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

FS 2018 79
PHYTOINHIBITION AND FORMULATION OF ALLELOPATHIC EXTRACT OF MIKANIA MICRANTHA KUNTH EX H.B.K. AS PRE-EMERGENT WEED SUPPRESSANT AGAINST ECHINOCHLOA COLONA (L.) LINK

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

June 2018
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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

By

LIM CHAW JIANG

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 ($\Phi_w$) 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 ºC 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

Pengerusi : Profesor Mahiran Basri, PhD
Fakulti : Sains

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 noksis. 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 noksis, 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 dipolialkoslato 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 isoterma kepada pecahan berat ($\Phi_w$) 0.8 dan 0.6. Dalam kajian reologi, sampel ini menunjukkan kelakuan penipisan ricih, viskoelastik linear (LVE) ($G'^{>}G''$) dan pelembutan terikan ($G'^{>} \sigma$). Dalam kajian mesomorfik, sampel E1, E2 dan E3 memaparkan vesikel multilamellar, fasa kubik bikontinyu dan fasa multilamellar 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 °C daripada ekstrak daun tanpa formulasi.

Bagi memudahkan aplikasi produk, formulasi pra-emulsi F1, F2 dan F3 dimuatkan ke serat lignoselulosa dimerseter 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 pelan awal kemudian berturut mencapai sejumlah 63.66-86.52% pada 168 j, manakala pembebasan fenolik daun 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.
ACKNOWLEDGEMENTS

First and foremost, I thank faithfully the God’s almighty blessings for academic accomplishment of my doctoral research. Pursuing a fresh topic and laborious PhD project has gained me plentiful knowledge and unique experience but with intense time, health and cost incurred through completing many harsh challenges. Frankly speaking, I was frequently stumbled down by unprecedented obstacles that hard to cope with in limited time. But, then self-resurged with my ambitious hope, curiosity, enthusiasm and faith, and took initiative and strived hardly with copious amounts of literature for grasping the newfound knowledges, and with repeating experiments in stepwise to figure out the solutions for obscure problems. As mentioned by the public expression of “no pain no gain”, I feel glad and thank to myself for completing the research works and treatise writing. Towards the end of research journey, struggling in tight timeframe has learnt me the every minute is precious at a glimpse of time. Meanwhile, the research findings within this thesis would never have been so fruitful without the help of these people who always stood beside me.

I would like to express my profound gratitude wholeheartedly to my supervisor, the late Prof. Dr. Mahiran Basri for her time and wisdom that were devoted to give professional guidance, constructive advices, corrective comments, unstinted support, encouragement and trust throughout the entire project. Under her supervision, it was my great pleasure to undertake this project in colloid field with rewarding experience. I am equally thankful to my supervisory committee members, Prof. Dr. Gwendoline Ee Cheng Lian and Prof. Dr. Dzolkhifli Omar, and a lecturer from Universiti Tunku Abdul Rahman, Dr. Lim Chan Kiang for sparing their valuable time to give expertise support, credible ideas, insightful guidance and generous help which have been a path-finding torchlight in my foundation work. Once again to express my utmost appreciation and salute for their ever-cooperating character, brainstorming discussions and intellectually stimulating research meetings. Besides that, I would like to sincerely acknowledge all the authors in the bibliography which I benefited from them in thesis writing and ultimately bringing up a quality dissertation in the present elegant form.

With warmest expression, I extend my heartfelt gratitude to all those dearest friends for being enormously involved in the tedious works including large scale collection of weeds, visiting in the paddy field for weed seeds collection, evaluation on in vitro screening bioassay and glasshouse study. Thank you for their great effort and a round of applause to them, Mahashanon Arumugam, Balakrishnan Shanmugam, Allegrendran Rajendran, Karthikumar Venkatchalam and Then Yoon Yee. Again thank to my closest friend, Mahashanon Arumugam who voluntarily assisted me and for being great friendship with his incessant inspiration and as a well-wisher. Additionally, I am very thankful to the chemical supplier from Laboratory & Scientific Enterprise, Mr. Tey Meng Tiong for his great help in ordering chemicals with fast service and voluntary technical support. Alongside thank you to Mr. William Ewbank from Ajinomoto OmniChem, Belgium, for providing me complimentary surfactant and oil samples, in making smooth my research progress.
I am cordially thankful to laboratory staffs and technicians who had given me precious assistance with laboratory instrument and facilities through their kindness and exemplary dedication towards part of my research works. They are UPM staffs, Madam Rusnani Amirudin for help in IR analysis, Ms. Nor Azlina Shari for assisting in TGA analysis (Department of Chemistry, Faculty of Science), Ms. Norhayati Yusuf for guiding in particle size and zeta potential analyses, Ms. Norsharina Ismail for aiding in UV analysis (Laboratory of Molecular Biomedicine, Institute of Bioscience), Madam Irmazian Abdul Shukor, Mr. Rafiuz Zaman Haroun and Mr. Azmi Mohd. Amban in handling TEM, FESEM and SEM analyses, respectively (Microscope Unit, Institute of Bioscience), Mr. Jarkasi Sarbini and Mr. Mohamed Zaki Yusoff for providing the guidance and facility in the glasshouse study (Toxicology Laboratory, Faculty of Agriculture), and also extended to non-UPM staffs including Madam Azney Zuhailly Md. Taib for attributing to LC-MS analysis (Proteomics and Metabolomic Lab, Malaysia Genome Institute) and Ms. Zaharah Harun for serving in GPC analysis (Polymer Research Centre, Universiti Kebangsaan Malaysia).

Likewise, it is my pleasure to take this opportunity to promote my heartfelt thank you to Ministry of Higher Education (MOHE) in financing me MyPhD scholarship (September 2011 to February 2015) under MyBrain 15 programme throughout my PhD study. My immense thank you to Universiti Putra Malaysia for about fourteen years (2004-2018) that I have spent here, this green campus has been impressive and memorable for harmony with nature. This memory will be always etched in my mind.

Last but certainly not least, I express my loving thanks and heartfelt adoration to my beloved parents and siblings for their pillars of support, unflinching love and care, endless patience, and countless blessings which injecting me with constant sources of inspiration, confidence and strength to fight all odds in taking up this PhD study. If without them, it would have not been possible with the best of my ability to finish this bitter but fantastic journey. I am very fortunate to have them and I feel forever indebted to my parents. I will always cherish you all.
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.

Members of the Thesis Examination Committee were as follows:

**Abdul Halim bin Abdullah, PhD**
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**Mansor b Hj Ahmad @ Ayob, PhD**
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

**Abdul Shukor b Juraimi, PhD**
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

**Abdul Khaliq, PhD**
Professor
University of Agriculture
Pakistan
(External Examiner)

RUSLI HAJI ABDULLAH, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 September 2018
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:

**Mahiran Basri, PhD**  
Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Gwendoline Ee Cheng Lian, PhD**  
Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

**Dzolkhifli Omar, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

**ROBIAH BINTI YUNUS, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________ Date: __________________

Name and Matric No.: Lim Chaw Jiang (Gs30407)
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) are adhered to.

Signature: __________________________________________
Name of Chairman of Supervisory Committee: Prof. Dr. Mahiran Basri

Signature: __________________________________________
Name of Member of Supervisory Committee: Prof. Dr. Gwendoline Ee Cheng Lian

Signature: __________________________________________
Name of Member of Supervisory Committee: Prof. Dr. Dzolkhifli Omar
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
</tr>
<tr>
<td>3</td>
<td>PHYTOINHIBITORY ACTIVITIES, PUTATIVE ALLELOCHEMICALS IDENTIFICATION AND EXTRACTION OPTIMISATION OF THE INVASIVE PLANT EXTRACTS AGAINST <em>ECHINOCLOA COLONA</em> (L.) LINK</td>
</tr>
</tbody>
</table>

| ABSTRACT | iii |
| ACKNOWLEDGEMENTS | v |
| APPROVAL | vii |
| DECLARATION | ix |
| LIST OF TABLES | xiv |
| LIST OF FIGURES | xvii |
| LIST OF SCHEMES | xxii |
| LIST OF ABBREVIATIONS | xxiii |

| 1.1 | Background of Study | 1 |
| 1.2 | Research Problems | 3 |
| 1.3 | Research Objectives | 4 |
| 1.4 | Scope of Study | 4 |
| 1.5 | Outlines of Thesis | 5 |

| 2.1 | Natural Products for Crop Protection | 7 |
| 2.1.1 | Alelopathy | 7 |
| 2.1.2 | Invasive Plants | 8 |
| 2.1.3 | Phytoactive Constituents | 11 |
| 2.1.4 | Mechanisms of Action of Allelochemicals | 15 |
| 2.1.5 | Optimisation of Extraction Processes | 18 |
| 2.2 | Formulation Development | 20 |
| 2.2.1 | Formulation Excipients | 21 |
| 2.2.2 | Safer Release Formulation | 28 |
| 2.2.3 | Nanodelivery Systems | 31 |
| 2.2.4 | Water-Dispersible Powder | 33 |
| 2.3 | Rice Weed | 34 |
| 2.4 | Summary | 37 |

| 3.1 | Introduction | 38 |
| 3.2 | Materials and Methods | 38 |
| 3.2.1 | Materials | 38 |
3.2.2 Phytotoxic Activity Study of Invasive Plant Extracts Against Rice Weed *E. colona* 39
3.2.3 Phytochemical Studies of the Top-Ranked Invasive Plant Extracts 42
3.2.4 Statistical Optimisation of Extraction Conditions of the Leaf Extracts Using Response Surface Methodology 46

3.3 Results and Discussion 49
3.3.1 Phytotoxic Activities of the Invasive Plant Extracts Against *E. colona* 49
3.3.2 Phytochemical Studies of the Top-Ranked Invasive Plant Extracts 54
3.3.3 Response Surface Optimisation of the Extraction Conditions for the Top-Ranked Phytotoxic Leaf Extracts 59

3.4 Conclusion 67

4 CONSTRUCTION AND PHYSICOCHEMICAL CHARACTERISATIONS OF PRE-EMULSIONS IN THE FORMULATION OF LEAF EXTRACT OF *MIKANIA MICRANTHA KUNTH EX H.B.K.*

4.1 Introduction 69
4.2 Materials and Methods 69
4.2.1 Materials 69
4.2.2 Surfactant Study 70
4.2.3 Development of Pre-Emulsion Systems 74
4.2.4 Preparation of Plant Extract Pre-emulsion Formulations 79

4.3 Results and Discussion 82
4.3.1 Concentrated Surfactant Systems 82
4.3.2 Formation of Pre-emulsion Systems 92
4.3.3 Plant Extract Pre-emulsion Formulation 112

4.4 Conclusion 116

5 FABRICATION, PHYSICOCHEMICAL CHARACTERISATIONS AND EFFICACY OF WATER-DISPERSIBLE POWDERS OF PRE-EMULSION FORMULATIONS AGAINST *ECHINOCHLOA COLONA* (L.) LINK AND PRODUCTIVITY OF *ORYZA SATIVA* L.

5.1 Introduction 118
5.2 Materials and Methods 118
5.2.1 Materials 118
5.2.2 Preparation of Lignocellulosic Fibre Template Loading
5.2.3 Preparation of Water-Dispersible Powder Formulations
5.2.4 Glasshouse Experiment
5.3 Results and Discussion
  5.3.1 Lignocellulosic Fibre as Loading Template
  5.3.2 Water-Dispersible Powders
  5.3.3 Glasshouse Experiment
5.4 Conclusion

6 CONCLUSIONS AND RECOMMENDATIONS
  6.1 Conclusions
  6.2 Recommendations for Future Research

REFERENCES
APPENDICES
BIODATA OF STUDENT
LIST OF PUBLICATIONS
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Fatty acid compositions of different rapeseed oils with iodine value, saponification value and solidification point</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>Experimental range of independent variables specified in coded and actual levels for extraction conditions of the leaf extracts using face-centered central composite design (FCCD)</td>
<td>47</td>
</tr>
<tr>
<td>3.2</td>
<td>Summary on the hormesis response of the <em>E colona</em> seedlings treated by the invasive plant extracts as compared to the untreated and blank controls</td>
<td>50</td>
</tr>
<tr>
<td>3.3</td>
<td>Phytochemical characterisation of the crude leaf extracts: <em>M. micrantha, C. hirta, D. linearis</em> and <em>A. conyzoides</em></td>
<td>55</td>
</tr>
<tr>
<td>3.4</td>
<td>Yield weights and total phenolic contents of the crude leaf extracts of <em>M. micrantha, C. hirta, D. linearis</em> and <em>A. conyzoides</em></td>
<td>56</td>
</tr>
<tr>
<td>3.5</td>
<td>Qualitative and quantitative LC-MS analysis of the identified aromatic/phenolic allelochemicals in the crude leaf extracts of <em>M. micrantha, C. hirta, D. linearis</em> and <em>A. conyzoides</em></td>
<td>57</td>
</tr>
<tr>
<td>3.6</td>
<td>Analysis of variance (ANOVA) determines the suitability of the developed quadratic model variables in fitting the experimental data which showing linear, quadratic and interaction terms on each response after backward elimination of the non-significant terms</td>
<td>60</td>
</tr>
<tr>
<td>3.7</td>
<td>Validation of the data set for the reduced quadratic models after backward elimination of the non-significant terms</td>
<td>63</td>
</tr>
<tr>
<td>3.8</td>
<td>Experimental data for the verification of predicted germination inhibition at the selected optimum conditions</td>
<td>67</td>
</tr>
<tr>
<td>4.1</td>
<td>Correlation between zeta potential and colloidal dispersion system of the solution</td>
<td>81</td>
</tr>
<tr>
<td>4.2</td>
<td>Physical characterisations on flow behaviour, consistency coefficient and refractive index of the mono- and binary surfactant systems</td>
<td>84</td>
</tr>
</tbody>
</table>
4.3 Mole fraction of PAFA surfactant in the mixed solute (α) and mixed micelles (X₁), experimental mixed CMC (CMC_{exp}), ideal mixed CMC (CMC_{mixed}), micellar interaction parameter (β) and activity coefficients (f₁ and f₂) for the binary PAFA-based surfactant systems, as determined by the surface tension measurement

4.4 Mean particle size, PDI and zeta potential of the individual/mixed surfactants at critical micelle concentration (CMC)

4.5 Chemical composition of the pre-emulsion concentrates chosen from ternary phase diagrams for nascent screening of the formation of nano-emulsions

4.6 The profiles of mean droplet size, PDI and zeta potential of the nano-emulsions produced resulting from the dilution and agitation of the pre-emulsion concentrates P1-P12

4.7 Chemical composition of the modified pre-emulsion concentrates containing surfactants, ROMEs and sodium silicate

4.8 Physical stability results of the pre-emulsions E1, E2 and E3 resulting from the centrifugal acceleration

4.9 Rheological parameters depict flow behaviour index and consistency coefficient of the pre-emulsion concentrates and in different diluted weight fractions of Φ_w(0.8), Φ_w(0.6), Φ_w(0.4) and Φ_w(0.2)

4.10 Oscillatory amplitude profiles of viscoelastic liquid crystal/gel emulsions including yield stress, strain amplitude and phase angle, at the cross points of stress moduli G’=G”

4.11 Mean droplet size, PDI and zeta potential values of the nano-emulsions NE1, NE2 and NE3

4.12 Chemical compositions of the pre-emulsion formulations of M. micrantha leaf extract (F1-F3) and the pure leaf extract of M. micrantha (EX)

4.13 Physicochemical stability of pre-emulsion formulations and a non-formulated leaf extract resulting from different accelerated storage conditions

5.1 Chemical compositions of the crude and mercerised fibres
5.2 Decomposition temperatures at various weight losses of the crude and mercerised fibres at different temperatures

5.3 Crystallinity index of the unmodified and mercerised fibres

5.4 Total phenolic content of the water-dispersible powders (TPC_{WDP}), pre-emulsion (TPC_{pre-emulsion}) and extract (TPC_{extract})

5.5 Crystallinity indices of the water-dispersible powders

5.6 The in vitro release kinetics parameters of phenolic constituents from the different water-dispersible powders

5.7 The particle sizes, polydispersity indices, zeta potentials and surface tensions promoted by the water-dispersible powders after dissolution in water

5.8 Extrapolated ED_{50}, ED_{80} and ED_{95} values of the formulated and unformulated water-dispersible powders on the germination inhibition of *E. colona*, from nonlinear regression fit of dose response curves within the assessed concentration (0 – 212.18 g WDP/m²) in order to determine the bioefficacy comparison between the samples

5.9 Summary on the inhibitory responses of the *E. colona* germination and seedling growth with treated water-dispersible powders, commercial Satunil® and non-treated weedy control

5.10 Summary on the physical development and grain yield of the rice variety MR219 treated by the water-dispersible powders in comparison with a commercial formulation, weedy and weed-free controls
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The different invasive plant species for use in phytotoxicity screening to seek their capability of inhibition against <em>E. colona</em></td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemical structure of the putative phenolic/aromatic allelochemicals</td>
<td>13</td>
</tr>
<tr>
<td>2.3</td>
<td>The central composite design (CCD) for three process factors (X, Y and Z) comprising of central, axial and factorial points. The different positions of the axial points classify the CCD types of (a) CCC ($\alpha&gt;1$, $\alpha&lt;-1$) and (b) FCC ($\alpha=1$)</td>
<td>19</td>
</tr>
<tr>
<td>2.4</td>
<td>The estimated response surface model displayed in (a) three dimensional (3D) and (b) contour plots using the FCC design in determining the optimum response with interactive effect between the two factors</td>
<td>19</td>
</tr>
<tr>
<td>2.5</td>
<td>Graphical illustration of a surfactant molecule containing the hydrophilic and hydrophobic portions</td>
<td>21</td>
</tr>
<tr>
<td>2.6</td>
<td>Formation of various self-assembly structures of amphiphilic molecules at different surfactant concentration</td>
<td>23</td>
</tr>
<tr>
<td>2.7</td>
<td>Chemical structure of the PAFA non-ionic surfactant containing alkyl chain length in x units, ethylene oxide (EO) in y units and propylene oxide (PO) in z units</td>
<td>24</td>
</tr>
<tr>
<td>2.8</td>
<td>Chemical structure of the alkyl sulfonate (AS) anionic surfactant containing alkyl chain ($R'=\text{C}<em>{14}\text{-C}</em>{17}$) and a sulfonate (anion) group</td>
<td>24</td>
</tr>
<tr>
<td>2.9</td>
<td>Chemical structure of the cocamidopropyl betaine (CB) zwitterionic surfactant containing alkyl chain ($R''=\text{C}<em>{5}\text{-C}</em>{18}$), amide, quaternary ammonium (anion) and carboxylate (cation) groups</td>
<td>25</td>
</tr>
<tr>
<td>2.10</td>
<td>Chemical structure of the alkyl polyglucosides (APG) non-ionic surfactant containing alkyl chain ($R'''=\text{C}<em>{9}\text{-C}</em>{11}$, DP=1.6) connected to a saccharide group with degree of polymerisation (DP)</td>
<td>25</td>
</tr>
<tr>
<td>2.11</td>
<td>Chemical structure of sodium silicate synthesised from the fusion of different proportions of silicon oxide ($\text{SiO}_2$) and sodium oxide ($\text{Na}_2\text{O}$)</td>
<td>28</td>
</tr>
</tbody>
</table>
2.12 Theoretical concentrations of pesticide release of the conventional and controlled release formulation in controlling pests, with the reference to minimum effective dose

2.13 Graphical illustration of densely packed multilamellar vesicles to form the vesicular gel emulsion. S = surfactant, O = oil and W = water

2.14 Graphical illustration of structural formation of O/W nano-emulsions where the oil droplets stabilised by surfactant molecules which dispersed in the bulk aqueous phase

2.15 Physical appearance of the predominant rice weed, *E. colona*

3.1 Inhibitory responses of (a) germination, (b) fresh weight, (c) shoot height and (d) radicle length (+SE) of *E. colona* at the concentration, 100 g BDWE/L.

3.2 Dose response curves represent the biological inhibition of *E. colona* by the top-ranked phytotoxic plant extracts in the concentration range, 0-100 g BDWE/L. The ED$_{50}$ values (g BDWE/L) of the leaf extracts were extrapolated: *A. conyzoides* (ED$_{50}$=72.48; $R^2=0.9904$), *C. hirta* (ED$_{50}$=51.61; $R^2=0.9188$), *D. linearis* (ED$_{50}$=70.64; $R^2=0.9622$) and *M. micrantha* (ED$_{50}$=29.14; $R^2=0.9955$)

3.3 Amount of the detected major allelochemicals: chlorogenic acid (CA), *p*-hydroxyphenylacetic acid (HPAA), gallic acid (GA) and protocatechuic acid (PA) as calculated from the leaf extracts of *A. conyzoides* (AC), *C. hirta* (CH), *D. linearis* (DL) and *M. micrantha* (MM) were listed: CA(AC) (91.48 mg/L) (2.58 x 10$^{-4}$ M), HPAA(AC) (58.54 mg/L) (3.85 x 10$^{-4}$ M), GA(CH) (94.94 mg/L) (5.58 x 10$^{-4}$ M), HPAA(DL) (116.05 mg/L) (7.63 x 10$^{-4}$ M), CA(MM) (37.85 mg/L) (1.07 x 10$^{-4}$ M) and PA(MM) (39.02 mg/L) (2.53 x 10$^{-4}$ M)

3.4 The three dimensional response surface plots (supported with contour plots) of germination inhibition % of *E. colona* with interaction effects: extraction time (min) versus stirring speed (rpm) for (a) *C. hirta* and (b) *A. conyzoides*; extraction time (min) versus methanol content (%) for (c) *M. micrantha*, (d) *D. linearis* and (e) *A. conyzoides*; stirring speed (rpm) versus methanol content (%) for (f) *C. hirta*, (g) *D. linearis* and (h) *A. conyzoides*
4.1 Phase diagram construction using different surfactant-oil ratios (SORs) and aqueous dilution of the pre-emulsions to produce disparate physical appearances of the emulsion systems

4.2 Apparent viscosity (ƞ) in correlation with shear rate (γ) of individual surfactants and PAFA-based mixed surfactants, for determination of Newtonian or non-Newtonian flow behaviour of the fluids

4.3 Surface tensions of mono- and binary surfactant systems at progressive increases in concentration: (a) PAFA and/or AS; (b) PAFA and/or CB, and (c) PAFA and/or APG

4.4 Variation of experimentally determined CMC_{exp} and theoretical calculated CMC_{mixed} of the binary surfactant mixtures of (a) PAFA-AS, (b) PAFA-CB and (c) PAFA-APG, as a function of mole fraction of PAFA

4.5 Ternary phase diagrams of ROMEs/PAFA-AS/water system at four MSRs of (a) 9:1, (b) 8:2, (c) 7:3 and (d) 6:4 (w/w)

4.6 Ternary phase diagrams of ROMEs/PAFA-CB/water system at four MSRs of (a) 9:1, (b) 8:2, (c) 7:3 and (d) 6:4 (w/w)

4.7 Ternary phase diagrams composed of ROMEs/PAFA-APG/water system at four MSRs of (a) 9:1, (b) 8:2, (c) 7:3 and (d) 6:4 (w/w)

4.8 Physical appearances of modified pre-emulsion concentrates E1, E2 and E3

4.9 Rheograms of apparent viscosity-shear rate relation of the pre-emulsion concentrates and in dilution at various weight fractions (Φ_w): (a) without dilution, (b) Φ_w(0.8), (c) Φ_w(0.6), (d) Φ_w(0.4) and (e) Φ_w(0.2)

4.10 Storage (G’) and loss (G’’) moduli as a function of strain amplitude (γ) at angular frequency (ω) of 1 rads^{-1}, for the colloidal liquid crystalline/gel phases: (a) E1 at Φ_w=0.8, (b) E2 at Φ_w=0.8, (c) E3 at Φ_w=0.8, (d) E1 at Φ_w=0.6, (e) E2 at Φ_w=0.6 and (f) E3 at Φ_w=0.6

4.11 Cole-Cole diagrams depict the dynamic-state rheological behaviour of the viscoelastic liquid crystal/gel emulsions E1-E3 of Φ_w=0.6 and 0.8 follow (a) the Maxwell model and (b) deviation from the Maxwell model. G’ is storage modulus and G’’ is loss modulus
4.12 Photomicrographs of the Maxwellian liquid crystal/gel emulsions (a) E1 of $\Phi_w=0.6$, (b) E2 of $\Phi_w=0.8$ and (c) E3 of $\Phi_w=0.6$

4.13 The formation of liquid crystal/gel emulsions at the bottom of glasses upon addition of water into the pre-emulsions E1, E2 and E3

4.14 Translucent bluish appearance of the O/W nano-emulsions NE1, NE2 and NE3

4.15 TEM micrographs demonstrate the morphological formation of nano-emulsions (a) NE1, (b) NE2 and (c) NE3 resulting from the aqueous dilution and isothermal agitation of the pre-emulsions E1, E2 and E3

4.16 Dark appearances of the pre-emulsion formulations (F1, F2 and F3) and the pure leaf extract of M. micrantha (EX)

5.1 Photographs depict the physical appearances of (a) glutinous aggregated crude fibre (UML) and (b) brittle fine mercerised fibre (MML)

5.2 FT-IR spectra of crude fibre (UML) and mercerised fibre (MML)

5.3 Thermal decompositions of crude (UML) and mercerised (MML) fibres by (a) thermogravimetric analysis and (b) differential thermogravimetry, in the temperature range of 50 °C to 1000 °C. (The highest peaks at UML=310.85 °C and at MML=326.65 °C)

5.4 XRD diffractograms of the crude fibre (UML) and the mercerised fibre (MML)

5.5 SEM micrographs of (a) crude fibre with smooth surface and (b) mercerised fibre with surface etching

5.6 Water absorption capacity of the crude (UML) and mercerised (MML) fibres

5.7 Coarse water-dispersible powders loading with different formulations of (a) WDP-F1, (b) WDP-F2 and (c) WDP-F3, and pure extract of (d) WDP-EX, were produced using the solvent percolation and evaporation (SPE) powderisation technique

5.8 XRD diffractograms of the water-dispersible powders
5.9 SEM micrographs of surface morphology of the water-dispersible powders (a) WDP-F1, (b) WDP-F2, (c) WDP-F3 and (d) WDP-EX

5.10 Water absorption capacities of the water-dispersible powders

5.11 *In vitro* phenolics release profiles from the water-dispersible powders over a period of 7 days

5.12 TEM micrographs depict the structural assembly and the particle release patterns from the water-dispersible powders of (i) WDP-F1, (ii) WDP-F2, (iii) WDP-F3 and (iv) WDP-EX

5.13 Dose response curves represent the biological inhibition of *E. colona* by the water-dispersible powders
LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Schematic diagram shows the biotic and abiotic factors which influencing the release of allelochemicals</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>A schematic overview of plant biosynthetic pathways leading to the formation of secondary metabolites</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic diagram of the routes of surfactant monomers to form saturation at air-water interface and spherical micelles in the bulk solution when the surfactant concentration reaching above the CMC</td>
<td>22</td>
</tr>
<tr>
<td>2.4</td>
<td>Synthesis of ROMEs from the raw rapeseed oils through transesterification process of triglycerides, with glycerol as side product</td>
<td>26</td>
</tr>
<tr>
<td>2.5</td>
<td>Formation of the LLC emulsions from the addition of water into the oil-surfactant mixtures with mixing to form variable morphological structures of the liquid crystals</td>
<td>29</td>
</tr>
<tr>
<td>2.6</td>
<td>Schematic representation of PIC procedure on the formation of O/W nano-emulsion resulting from the dilution of lamellar mesophase through changing of the surfactant spontaneous curvature using low intensity emulsification</td>
<td>33</td>
</tr>
<tr>
<td>2.7</td>
<td>The chemical structures of cellulose, hemicellulose and lignin which are the main components presented in the lignocellulosic fibre waste from the leaves of <em>M. micrantha</em> after solvent removal of the extractives</td>
<td>35</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
</tr>
<tr>
<td>ACO</td>
<td>1-aminocyclopropane-1-carboxylic acid oxidase</td>
</tr>
<tr>
<td>ACS</td>
<td>1-aminocyclopropane-1-carboxylic acid synthase</td>
</tr>
<tr>
<td>ANN</td>
<td>artificial neural networks</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ANT</td>
<td>adenine nucleotide translocase</td>
</tr>
<tr>
<td>APG</td>
<td>alkyl polyglucosides</td>
</tr>
<tr>
<td>AS</td>
<td>alkyl sulfonates</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>a.u.</td>
<td>arbitrary unit</td>
</tr>
<tr>
<td>BWDE</td>
<td>biomass dry weight equivalent</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CB</td>
<td>cocamidopropyl betaine</td>
</tr>
<tr>
<td>CCC</td>
<td>circumscribed central composite</td>
</tr>
<tr>
<td>CCD</td>
<td>central composite design</td>
</tr>
<tr>
<td>C-CD</td>
<td>charge-coupled device</td>
</tr>
<tr>
<td>CMC</td>
<td>critical micelle concentration</td>
</tr>
<tr>
<td>CRD</td>
<td>completely randomized design</td>
</tr>
<tr>
<td>CRF</td>
<td>controlled release formulation</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>DAS</td>
<td>day after sowing</td>
</tr>
<tr>
<td>DAT</td>
<td>days after treatment</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Term</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>DoE</td>
<td>design of experiments</td>
</tr>
<tr>
<td>DP</td>
<td>dust powder</td>
</tr>
<tr>
<td>DSR</td>
<td>direct-seeded rice</td>
</tr>
<tr>
<td>DTG</td>
<td>differential thermogravimetry</td>
</tr>
<tr>
<td>EC</td>
<td>emulsifiable concentrate</td>
</tr>
<tr>
<td>ED</td>
<td>effective dose</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FCC</td>
<td>face-centered central composite</td>
</tr>
<tr>
<td>FCCD</td>
<td>face-centered cube design</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier-transform infrared</td>
</tr>
<tr>
<td>FTR</td>
<td>flooded transplanted rice</td>
</tr>
<tr>
<td>GA</td>
<td>gibberellic acid</td>
</tr>
<tr>
<td>GAE</td>
<td>gallic acid equivalent</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>HIPE</td>
<td>high internal phase emulsion</td>
</tr>
<tr>
<td>HLB</td>
<td>hydrophile-lipophile balance</td>
</tr>
<tr>
<td>HR-TEM</td>
<td>high-resolution transmission electron microscopy</td>
</tr>
<tr>
<td>IAA</td>
<td>indole-3-acetic acid</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometer</td>
</tr>
<tr>
<td>LLC</td>
<td>lyotropic liquid crystal</td>
</tr>
<tr>
<td>LVE</td>
<td>linear viscoelastic</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MDS</td>
<td>molecular dynamic simulation</td>
</tr>
<tr>
<td>MLVs</td>
<td>multilamellar vesicles</td>
</tr>
<tr>
<td>MoAs</td>
<td>mode of actions</td>
</tr>
<tr>
<td>MSR</td>
<td>mixed surfactant ratio</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>O/W</td>
<td>oil-in-water</td>
</tr>
<tr>
<td>PAFA</td>
<td>polyalkoxylated fatty alcohols</td>
</tr>
<tr>
<td>PAL</td>
<td>phenylalanine ammonia-lyase</td>
</tr>
<tr>
<td>PALS</td>
<td>phase analysis light scattering</td>
</tr>
<tr>
<td>PCS</td>
<td>photon correlation spectroscopy</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PIC</td>
<td>phase inversion composition</td>
</tr>
<tr>
<td>PIT</td>
<td>phase inversion temperature</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PPOs</td>
<td>polyphenol oxidases</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>RCBD</td>
<td>randomized complete block design</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>ROMEs</td>
<td>rapeseed oil methyl esters</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RSM</td>
<td>response surface methodology</td>
</tr>
<tr>
<td>RST</td>
<td>regular solution theory</td>
</tr>
<tr>
<td>SAR</td>
<td>structure-activity relationship</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SL</td>
<td>soluble liquid</td>
</tr>
<tr>
<td>SOR</td>
<td>surfactant-oil ratio</td>
</tr>
<tr>
<td>SPE</td>
<td>solvent percolation and evaporation</td>
</tr>
<tr>
<td>SRF</td>
<td>sustained release formulations</td>
</tr>
<tr>
<td>TGA</td>
<td>thermogravimetric analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TPC</td>
<td>total phenolic content</td>
</tr>
<tr>
<td>UPLC</td>
<td>ultra-performance liquid chromatography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VOCs</td>
<td>volatile organic components</td>
</tr>
<tr>
<td>W/O</td>
<td>water-in-oil</td>
</tr>
<tr>
<td>WP</td>
<td>wettable powders</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
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).

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, clefloxidym, 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 phytotoxic inhibitory 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.
REFERENCES


Krishnaswamy, K., Orsat, V., Gariépy, Y. and Thangavel, K. (2013). Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (*Vitis vinifera*). *Food and Bioprocess Technology*, 6(2):441-455.


