UNIVERSITI PUTRA MALAYSIA

PHYTOINHIBITION AND FORMULATION OF ALLELOPATHIC EXTRACT OF MIKANIA MICRANTHA KUNTH EX H.B.K. AS PRE-EMERGENT WEED SUPPRESSANT AGAINST ECHINOCHLOA COLONA (L.) LINK

LIM CHAW JIANG

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By

LIM CHAW JIANG

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

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PHYTOINHIBITION AND FORMULATION OF ALLELOPATHIC EXTRACT OF *MIKANIA MICRANTHA* KUNTH EX H.B.K. AS PRE-EMERGENT WEED SUPPRESSANT AGAINST *ECHINOCHLOA COLONA* (L.) LINK

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June 2018

Chairman : Professor Mahiran Basri, PhD
Faculty : Science

Allelopathy delivers the concept of using allelochemicals elicited from donor plants into environment to influence the survival of receptor plants. Invasive plants having allelochemicals could provide low-cost, abundant and eco-friendly materials for controlling noxious weeds. In spite of that, the direct use of invasive plant extracts encounters major hurdles such as variation of bioactive chemicals not being identified, lack of optimal extraction, inadequate physicochemical properties and poor delivery system resulting in low efficacy of the natural products. This study aims to investigate the phytotoxicity, putative allelochemicals, extraction optimisation, pre-emulsion formulations and water-dispersible powders of the leaf extract of *Mikania micrantha* Kunth ex H.B.K. against a noxious weed, *Echinochloa colona* (L.) Link and the impact on productivity of rice plant, *Oryza sativa* L..

In phytotoxicity study, the leaf, stem and root (except *M. micrantha*) extracts of nine invasive plant species were evaluated against *E. colona*. Among the plant extracts, the leaf extract of *M. micrantha* showed 100% inhibition of germination of *E. colona* at the concentration of 100 g BDWE/L. The leaf extract was subjected to liquid chromatography-mass spectrometer (LC-MS) analysis to identify phytochemicals with possible inhibitory effect. Joint putative allelochemicals consisting of 16 phenolics and 4 aromatics were detected and protocatechuic acid was found contributed to 15.39% inhibition of germination. The leaves of *M. micrantha* were processed through extraction optimisation using response surface methodology (RSM). The optimal extraction condition was found at an extraction time of 262 min, a stirring speed of 259 rpm and an aqueous methanol of 95% v/v.

In the development of pre-emulsion formulations, three polyalkoxylated fatty alcohol (PAFA)-based mixed surfactants PAFA-AS (alkyl sulfonate), PAFA-CB

(cocamidopropyl betaine) and PAFA-APG (alkyl polyglucosides) were used to construct the pre-emulsions E1, E2 and E3 containing rapeseed oil methyl esters (ROMEs), water and sodium silicate. The pre-emulsions were diluted with water and agitated with an isothermal shaker to the weight fractions (Φ_w) of 0.8 and 0.6. In rheology study, these samples showed shear thinning, linear viscoelastic (LVE) (G'>G") and strain softening (G">G') characters. In mesomorphic study, the samples E1, E2 and E3 promoted multilamellar vesicles, bicontinuous cubic phase and multilamellar phase, respectively. The pre-emulsions E1, E2 and E3 were incorporated with the leaf extract of *M. micrantha* to form the pre-emulsion formulations F1, F2 and F3. These pre-emulsion formulations exhibited higher stability against heat at a temperature of 54 ^{o}C than the non-formulated leaf extract.

For product ease of application, the pre-emulsion formulations F1, F2 and F3 were loaded onto mercerised lignocellulosic fibre to produce water-dispersible powders WDP-F1, WDP-F2 and WDP-F3, respectively, and the non-formulated leaf extract powder WDP-EX was prepared for comparison. In release kinetics study, the formulated powders demonstrated initial burst and subsequent sustained release of phenolics achieved the amounts of 63.66 to 86.52% at 168 h, whereas phenolic release from the non-formulated powder was at the lowest amount of 41.98%. The particle releases from the formulated powders showed mean particle sizes were in the range of 87.56 to 103.27 nm whereas the mean particle size of 423.93 nm was observed from the non-formulated powder. In controlling the germination of *E. colona*, the formulated powders gave lower ED_{50} values in the range of 25.97-33.66 g WDP/m^2 than the non-formulated powder at 132.00 g WDP/m^2.

In the glasshouse study, the formulated powders WDP-F1, WDP-F2 and WDP-F3 exhibited higher inhibition of germination, shoot height, fresh weight and dry weight of *E. colona* than the non-formulated powder WDP-EX and non-treated weedy control NWC. Notably, the sample WDP-F2 demonstrated the greatest inhibition of *E. colona* and statistically equivalent to commercial Satunil® and weed-free non-treated control NWF. Due to efficient controlling of *E. colona* by the formulated powders, the tiller height, tiller number, panicle number, fresh weight, dry weight, grain number and grain weight of *O. sativa* were increased in comparison to the non-formulated powder WDP-EX and non-treated weedy control NWC. The potential exploitation and formulation of the phytotoxic leaves of *M. micrantha* could pave the alternate to synthetic herbicide use in forging eco-friendly weed management.

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

PERENCATAN FITO DAN FORMULASI EKSTRAK ALELOPATI *MIKANIA MICRANTHA* KUNTH EX H.B.K. SEBAGAI RACUN RUMPAI PRA-CAMBAH TERHADAP *ECHINOCHLOA COLONA* (L.) LINK

Oleh

LIM CHAW JIANG

Jun 2018

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Alelopati menyampaikan konsep penggunaan alelokimia yang dilepaskan daripada tumbuhan penderma ke alam sekitar untuk mempengaruhi kehidupan tumbuhan penerima. Tumbuhan invasif mempunyai alelokimia dapat menyediakan bahan-bahan murah, banyak dan mesra alam bagi mengawal rumpai noksius. Walau bagaimanapun, penggunaan secara langsung ekstrak tumbuhan invasif menghadapi halangan utama seperti variasi sebatian kimia bioaktif tidak dikenalpasti, kekurangan pengekstrakan optimum, sifat fizikokimia tidak mencukupi dan sistem penyampaian lemah menyebabkan keberkesanan rendah produk semula jadi. Kajian ini bertujuan untuk menyiasat kefitotoksikan, alelokimia putatif, pengoptimuman ekstrakan, formulasi pra-emulsi dan serbuk serak air untuk ekstrak daun *Mikania micrantha* Kunth ex H.B.K. terhadap rumpai noksius, *Echinochloa colona* (L.) Link dan kesan ke atas produktiviti tanaman padi, *Oryza sativa* L..

Dalam kajian kefitotoksikan, ekstrak daun, tangkai dan akar (kecuali *M. micrantha*) daripada sembilan spesies tumbuhan invasif telah dinilai terhadap *E. colona*. Antara ekstrak tumbuhan, ekstrak daun *M. micrantha* menunjukkan 100% perencatan percambahan *E. colona* pada konsentrasi 100 g BDWE/L. Ekstrak daun ini tertakluk kepada analisis kromatografi cair-spektrometer massa (LC-MS) untuk mengenal pasti fitokimia berkemungkinan memberi kesan perencatan. Sekumpulan alelokimia putatif terdiri daripada 16 fenolik dan 4 aromatik dikesan dan asid protokatesik didapati menyumbang 15.39% perencatan percambahan. Daun *M. micrantha* telah diproses melalui pengoptimuman ekstrakan menggunakan kaedah gerak balas permukaan (RSM). Kondisi ekstrakan optimum didapati adalah pada masa ekstrakan 262 min, kelajuan kacau 259 rpm dan metanol akueus 95% v/v.

Dalam pembangunan formulasi pra-emulsi, tiga surfaktan campuran berasaskan dipolialkosilat lemak alkohol (PAFA) iaitu PAFA-AS (alkil sulfonat), PAFA-CB (kokamidopropil betain) dan PAFA-APG (alkil poliglukosida) digunakan untuk membina pra-emulsi E1, E2 dan E3 yang mengandungi ester metil minyak rapa (ROMEs), air dan natrium silikat. Pra-emulsi itu telah dicairkan dengan air dan dikacau dengan mesin pengoncang isotermal kepada pecahan berat (Φ_w) 0.8 dan 0.6. Dalam kajian reologi, sampel ini menunjukkan kelakuan penipisan ricih, viskoelastik linear (LVE) (G'>G") dan pelembutan terikan (G"> G'). Dalam kajian mesomorfik, sampel E1, E2 dan E3 mempamerkan vesikel multilamelar, fasa kubik bikontinyu dan fasa multilamelar masing-masing. Pra-emulsi E1, E2 dan E3 telah diperbadankan dengan ekstrak daun *M. micrantha* bagi membentuk formulasi pra-emulsi F1, F2 dan F3. Formulasi pra-emulsi ini menunjukkan kestabilan lebih tinggi terhadap haba pada suhu 54 ^oC daripada ekstrak daun tanpa formulasi.

Bagi memudahkan aplikasi produk, formulasi pra-emulsi F1, F2 dan F3 dimuatkan ke serat lignoselulosa dimerser untuk menghasilkan serbuk serak air WDP-F1, WDP-F2 dan WDP-F3 masing-masing, dan serbuk ekstrak daun tanpa formulasi WDP-EX disediakan untuk perbandingan. Dalam kajian kinetik pelepasan, formulasi serbuk menunjukkan pembebasan fenolik secara pecut awal kemudian berterusan mencapai sejumlah 63.66-86.52% pada 168 j, manakala pembebasan fenolik daripada serbuk tanpa formulasi mencapai jumlah terendah iaitu 41.98%. Pembebasan zarah daripada formulasi serbuk menunjukkan saiz zarah purata dalam lingkungan 87.56 hingga 103.27 nm manakala saiz zarah purata sebanyak 423.93 nm diperhatikan daripada serbuk tanpa formulasi. Dalam mengawal percambahan *E. colona*, formulasi serbuk memberikan nilai ED_{50} lebih rendah dalam lingkungan 25.97-33.66 g WDP/m^2 daripada serbuk tanpa formulasi pada 132.00 g WDP/m^2.

Dalam kajian rumah kaca, formulasi serbuk WDP-F1, WDP-F2 dan WDP-F3 mempamerkan perencatan percambahan, ketinggian tangkai, berat segar dan berat kering *E. colona* yang lebih tinggi daripada serbuk tanpa formulasi WDP-EX dan kawalan rumpai tanpa rawatan NWC. Terutamanya, sampel WDP-F2 menunjukkan perencatan *E. colona* tertinggi dan statistik bersamaan dengan komersial Satunil® dan kawalan rumpai bebas tanpa rawatan NWF. Oleh sebab pengawalan *E. colona* yang cekap oleh formulasi serbuk, ketinggian anakan, bilangan anakan, jumlah malai, berat segar, berat kering, bilangan bijirin dan berat bijirin *O. sativa* telah meningkat berbanding dengan serbuk tanpa formulasi WDP-EX dan kawalan rumpai tanpa rawatan NWC. Potensi eksploitasi dan formulasi daun fitotoksik *M. micrantha* dapat membuka alternatif kepada penggunaan herbisida sintetik dalam memupuk pengurusan rumpai mesra alam.

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I certify that a Thesis Examination Committee has met on 26 June 2018 to conduct the final examination of Lim Chaw Jiang on his thesis entitled "Phytoinhibition and Formulation of Allelopathic Extract of *Mikania micrantha* Kunth ex H.B.K. as Pre-Emergent Weed Suppressant Against *Echinochloa colona* (L.) Link" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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ABA	abscisic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ACS	1-aminocyclopropane-1-carboxylic acid synthase
ANN	artificial neural networks
ANOVA	analysis of variance
ANT	adenine nucleotide translocase
APG	alkyl polyglucosides
AS	alkyl sulfonates
ATP	adenosine triphosphate
ATR	attenuated total reflectance
a.u.	arbitrary unit
BWDE	biomass dry weight equivalent
CAT	Catalase
CB	cocamidopropyl betaine
CCC	circumscribed central composite
CCD	central composite design
C-CD	charge-coupled device
CMC	critical micelle concentration
CRD	completely randomized design
CRF	controlled release formulation
CV	coefficient of variation
DAS	day after sowing
DAT	days after treatment
DLS	dynamic light scattering
DNA	deoxyribonucleic acid

DoE	design of experiments
DP	dust powder
DSR	direct-seeded rice
DTG	differential thermogravimetry
EC	emulsifiable concentrate
ED	effective dose
FAO	Food and Agriculture Organization
FCC	face-centered central composite
FCCD	face-centered cube design
FT-IR	Fourier-transform infrared
FTR	flooded transplanted rice
GA	gibberellic acid
GAE	gallic acid equivalent
GPC	gel permeation chromatography
HIPE	high internal phase emulsion
HLB	hydrophile-lipophile balance
HR-TEM	high-resolution transmission electron microscopy
IAA	indole-3-acetic acid
LC-MS	liquid chromatography-mass spectrometer
LLC	lyotropic liquid crystal
LVE	linear viscoelastic
MDA	Malondialdehyde
MDS	molecular dynamic simulation
MLVs	multilamellar vesicles
MoAs	mode of actions
MSR	mixed surfactant ratio
NADH	nicotinamide adenine dinucleotide

O/W	oil-in-water
PAFA	polyalkoxylated fatty alcohols
PAL	phenylalanine ammonia-lyase
PALS	phase analysis light scattering
PCS	photon correlation spectroscopy
PDI	polydispersity index
PIC	phase inversion composition
PIT	phase inversion temperature
POD	Peroxidase
PPOs	polyphenol oxidases
PTFE	Polytetrafluoroethylene
RCBD	randomized complete block design
RNA	ribonucleic acid
ROMEs	rapeseed oil methyl esters
ROS	reactive oxygen species
RSM	response surface methodology
RST	regular solution theory
SAR	structure-activity relationship
SEM	scanning electron microscopy
SL	soluble liquid
SOR	surfactant-oil ratio
SPE	solvent percolation and evaporation
SRF	sustained release formulations
TGA	thermogravimetric analysis
THF	Tetrahydrofuran
TPC	total phenolic content
UPLC	ultra-performance liquid chromatography

UV	Ultraviolet
VOCs	volatile organic components
W/O	water-in-oil
WP	wettable powders
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The world's population has exceeded 7 billion people in 2012 and the burgeoning population growth is forecasted to continually swell by 30% and is expected to rise to 9.2 billion by the year 2050 (Popp et al., 2013). To cater for an extra 2.2 billion people in the developing world, and the pressure on ensuing rise in food demand with better nutrition, broader dietary habits and high-value crops are expected to be increased to between 50% to 100% by the year 2050 (Godfray et al., 2010; Maienfisch and Stevenson, 2015). To cope with the disconcerting possibility of tightening food supply owing to the expanding population, more concerted efforts are needed to search for the holistic pest management strategies which are influenced by a wide array of factors such as prolific crop pests, weather and climate changes, soil fertility, labour force shortage, knowledge and technology access, market prices and government policies (Speranza et al., 2008; Campos et al., 2014).

In crop protection practices, pesticide use is immensely implemented to eradicate pest outbreaks including weeds, insects and pathogens, and minimise crop yield losses (Enserink et al., 2013; Delcour et al., 2015). Chemical pesticides have been ushered in since the mid-twentieth century, in the obligatory need to govern effective pest control, as exemplified by 50-fold increase of the culminated pesticide use to gravely improve crop productivity in modern agriculture (Lamberth et al., 2013). The heavy reliance of chemical pesticides by farmers is due to the fact that the chemical adoption is the easiest and most cost-effective method to proffer a sizeable increase in crop production using an application rate as low as gram per hectare and a good selectivity towards crops. This has resulted in alleviating farm labour shortage, less time consuming and lessen tedious work in manual weeding and lessen drudgery in minimising soil erosion, nutrient and water run-off, greenhouse gas emission and fuel consumption (Gianessi, 2013; Kraehmer et al., 2014).

In the aspect of weed control, the crop yield losses inflicted by weeds (~34%) are significantly greater than those by insect pests (18%) and pathogens (15%) (Jabran et al., 2015; Van Evert et al., 2017). Weed infestation in paddy field is one of the chief impediments in high yield rice production (Baki et al., 2012; Burhanuddin Al-Helmy et al. 2015). Rice (*Oryza sativa* L.) is the main staple food source for the majority of the world's population, and about 90% world rice is produced in Asia (FAO, 2014; USDA, 2016). The insidious spread of weeds which interfere with the rice crop with uptake of available resources such as light, space, nutrient and water could impinge on the rice yield decline achieving up to 95% (Wang et al., 2007a; Rabbani et al., 2011). As a consequence, the scenario of herbicide-based weed management prevails in the rice cultivation to safeguard the sustainable rice production (Busi et al., 2017).

The herbicide consumption in Malaysian agriculture particularly the rice crops cost ~$US10.96 million (1984–2014) (DOS, 2015).

In Asian countries today, the direct-seeded rice (DSR) system has majorly superseded the flooded transplanted rice (FTR) method due to looming water scarcity, rural labour shortage, climbing labour wages and land competition for transplanting rice (Chauhan et al., 2012; Joshi et al., 2013). In the DSR system, the rice seeds sown directly into fields in the absence of standing water and aerobic soil conditions are highly conducive to weed germination at monumental scale and concomitant emergence in cohorts of the weeds with rice seedlings leading to harsh competition for available resources (Khaliq and Matloob, 2011; Chauhan, 2012a,b). More than a thousand weed species has encroached in the global rice fields, with thirteen weed species causing the most severe effects, among which includes *Echinochloa colona* (L.) Link (jungle rice) (Holm et al., 1991; Aliotta et al., 2006). The advent of DSR system has prompted farmers to enormously use chemical herbicides to counteract most weed intervention (Farooq et al., 2011; Chauhan, 2013).

In recent years, the agrochemical industry has boosted an interest to develop eco-friendly pesticides owing to a steadily growing demand for food safety and stringent restrictions on the use of toxic chemicals (Yusoff et al., 2016). In escalating the involvement of natural products in sustainable weed management, plant emancipated allelochemicals having interfering effect on recipient plants with non-toxic mechanisms could be used to suppress weeds in agroecosystems. This might coincidentally enrich the environment (Dayan et al., 2009a; Ladhari et al., 2013a,b). In forest ecosystems, invasive plants exude allelochemicals which have been implicated as phytochemical inhibitor to outcompete indigenous species, pests and disease resistance (Pisula and Meiners, 2010). The invasive plants have high fecundity which can be economically invoked for potential use as weed growth retardant.

Implementing new scientific knowledge to create innovative formulation technology is crucially important in ameliorating crop protection products' physicochemical properties, delivery performance and bioefficacy thereby bringing the environment and ecological systems to the least detrimental impacts. The eco-friendly benign formulation should have distinctive properties which include low toxicity, high biodegradability, good biocompatibility, cost effectiveness, abundant and renewable source and ease of preparation and application (Yusoff et al., 2016). For soil-applied products, many researchers have triggered the use of sustained release formulations (SRF) in which the pesticides are incorporated to a matrix for sustained release, thus retarding mobility of the pesticides in soil for consistent release and prolonged uptake by weedy plants (Yusoff et al., 2016).

Recently, liquid crystal emulsions have emerged as potential colloidal delivery matrix for diffusion controlled release with a more superior moisturising effect than the conventional liquid emulsion (Zhang and Liu, 2013; Jia et al., 2018). The liquid crystal-based emulsions are formed from mutual co-existence of the excess oil and water, with the surfactant in the liquid crystals possessing the capability to suspend

substances for sustained release (Rodriguez et al., 2007; Alam, 2009). Upon the emulsification process, the aqueous dilution of liquid crystalline phase could form nano-emulsions (Solans and Sole, 2012; Gohtani and Prasert, 2014). The green nano-emulsions have been gaining an accolade for efficient substance delivery in the fields of pharmaceutical (Jaiswal et al., 2015; Singh et al., 2017), cosmetics (Sharma and Sarangdevot, 2012), food (McClements and Rao, 2011) and pesticide formulation (Lim et al., 2012a,b,c; Lim et al., 2013). However, development of the SRF-based system for allelopathic plant extract is a new breakthrough in the weed control.

In product formulation, the plant extract has to be converted into powder form instead of an amorphous paste for ease of handling and application. The lignocellulosic fibre wastes could be an ideal loading template to well support the formulated paste extract. Lignocellulose fibres are perceived as physiologically inert, low cost, light weight, renewable, biodegradable and readily available substances which can be efficiently exploited to generate the high value added products (Yan et al., 2012; Santana-Meridas et al., 2012). The smart utilisation of plant leaf residues rich in lignocellulose content is of great endeavour towards valorisation of the agro-wastes (Daud et al., 2014). For instance, the development of neem leaf powder in the alginate beads has been reported in the pesticide controlled release formulations (Singh et al., 2010). The lignocellulosic fibres are hydrophilic in nature exhibiting a high tendency to absorb moisture (Dhakal et al., 2007), thus allowing the polar substances to be released from the fibre matrix through percolation of water.

1.2 Research Problems

The perpetuating dependent use of chemical pesticides has triggered the outbreak of recalcitrant herbicide-resistant weeds increasing in a rapid rate (Bhatti et al., 2013; Busi et al., 2013). About 250 species of noxious weeds have built resistance and are able to withstand the known mode of actions (MoAs) by herbicide detoxification worldwide (Heap, 2016). The herbicide-resistant *E. colona* has evolved in a plentiful amount and is becoming less effective to chemical herbicides including ametryn, atrazine, clefoxidym, cyhalofop-butyl, metribuzin, propanil, glyphosate, quinclorac, bispyribac-sodium, imazapyr, triazine and acetyl coenzyme A carboxylase (ACCase) inhibitor (Valverde, 2007; Peerzada et al., 2016). Such dire scourges have awakened researchers to search for alternatives to complement the obsolete herbicides (Sparks and Lorsbach, 2017).

Natural products have gained a recent surge in popularity and could provide a fulgurant array of structural diversity of natural phytotoxins (Dayan and Duke, 2014; Sparks et al., 2017). Current paradigm on herbicide-resistant problems can be allayed through advocating the natural product use of allelopathy as one of the weed management options (Tesio and Ferrero, 2010). The allelopathy concept could offer a green alternative route for the apparent impasse, with the standpoints of crop safety, cost-effectiveness, safe environmental and toxicological profiles. Plant allelochemicals have multi-site actions which function through the MoAs not owned

by commercial herbicide and their use could be suitable in preventing weeds from developing resistance (Dayan et al., 2012).

In fact, plants produce small quantities of diverse bioactive compounds which constitute assorted allelochemicals working in synergistic manners (Zuo et al., 2016). Sometimes, isolation and purification of the allelochemical complexes may lead to loss in bioactivity origin. Plant extracts could be the ideal preference as natural-low-cost phytoactives for ease of use while maintaining the chemical integrity. Despite plant extracts having low environmental impact, they encounter major hurdles such as variation of bioactive chemicals not being fully identified, limited selectivity to preserve the crop of interest, low efficacy at a large quantity of plant biomass, inadequate physicochemical properties and lack of product development, inferior storage stability, poor solubility and delivery system, environmental instability and no standard protocol for quality control (Moshi and Matoju, 2017). These serve to hamper the product quality and restriction in use.

1.3 Research Objectives

The present study conveys the research objectives:

1. To assess the phytoinhibition activities of invasive plant extracts and putative allelochemicals against a noxious weed, *E. colona*.
2. To optimise the extraction method for invasive plants using response surface methodology (RSM).
3. To construct, formulate and characterise the pre-emulsions with tendency to form liquid crystalline/gel phase for nano-emulsion delivery of the leaf extract of *M. micrantha*.
4. To modify and characterise the lignocellulosic fibre for conversion of the pre-emulsion formulations into water-dispersible powders.
5. To characterise the physicochemical properties, release kinetics and dose response study of the water-dispersible powder formulations.
6. To evaluate the efficacy of the water-dispersible powder formulations against *E. colona* and the productivity of *O. sativa* in the glasshouse study.

1.4 Scope of Study

Looking towards an eco-sustainable approach, a plethora of invasive plant products were harnessed to be potent plant growth regulators in controlling the rice weed, *E. colona*. Invasive plants have the tendency to exude allelochemicals which can be utilised to impede the weed growth. Herein, a phytotoxicity examination of the invasive plant products was conducted to assess their capability of inhibition against the rice weed. Those invasive plants with top-ranked phytotoxic activities were chosen to study the putative allelochemicals including the aromatic and phenolic constituents to support the phytotoxicity outcome. In the plant extraction study, statistical optimisation of the process parameters such as extraction time, mechanical energy use and organic solvent consumption was performed to evaluate the optimal plant

throughput method in the perspectives of biological activity, cost-effectiveness and environmental impact.

In the eco-friendly product development of plant extract, surfactants and oil were chosen as the principal excipients in developing pre-emulsion system. Surfactant acts as emulsifying agent to incorporate oil into water, with reducing interfacial and surface tensions to exert better extract dispersion and penetration. Oil helps in solubilising the hydrophobic constituents contained in the plant extract and provide better adherence on the waxy seed coat. At first, the binary surfactant systems were developed and the physicochemical properties and mutual interactions were investigated. Later, the mixed surfactants were used to develop pre-emulsions by incorporating the rapeseed oil methyl esters (ROMEs) into aqueous phase. In the phase behaviour study, the centrifugally stable pre-emulsions which were able to form liquid crystalline phase (for sustained release) and the subsequent nano-emulsion dispersion (for delivery) were chosen with further modification to improve the sample long-term physical stability.

In the pre-emulsion formulation study, the leaf extract of *Mikania micrantha* Kunth ex H.B.K. was incorporated with the modified pre-emulsions to form the paste formulations. For product ease of application, the paste formulations were amended into the water-dispersible powder form. Lignocellulosic fibre waste of *M. micrantha* resulting from the solvent extraction was used as coating template for the paste formulations. The leaf fibre waste was modified through mercerisation to improve its surface characteristics for efficient paste coating. The water-dispersible powder formulations were subjected to physicochemical characterisation, sustained release profiling and dose response study. In the glasshouse study, the water-dispersible powder formulations were applied along with a commercial formulation for phytotoxicity comparison against the weed *E. colona* and the impact on the productivity of the rice *O. sativa*.

1.5 Outlines of Thesis

This thesis is mainly divided into six chapters. Chapter 1 describes the background of study, research problems, research objectives, scope of study and outlines of thesis about the importance of pesticides in escalating crop productivity, main challenges of natural product use in weed control and the focus of study on allelopathic plant extracts and the chosen of leaf extract of *Mikania micrantha* H.B.K. for pre-emulsion formulation in controlling a rice weed, *Echinochloa colona* (L.) Link. Chapter 2 compiles the literature review related to natural products on allelopathy of invasive plants including *M. micrantha*, statistical optimisation technique of the plant material extraction, development of formulation excipients from surfactants to liquid crystal emulsions to nano-emulsion delivery systems, potential use of lignocellulosic fibre as water-dispersible powder and brief introduction on the rice weed, *E. colona*. Chapter 3 discusses the phytoinhibitory activities of invasive plant extracts against the seed germination and seedling growth of *E. colona*, phytochemical tests and spectroscopic identification of putative allelochemicals and statistical optimisation of extraction

method of the invasive plant materials. Chapter 4 discusses the formulation excipients from surfactants to pre-emulsions to liquid crystal/gel emulsions to nano-emulsions and the pre-emulsion formulation of leaf extract of *M. micrantha*. Chapter 5 discusses the modification of lignocellulosic fibre from the leaf waste of *M. micrantha* as water-dispersible powder of the pre-emulsion formulations, physicochemical characterisations and bioefficacy performance of the water-dispersible powders against *E. colona* in glasshouse study. Chapter 6 concludes the general findings which achieved the main objectives and recommendations for future studies.

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