MALAYSIA

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FP 2022 45

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By

MST. MOTMAINNA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

August 2021

DEDICATION

This thesis is dedicated to
my beloved husband Md. Mahmudul Hasan
and my beloved parents Muhsin Ali Basunia & Roushan Ara Basunia
and mother-in-law Most. Gulshan Ara Begum

With love, respect and a bunch of memories
indeed, we belong to Allah and indeed to Him we will return.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

DETERMINATION OF ALLELOPATHIC NOXIOUS WEED SPECIES AS POTENTIAL BIOHERBICIDE FOR WEED CONTROL IN MALAYSIA

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Chairman : Professor Dato' Abdul Shukor bin Juraimi, PhD
Faculty : Agriculture

Continuous and indiscriminate use of synthetic herbicides has caused environmental pollution and the development of herbicide resistance weed. Allelopathic plants as natural herbicides could be a novel solution to reduce reliance on synthetic herbicides while also improving crop weed management. Noxious weeds have the potential to convey allelochemicals and influence the organisms around them. There is relatively little information available about the allelopathic activity of Malaysian noxious weed species. The discovery of unknown allelopathic noxious weeds in Malaysia could provide the way to develop new natural herbicides. In this regard, a variety of experiments were carried out in the lab and glasshouse to achieve the following goals; i) to assess the allelopathic activity of 30 noxious weed species in Malaysia ii) to determine the effects in laboratory and glasshouse; iii) to identify their allelopathic substance(s); iv) to determine the efficacy in comparison to the commercial herbicides, v) to evaluate the effect on physiological and biochemical changes of weeds. The experiment was conducted at the Seed Technology Laboratory, Crop Science, and Ladang 15, Universiti Putra Malaysia. A complete randomized design (CRD) with five replications and randomized complete block design (RCBD) with four replications were laid out for laboratory and glasshouse experiments. Experiment on the effects of methanolic extracts of 30-Malaysian noxious weed species (9 families) on the seeds survival rate and seedlings growth of Weedy rice (*Oryza sativa* f. *spontanea* Roshev**)** in the laboratory has been conducted. Five concentrations (6.25, 12.5, 25, 50 and 100 g L^{-1}) of extracts were compared with control (distilled water). *Parthenium hysterophorus* L., *Cleome rutidosperma* DC. and *Borreria alata* (Aubl.) DC. proved strongly allelopathic compared to the other tested extracts. The phytotoxic effects of *P. hysterophorus*, *C. rutidosperma* and *B. alata* extract concentrations were further investigated and compared on survival rate and growth of crops (*Zea mays* L., *Oryza sativa* L., *Abelmoschus esculentus* L. Moench, *Amaranthus gangeticus* L.) and weeds (weedy rice, *Echinochloa colona* L. Link., *Euphorbia hirta* L. and *Ageratum conyzoides* L.) under laboratory and glasshouse conditions. Test plants were less sensitive to *C. rutidosperma* and *B. alata* than *P. hysterophorus* extract in both conditions (lab and glasshouse).

Ageratum conyzoides, E. hirta, *A. esculentus* and *A. gangeticus* were mostly injured by *P. hysterophorus* extract at 100 g L^{-1} in the glasshouse. The LC-QTOF-MS/MS analysis has confirmed the presence of phenolic compounds (flavonoids, phenols, coumarins, carboxylic acids, benzoic acids), terpenoid, alkaloids, amino acids, fatty acids, piperazines, benzofuran, indole, amines, azoles, sulfonic acid and other unknown compounds in *P. hysterophorus, C. rutidosperma* and *B. alata*. The result indicated that methanol extract of *C. rutidosperma* and *B. alata* had fewer known phytotoxic substances than *P. hysterophorus*. A comparison study was conducted between *P. hysterophorus* extract (20, 40, and 80 g L^{-1}), synthetic herbicide (glyphosate and glufosinate-ammonium at 2 L ha^{-1}) as positive control and no treatment (negative control) on *A. conyzoides,* Weedy rice *and Cyperus iria.* No significant difference was obtained when *P. hysterophorus* extract (80 g L^{-1}) and synthetic herbicides (glyphosate and glufosinate-ammonium) were applied on *A. conyzoides.* The response of physiological and biochemical properties of *A. conyzoides,* Weedy rice *and C. iria* was also investigated by the foliar application of *P. hysterophorus* at different concentrations (20, 40 and 60 g L^{-1}). Physiological and biochemical properties of *A. conyzoides* were more sensitive to *P. hysterophorus,* especially at higher concentrations (60 g L^{-1}). These results confirmed that *P. hysterophorus* have significant herbicidal effects for weed control. Hence it has the potential to be used as a bioherbicide to control weed in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGENALPASTIAN SPESIS RUMPAI BAHAYA BERALELOPATI SEBAGAI BIOHERBISIDA YANG BERPOTENSI UNTUK KAWALAN RUMPAI DI MALAYSIA

Oleh

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Pengerusi : Profesor Dato' Abdul Shukor bin Juraimi, PhD
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Penggunaan racun rumpai sintetik yang berterusan dan sembarangan telah menyebabkan pencemaran alam sekitar dan pembentukan rumpai rintang herbisida. Tumbuhan alelopati sebagai racun herba semula jadi boleh menjadi penyelesaian baru untuk mengurangkan pergantungan pada racun rumpai sintetik di samping menambah baik pengurusan rumpai tanaman. Rumpai berbahaya mempunyai potensi untuk menyampaikan alelokimia dan mempengaruhi organisma di sekelilingnya. Terdapat sedikit maklumat yang tersedia tentang aktiviti alelopati spesies rumpai berbahaya Malaysia. Penemuan rumpai berbahaya alelopati yang tidak diketahui di Malaysia boleh menyediakan jalan untuk membangunkan racun herba semula jadi yang baharu. Beberapa eksperimen telah dijalankan di makmal dan rumah kaca untuk mencapai matlamat berikut; i) untuk menilai aktiviti alelopati 30 spesies rumpai berbahaya di Malaysia ii) untuk menentukan kesannya di makmal dan rumah kaca; iii) untuk mengenal pasti bahan alelopati rumpai tersebut; iv) untuk menentukan keberkesanan berbanding dengan racun rumpai komersial, v) untuk menilai kesan ke atas perubahan fisiologi dan biokimia rumpai. Eksperimen telah dijalankan di Makmal Teknologi Benih, Sains Tanaman, dan Ladang 15, Universiti Putra Malaysia. Reka bentuk rawak lengkap (CRD) dengan lima replikasi dan reka bentuk blok lengkap rawak (RCBD) dengan empat replikasi telah disusun atur untuk eksperimen makmal dan rumah kaca. Eksperimen terhadap kesan ekstrak metanol 30-spesies rumpai Malaysia yang berbahaya (9 famili) terhadap kadar kemandirian benih dan pertumbuhan anak benih padi angin (*Oryza sativa* f. *spontanea* Roshev) di makmal telah dijalankan. Lima kepekatan (6.25, 12.5, 25, 50 dan 100 g L^{-1}) ekstrak dibandingkan dengan kawalan (air suling). *Parthenium hysterophorus* L., *Cleome rutidosperma* DC. dan *Borreria alata* (Aubl.) DC. telah terbukti mempunyai alelopati yang kuat berbanding dengan ekstrak lain yang diuji. Kesan fitotoksik kepekatan ekstrak *P. hysterophorus*, *C. rutidosperma* dan *B. alata* telah disiasat selanjutnya dan dibandingkan dengan kadar survival dan pertumbuhan tanaman (*Zea mays* L., *Oryza sativa* L., *Abelmoschus esculentus* L. Moench, *Amaranthus gangeticus* L.) dan rumpai (padi angin, *Echinochloa colona* L. Link.,

Euphorbia hirta L. dan *Ageratum conyzoides* L.) di dalam suasana makmal dan rumah kaca. Tanaman-tanaman yang diuji didapati kurang sensitif kepada *C. rutidosperma* dan *B. alata* berbanding ekstrak *P. hysterophorus* dalam kedua-dua keadaan (makmal dan rumah kaca). *Ageratum conyzoides*, *E. hirta*, *A. esculentus* dan *A. gangeticus* kebanyakannya dapat dicederakan oleh ekstrak *P. hysterophorus* pada 100 g L^{-1} dalam rumah kaca. Analisis LC-QTOF-MS/MS telah mengesahkan kehadiran sebatian fenolik (flavonoid, fenol, kumarin, asid karboksilik, asid benzoik), terpenoid, alkaloid, asid amino, asid lemak, piperazine, benzofuran, indole, amina, azoles, asid sulfonik dan sebatian lain yang tidak diketahui dalam *P. hysterophorus*, *C. rutidosperma* dan *B. alata*. Keputusan tersebut menunjukkan bahawa ekstrak metanol *C. rutidosperma* dan *B. alata* mempunyai sedikit bahan fitotoksik yang diketahui berbanding *P. hysterophorus*. Kajian perbandingan telah dijalankan antara ekstrak *P. hysterophorus* (20, 40, dan 80 g L^{-1}), racun herba sintetik (glifosat dan glufosinate-ammonium pada kadar 2 L ha^{-1}) sebagai kawalan positif dan tiada rawatan (kawalan negatif) pada *A. conyzoides*, padi angin dan *Cyperus iria*. Tiada perbezaan ketara diperoleh apabila ekstrak P. *hysterophorus* (80 g L^{-1}) dan racun herba sintetik (glyphosate dan glufosinate-ammonium) diberikan pada *A. conyzoides*. Tindak balas sifat fisiologi dan biokimia *A. conyzoides*, padi angin dan *C. iria* juga telah disiasat dengan memberikan semburan dedaun *P. hysterophorus* pada kepekatan yang berbeza (20, 40 dan 60 g L^{-1}). Sifat fisiologi dan biokimia *A. conyzoides* didapati lebih sensitif terhadap *P. hysterophorus*, terutamanya pada kepekatan yang tinggi (60 g L^{-1}). Hasil kajian ini mengesahkan bahawa *P. hysterophorus* mempunyai kesan herbisida yang ketara untuk kawalan rumpai. Oleh itu, rumpai ini berpotensi untuk digunakan sebagai bioherbisida untuk mengawal rumpai di Malaysia.

ACKNOWLEDGEMENTS

Alhamdulillah, I am grateful and thankful to the Almighty Allah S.W.T for giving me strength, courage, patience and determination in preparing and completing this research successfully. I would like to express my deepest gratitude to my supervisor, Professor Dato' Dr. Abdul Shukor bin Juraimi for his continuous supervision, assistance, guidance, opportunity and trust for the accomplishment of this research. I am grateful for his generous and friendly approach that made my study enjoyable. Without his counsel, this thesis would not be completed and not able to achieve its objectives.

My earnest appreciation also goes to the members of the supervisory committee Associate Professor Dr. Md. Kamal Uddin, Dr. Norhayu binti Asib and Professor Dr. A. K. M. Mominul Islam for their constructive suggestions and guidance in the formation and execution of the research projects and critical reviews of the manuscript.

Special thanks to all the staff of the Department of Crop Science, Faculty of Agriculture, UPM. Special thanks to my friends, colleagues, and students of Weed Science Laboratory for their continuous help, cooperation, and valuable suggestions during my research work.

Last but not least, I am greatly indebted to my parents and mother-in-law for their sincere love and care. I will be ever grateful to my husband, Md. Mahmudul Hasan for his endless sacrifice, support, encouragement, and enthusiasm and countless sacrifices and allowing me to complete this study successfully.

I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Mst. Motmainna on her PhD thesis entitled "Determination of Allelopathic Noxious Weed Species as Potential Bioherbicide for Weed Control in Malaysia" in accordance with the universities and university college act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15th March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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DNA	Deoxyribonucleic acid
LAI	Leaf area index
PSII	Photosystem II
HPPD	Hydroxyphenylpyruvate dioxygenase
ROS	Reactive oxygen species
CAT	Catalase
MDA	Malondialdehyde
POD	Peroxidase
SOD	Superoxide dismutase
LC-MS	Liquid chromatography–mass spectrometry
ANOVA	Analysis of variance
CRD	Completely randomized *design*
RCBD	Randomized complete block *design*
RT	Retention time
ppm	Part per million
ND	Not detected
LC-MS QTOF	Liquid chromatography–mass spectrometryquadrupole time-of-flight
SAS	Statistical Analysis Software
SPAD	Soil Plant Analysis Development Chlorophyll Meter
g	Gram
Kg	Kilogram
µ mole	Micromole
%	Percentage

^{0}C	Degree Celsius
µ	Micron
cm	Centimeter
g	Gram
m	Meter
L	Liter
mg	Milligram
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background

Any plant that has been designated as being harmful to agriculture, horticulture, natural habitats or ecosystems, humans or livestock by an agricultural or other governing authority is referred to as a noxious weed. The term "noxiousness" refers to both the desirability and difficulty of controlling a plant (González *et al*., 2017). Some noxious weeds are indigenous to the area. Typically, these plants grow aggressively, reproduce rapidly in the absence of natural controls (native herbivores, soil chemistry, etc.), and exhibit harmful effects when they come into contact with or are consumed. The presence of noxious weeds poses a threat to both our environment and our economy. Due to the introduction of these noxious species, our region has suffered millions of dollars in lost agricultural production, environmental deterioration, and increased maintenance expenses (Nghiem *et al*., 2013) The moderately warm to high temperature and high humidity in tropical Asian regions are ideal for year-round luxuriant weed growth. Noxious weed seeds frequently contaminate crop seeds in rice fields (Chauhan and Mahajan, 2012). They are spread between fields via farm machinery and on the feet, fur, feathers, and skin of rodents, birds, and larger animals, including humans.

Allelopathy is known as the effect of plants on associated plants or microbes through the release of chemical compounds (Latif *et al*., 2017). These chemical compounds are recognized as allelochemicals. They are present in plant tissues and produced by secondary metabolism (Iqbal *et al*., 2019). Most of the allelochemicals are synthesized through the shikimate pathway (Hussain *et al*., 2011). In bacteria, archaea, fungus, algae, certain protozoans, and plants, the shikimate pathway is used to synthesise folates and aromatic amino acids (tryptophan, phenylalanine, and tyrosine). Allelochemicals are released into the environment by root exudation, leaching, volatilization and evaporation. The presence of allelochemicals is not harmful to bio-ecosystems and human health. Thus, allelochemicals can be applied as insecticides, herbicides, and antimicrobial crop production and growth regulators. Soltys *et al*. (2013) reported that the mode of actions of allelochemicals is similar to herbicides and most of them are water-soluble. Thus, surfactants are not essential to apply allelochemicals (Lucia and Guzmán, 2020). Allelochemicals were applied directly in many cases as natural herbicides.

According to Dayan *et al*. (2015) several researchers have already produced some bioherbicides to control weeds. Alpha terpineol is the active compound of Bioweed herbicide and is derived from the pine tree. It is a non-systemic and pre-emergence herbicide (Kawuma, 2019). Sarmentine isolated from Piper species is known as a natural contact herbicide with broad-spectrum activity (Dayan *et al*., 2015; Dayan *et al*., 2012). Similarly, pelargonic acid acts as a natural commercial herbicide that has a new mode of action for controlling a wide range of weeds (Dayan *et al*., 2012). Clove oil, another

plant-derived natural herbicide, is produced by steam distillation of the clove leaves. There are several terpenoid compounds and eugenol, which are strongly suppressive in clove oil (Perez-Roses *et al*., 2016). Triketone herbicides were identified from bottlebrush (*Calistemon* spp). This new chemical class could become a commercial herbicide through bleaching of plant tissues (Dayan *et al*., 2012; Dayan and Duke, 2014). The commercial products of natural herbicides introduced new chemical classes of compounds with novel mechanisms of action (Dayan *et al*., 2012). Since 2004, 11% of the global agricultural pesticides are natural products or compounds that trace their discoveries back to bioactive natural products (Dayan *et al*., 2012). Although, 35.7% of all registered active pesticide ingredients between 1997-2010 approved by the U.S. Environmental Protection Agency were natural products followed by synthetic with 30.7%, biological with 27.4%, and synthetic natural derived with 6.1% (Cantrell *et al*., 2012).

1.2 Problem statement

Weed management in Malaysian agro-ecosystems is very much herbicide-based (Yaduraju and Rao, 2013). The regular use of synthetic herbicides increases the resistance of weeds against herbicide and ecological imbalance, and environmental pollution. Synthetic herbicides can create the opportunity for other harmful organisms to cause disease by killing beneficial pathogens and organisms (Lushchak *et al*., 2018). Hence, it is essential to replace agrochemicals with natural products to achieve sustainable agriculture. In this relation, scientists have tried to invert the reliance on chemical weed management by developing effective natural herbicides (Korres *et al*., 2019). The allelopathic potential of noxious weeds could help the development of natural herbicides for long-term, environmentally friendly weed control. Noxious weeds are toxic to many or most crops; however, they may not be toxic to all crops. A heavy infestation of these weeds adversely affects the growth of adjacent plants (Davies *et al*., 2010). But little information is available regarding the allelopathic potential of these noxious weeds in Malaysia. Understanding these effects could considerably improve the management in farmlands and significantly reduce its effects on crop productivity. Therefore, it is important to identify potential growth inhibitor compounds to develop a natural herbicide based on natural compounds derived from noxious weed species. We expect that screening of noxious weed species in Malaysia will provide a unique opportunity to discover the novel phytotoxic compounds with a novel mode of action.

1.3 Objectives

The study aimed to evaluate the potential allelopathic weed from 30 noxious weed species in Malaysia for controlling the weed.

The specific objectives of the study were:

1. To assess the allelopathic activity of 30 noxious weed species in Malaysia on seed germination and seedling growth of *Oryza sativa* f. *spontanea* Roshev (weedy rice).
2. To determine the effects of most allelopathic potential weed(s) extract on the seed germination and seedling growth of weeds and crops in different conditions (laboratory and glasshouse).
3. To identify the allelopathic substance(s) from the most allelopathic potential weed (s) extract.
4. To determine the efficacy of most allelopathic potential weed(s) extract in comparison for weed management to the commercial herbicides.
5. To evaluate the effect of most allelopathic potential weed(s) extract on physiological and biochemical changes of weeds for management.

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