PROFILING OF PARTHENIUM (*Parthenium hysterophorus* L.) ACTIVE COMPOUNDS AND ALLELOPATHIC SUPPRESSION OF CROPS AND WEEDS IN BAMBARA GROUNDNUT (*Vigna subterranea* L.) CULTIVATION

By

H. M KHAIRUL BASHAR

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

September 2022

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DEDICATION

To my pretty father, Md. Fazlur Rahman, late mother, Mst. Rokeya Begam, my father in-law, Md. Abdur Rahim Mia, Mother-in-law, Tara Banu and my beloved wife Ferdoushi Rahaman, my adorable son Zubair Bin Bashar, my daughter Ayesha Al Humayera and Fatima Tuz Zohra who always kept praying for me day and night and sacrifice to achieve my goal. Specially thanks to all of my family members

and

To all of my friends who supported me all these years.

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

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

Parthenium hysterophorus L. is a noxious herb with pharmaceutical properties and has allelopathic potentiality to retards the growth of many species. Hence, research was undertaken with the aim to identified phytotoxic compounds and determine the possibility of using aerial parts (leaf, stem and flower) of *P. hysterophorus* as a potential bioherbicide to control weed in Bambara groundnut field. The experiment was conducted in laboratory and glasshouse condition at University Putra Malaysia (UPM) using complete randomize design (CRD) and randomize complete block design (RCBD) accordingly. Germination of seeds and seedling growth of selected crops and weed species were tested with six concentrations of methanol, hexane, acetone, and water extracts (25, 50, 75, 100, and 150 g L^{-1}) and distilled water as the control. The results showed that the phytotoxicity effects were significantly higher by leaf extract and methanol solvent compared to other treatments. The methanol extract of *P. hysterophorus* leaf sprayed as post-emergence applications on bambara groundnut (*V. subterranean* L.), maize (*Z. mays*), *D. sanguinalis, E. indica, A. conyzoides, C. iria, E. hirta, C. difformis* showed that the efficacy of the treatments were in the following trend; leaf>flower>stem and methanol>acetone>water>hexane. The liquid chromatography-mass spectrometry (LC-MS) analysis exposed the presence of 67 and 196 compounds in methanol, 60 and 191 in hexane, 75 and 77 in acetone, and 52 and 184 from water in both positive and negative polarity analysis. Seven phytotoxic derivatives were identified from the methanol solvents of leaf and flower but two and three compounds in acetone, hexane, and water extract respectively. On the other hand, high-performance liquid chromatography (HPLC) analysis confirmed methanol with leaf extracts contained a higher number and amount of chemical compounds than did those of the other solvent extracts. Caffeic acid, chlorogenic acid, and parthenin (highest allelopathic activity) were detected in the highest concentrations in the methanol leaf extract, but this compound was not found in the other extracts. *P. hysterophorus* methanol leaf extract administered to the test species at doses of 100 g L^{-1} showed a reduction in photosynthesis rate (22-83.37%), stomatal conductance (17-120.40%), transpiration

(27.41%), chlorophyll content (13-143.87%), and carotenoid content (15-117.72%) at 6 hours after spray (HAS). The activities of antioxidant enzymes, malondialdehyde (MDA)(183-596%), Proline content (150-500%), superoxide dismutase (SOD)(76-95%), catalase (CAT)(98-208%), and peroxidase (POD)(94-212%) were also increased with the same concentrations at 72 HAS in different weeds. Present findings confirm that the methanol leaf extract of *P. hysterophorus* can disrupt the physiological and biochemical mechanism of target weeds. For field evaluation, treatment of *P. hysterophorus* leaf methanol extract (75, 100, and 150 g L^{-1}) along with five chemical herbicides [chemical name % a.i. (Fusilade Forte 150 EC), chemical name % a.i. (Prowl ® 3.3 EC), chemical name % a.i. (Hextar Diuron 80wp), glyphosate 41% a.i. (Roundup®) and glufosinate-ammonium 13.5% a.i. (Basta®)] at 1 L ha^{-1}, no herbicide treatment and weeding manually was done. It was observed that *P. hysterophorus* leaf methanol extract at the rate of 150 g L^{-1} and all chemical herbicides killed Bambara groundnut and most weeds. However, Bambara groundnut yielded the most when sprayed with *P. hysterophorus* leaf methanol extract at a rate of 100 g L^{-1} followed by manual (hand weeding). As a result, 100 g L^{-1} of *P. hysterophorus* leaf methanol extract could be sprayed to manage weeds without reducing Bambara groundnut yield. Based on-these findings, *P. hysterophorus* leaf methanol extract can be utilized as a natural post-emergent herbicide in Bambara groundnut fields to suppress weeds, as well as for the development of novel herbicides based on phytotoxic chemicals generated by this plant.

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

PROFIL PARTHENIUM (*Parthenium hysterophorus* L.) SEBATIAN AKTIF DAN PENINDASAN ALELOPATIK TANAMAN DAN RUMPAI DALAM PENANAMAN KACANG BAMBARA (*Vigna subterranea* L.)

Oleh

H. M KHAIRUL BASHAR

September 2022

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.

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I certify that a thesis examination committee has met on 22 September 2022 to conduct the final examination of H M Khairul Bashar on his Doctor of Philosophy thesis entitled "Profiling of Parthenium (*Parthenium hysterophorus*) Active Compounds and Allelopathic Suppression of Crops and Weeds in Bambara groundnut (*Vigna subterranea* L.) Cultivation" in accordance with Universiti Pertanian Malaysia (Higher Degree) act 1980 and Universiti Pertanian Malaysia (Higher Degree) regulations 1981. The committee recommends that the candidate be awarded the relevant degree.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
FW	Fresh weight
DW	Dry weight
cm	Centimetre
CRD	Completely randomize design
G	Gram
H	Hour
ha^{-1}	Hectare
Kg	Kilogram
L	Liter
RT	Retention time
m	Meter
mg/L	Milligram per liter
ml	Milliliter
mm	Millimeter
ppm	Part per millions
ppt	Part per thousands
SD	Standard Division
Sp.	Species
T	Treatment
UPM	Universiti Putra Malaysia
USA	United States of America
^{0}C	Degree Celsius
%	Percentage

<	Less than
>	More than
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
MDA	Malondialdehyde
HAS	Hours after spray
ROS	Reactive oxygen species
RL	Root length
SL	Shoot length
Ger	Germination

CHAPTER 1

INTRODUCTION

Bambara groundnut (*Vigna subterranea* L.) is an indigenous African legume crop primarily grown for its seeds. It is becoming popular as a food crop in rural areas of many countries in Africa (Jeklin et al., 2011). As human food, it is considered as a complete food crop since it has sufficient levels of the N, P, K macronutrients. Compared to intensively grown legumes such as groundnut and soybean, it has a high carbohydrate content (55%), low oil levels (5-6%), and a protein content of 22% (Oyeleke et al., 2012), with higher concentrations of essential amino acids like methionine and lysine (Mazahib et al., 2013). Bambara groundnut is a crop similar in morphology and growth habit to groundnut (*Arachis hypogaea* L.) but with high level of nutritional qualities and is drought tolerant (Feldman et al., 2019).

Parthenium *(Parthenium hysterophorus* L.) weed is a mystic annual invasive plant prevalent in several tropical and sub-tropical countries (Cowie et al., 2020), threatening natural ecosystems and agroecosystems in over 30 countries worldwide (Adkins & Shabbir, 2014). *Parthenium hysterophorus* is a plant which has positive and negative impacts on human being. This plant first come out in Australia and later spread to the whole world (Khan & Fahad, 2020). Parthenium weed is now spread from India to most of its neighbouring countries. It is suspected that this weed spread to Nepal, Bhutan, Bangladesh and Pakistan through vehicles or as a contaminant of seed lots (Shabbir et al., 2012). This plant is a strongly allelopathic species (Singh et al., 2005) and this trait is important in its invasion and persistence in a wide range of native and non-native ecosystems. Parthenium weed contains a number of potential allelochemicals within its aerial parts (e.g. leaves) and in its roots, which can inhibit the germination and growth of a wide range of plant species, including native plants, as well as various crops and pasture species (Singh et al., 2005).

Allelopathy is a mechanism in which the chemical produced by the plant may increase or decrease the plant growth (Sturm et al., 2016). Allelopathy is considered as an effective, economical and environment friendly weed management approach. The release of allelopathic compounds from leaves, flowers, seeds, stems and roots of living and decomposing plant materials can influence weed density and growth (Sathishkumar et al., 2020). The harmful chemicals released by allelopathic plants are known as allelochemicals. Some allelochemicals change the amount of chlorophyll production in a plant and it can be used to control weeds in agricultural systems by using allelopathic crops for intercropping, crop rotation, or mulching (Rehman et al., 2019). The allelopathic compounds can be used as natural herbicides and other pesticides; they are less disruptive to the global ecosystem than synthetic agrochemicals (Shahena et al., 2020).

Khan et al., (2012) remarked the inhibited seed germination and the seedling growth of wheat cultivar due to allelopathic effects of root, stem and leaf extracts of parthenium.

Hassan et al., (2018) reported that with increasing concentration of Parthenium weed extract, the germination, root length and shoot length of weeds decreased significantly. Leaves of Parthenium may be a source of natural weedicide which can help to control invasive plants (Maharjan et al., 2007). (Msafiri et al., 2013) revealed that significant allelopathic effects of aqueous extract of *P. hysterophorus* leaf and seed on seed germination, roots and shoot length, fresh and dry weight of crops. Aqueous extract of *P. hysterophorus* and *D. alba* decreased the seed germination, shoot length, fresh and dry biomass of different crops (Afridi & Khan, 2015). The higher concentration of Parthenium leaf extract decreased the photosynthetic pigments, carbohydrate, and enzymes in *Vigna aconitifolia* L. (Patil Bhimarao & Khade Hemlata, 2017). Seed germination and growth performance of chickpea were low at high concentrations of leaf extract of Parthenium (Shikha & Jha, 2016).

With the increasing trend of world population and decreasing available resources, weed management to protect crops is even more important as well as a challenging job. Weed management can be done mechanically, chemically or biologically. Mechanical weed control is very expensive, chemical weed control leads to environmental pollution and results in the evolution of herbicide resistance in weeds (Sharma, Kumar, et al., 2019). Biological weed control especially using the mechanism of allelopathy is well-adapted and a promising science (Petit et al., 2018). Shafiq et al., (2020) conducted an experiment with different parts of Parthenium on seed germination and biomasses of *Cicer arietinum* L and found that aqueous extracts of root, leaves and flowers significantly reduced the germination and seedling growth. Devi et al., (2014) investigated the allelopathic effect of Parthenium on growth and productivity of *Z. mays* L. and its phytochemical screening they observed that increasing concentration of *P. hysterophorus* whole plant extract significantly inhibited the germination, shoot length, root length, seedling length and SVI of crops. Hassan et al., (2018) recorded allelopathic effect of Parthenium extract and residue on some agronomic crops and weeds. There is inadequate data with respect to the effect of Parthenium plant extract on the growth and seed quality of Bambara groundnut.

Bambara groundnut is a new crop for Malaysia and Bangladesh and there is no information available on the allelopathic suppression of weeds on Bambara groundnut. Another one, the studies on profiling of active compounds and physio-biochemical responses of Parthenium allelochemicals are scarce. An accurate and sustainable weed control is compulsory for maintaining food security throughout the world. Weeds caused a 30 percent yield loss on average, but inadequate management circumstances resulted in yield losses of up to 60 percent (Mhlanga et al., 2016). In case of Bambara groundnut yield losses of 35 to 80 percent, as well as lower pod and haulm yields, according to Priya et al., (2013).

The overuse of chemical herbicides not only causes the environmental pollution but also poses many other threats to human health. Most importantly, herbicides, with few exceptions, cannot be applied in fields in which crops are being grown organically. The use of bio-herbicides may serve to keep weed infestations low and improve the crop productivity across the globe. This technique will help to reduce the dependence on chemical herbicide, reducing the chances of herbicide resistance development in weeds, reduce health hazards, environmental damage and strengthen national economy as well. Hence, exploring allelopathic potential of Parthenium, a combination of the laboratory and green house trials to investigate the allelopathic suppression of weeds by Parthenium in Bambara groundnut is a new avenue of research. This research is enriching the data pool and provides information to future study. With these ends in views the studies were conducted with the following objectives:

1. To evaluate pre- and post-herbicidal effects of various parts of Parthenium extracts in different solvents on weeds and crops.
2. To identify and quantify potential phytotoxic compounds in different parts of Parthenium weed under different solvents by HPLC analysis.
3. To evaluate phytotoxic potential of the identified compounds on seed germination and initial growth of weeds.
4. To determine the mode of action of Parthenium allelochemical in inhibiting plant growth.
5. To develop potential formulation of Parthenium extract and to compare its efficacy to commercial herbicides for weed control in Bambara groundnut.

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