Studies on Low-Toxic Sugar Ester Based Pesticides against *Rhyzopertha Dominica*

A dissertation submitted in fulfillment of the requirement for the degree of Master of Philosophy

by

Hsin-Yi Sheena Chen

August 2015

School of Chemical Engineering
Faculty of Engineering, Computer and Mathematical Sciences
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>i</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
</tbody>
</table>

CHAPTER 1

Background and Literature Review

1.1 Post-Harvest Losses          | 1  |
1.2 Rhyzopertha dominica         | 3  |
1.3 Integrated Pest Management   | 5  |
  1.3.1 Australian strategies and perspective for pest control of stored grain | 8  |
1.4 Chemical Insecticides        | 8  |
1.5 Diatomaceous Earth           | 12 |
  1.5.1 Effect of experimental conditions | 14 |
  1.5.2 Effect of insects         | 15 |
  1.5.3 Effect of DE properties   | 16 |
  1.5.4 Advantages and disadvantages | 17 |
1.6 Sugar fatty acid esters      | 18 |
  1.6.1 Insecticidal activity     | 18 |
  1.6.2 Extraction from natural sources | 22 |
  1.6.3 Synthetic methods         | 24 |
    1.6.3.1 Transesterification method | 24 |
    1.6.3.2 Acyl chloride esterification method | 27 |
CHAPTER 2

Experimental design and methodology

2.1 Chemicals and Materials 55
   2.1.1 Diatomaceous earth 55

2.2 Equipment 56

2.3 Synthesis of Sugar Esters 56

2.4 Characterisation of Sugar Esters 57
   2.4.1 FTIR 57
   2.4.2 NMR 58
   2.4.3 Contact Angle 59

2.5 Insecticide Bioassays 59
   2.5.1 Preparation of grain 59
   2.5.2 Selection of insects 60
   2.5.3 Comparative bioassays 61
   2.5.4 Lethal dose bioassays 62
   2.5.5 Surface bioassay 63

2.6 Grain bulk density 63

2.7 Angle of Repose 64

2.8 Optical Microscopy 64

2.9 Scanning electron microscopy (SEM) 65

References 67
CHAPTER 3

Synthesis and characterisation of sugar esters

3.1 Introduction 68
3.2 Materials and methods 69
  3.2.1 Synthesis and purification of sugar esters 69
  3.2.2 Characterisation 69
    3.2.2.1 FTIR 69
    3.2.2.2 NMR 69
    3.2.2.3 Contact angle 69
3.3 Results and Discussion 70
  3.3.1 Product yield 70
  3.3.2 FTIR 71
  3.3.3 Proton NMR 74
  3.3.4 Determine the HLB value 76
  3.3.5 Contact Angle 77
3.4 Conclusions 82

References 83

CHAPTER 4

Insecticidal activity of sugar esters against *Rhizopertha dominica*

4.1 Introduction 85
4.2 Materials and methods 86
  4.2.1 Sugar esters 86
  4.2.2 Bioassay 86
  4.2.3 HLB value calculation 86
  4.2.4 Contact angle 86
4.3 Results and Discussion 87
  4.3.1 Size of the sugar molecule, chain length of fatty acid 87
and their impact on insecticidal activity

4.3.2 Purification and the impact on insecticidal activity

4.3.3 Investigation of the mode of action of sugar esters

4.2.3.1 Contact angle

4.2.3.2 Hydrophilic-lipophilic balance value

4.2.3.3 Mode of feeding

4.2.3.4 Electron microscopic study

4.4 Conclusion

References

CHAPTER 5

Insecticidal activity of sorbitol octanoate combining with diatomaceous earth against \textit{Rhizopertha dominica}

5.1 Introduction

5.2 Materials and methods

5.2.1 Sorbitol octanoate

5.2.2 DE and Dryacide

5.2.3 Comparative bioassay

5.2.4 Lethal dose bioassay

5.3 Results and discussion

5.3.1 Efficacy of combined formulations of DE and sC8

5.3.2 Mode of insecticidal action

5.3.3 Effect of formulation treatments on grain properties

5.3.3.1 Grain colour

5.3.3.2 Bulk density

5.3.3.3 Angle of repose

5.4 Conclusion

References
Conclusions and Future Perspectives

6.1 Conclusion

6.2 Future direction

Appendix 1 IRAC MoA Classification v 7.3, February 2014

Appendix 2 NMR spectra of sugar esters

Appendix 3 Supplementing bioassay data

Appendix 4 Contact angles of sugar esters

Appendix 5 SEM images showing insect parts including mandible, tibia, tarsal segments and abdominal sternites after treated by DE-SO combined formulation
ABSTRACT

*Rhyzopertha dominica* is a destructive, cosmopolitan pest of major concern to post-harvest storage of grains and other products. Failing in control of primary insect pest such as *R. dominica* assist the attack of cereal commodities by secondary pests and thus greater damages and losses. Conventional treatments rely heavily on rapid eradication of pest insects using synthetic organic chemicals. However, serious trade-off was only learnt after the broad-spectrum toxicity threatens our eco-system as well as health and the emerging of insect resistance. Insecticide research during the past decades have shift focus to seek for safer alternatives, preferably from natural sources with low toxicity, to ensure the biosecurity of stored grains world-wide. Inert dusts such as diatomaceous earths (DE), the fossil sediments of marine algae mainly composed of silica dioxides, have been used as grain protectants. This biosilica with delicate microstructures has demonstrated control on several insect species including *R. dominica* without the development of resistance. The inhibition mechanism of these effects was believed to be the absorption of cuticle wax by the micro- and nano-pores on DE which leads to dehydration then death of target insects. However, application of DE on stored grains has not been widely adopted. This is mainly due to its negative influences on grain appearance and processing as a result of relatively large effective amounts required. Research in this field has been worked on ways to address this challenge and increase their market acceptance. One promising approach is to combine DE with other safe additives, such as sugar esters. Synthetic sugar ester is a group of low-toxic insecticides produced from natural sources. Previous studies have demonstrated this compound was capable in killing insects without causing any adverse effects to other organisms. Owing to the surfactant nature, these compounds were believed to desiccate target insects via interrupting the cuticular wax layer. In this research, sugar esters were for the first time assayed against *R. dominica*. The synergistic effect from combining with DE has also been evaluated. A series of esters of fatty acids with polyols have been synthesized via direct esterification using an environment-friendly solvent-less
method. These crude composites, namely sorbitol octanoate, sorbitol decanoate, sorbitol laurate, xylitol octanoate, xylitol decanoate and xylitol laurate were then purified using a two steps process. Qualitative analysis of the products was conducted using Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy methods. FTIR provided rapid and reliable identification for the characteristic functional group of sugar esters showing absorption bands. NMR spectra agree with the FTIR results, which have confirmed the formation of expected compounds. The insecticidal activity of these compounds was investigated where sorbitol octanoate was found to be the most effective at the concentration of 4000 ppm. No strong correlation of Hydrophilic-lipophilic balance values (HLB, calculated based on NMR spectra) associated with sugar ester’s killing effect was observed as suggested by other research groups. It was also realized that purification was essential in order to enhance insect mortality. Further study has revealed that the mechanism of this compound on killing the insects is by contact rather than ingestion. Combination of sorbitol octanoate with DE was also studied. Data analysis showed that while there was an improvement in the quantity of DE required, the effect is not quiet synergistic where combined is less effective than expected. Lethal dosage of DE that were required to kill over 90% of insect population have been reduced from 700 ppm to 100 ppm when combined with 4000 ppm of sugar ester. Bulk density tests indicated that this formulation can increase grain hectoliter mass by 2-fold and reduced the angle of repose from 34˚ to 31˚ as opposed to 36˚ by 100ppm DE alone. Addition of sugar ester has effectively reduced the friction between DE treated grains which was proven to be a favourable strategy. Adherence of DE on R. dominica’s exoskeleton were investigated by both optical microscope and scanning electron microscope (SEM). Both techniques have shown a significant reduction of DE attachment on insect body when combined with sugar esters. Our finding supports the theory that inhibition mechanism may be due to penetration of the lipophile section of sugar ester in the cuticular layer.
DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Hsin-Yi Sheena Chen

______________________

Date: _____________
ACKNOWLEDGEMENT

First and foremost, I would like to express my most sincere gratitude to my advisory team; Dr. Lucas Johnson, Prof. Dusan Losic, Dr. Patrick Collins and Prof. John Jennings, for their invaluable and continuous guidance of my MPhil research and study, without which it would be impossible to have these achievements during my M.Phil period. I would also like to give my thanks to Mr. Angus Netting, Ms. Ruth Williams, Mr. Ken Neubauer and the team from Adelaide Microscopy for their technical support during my research.

Besides, I would like to give my heartfelt thankfulness to my beloved family. Words cannot express my gratitude to my husband, Dr. Ji Liang, for his sacrifice, firm support and sustained encouragements during the last four years of my M.Phil. This thesis would not be possible without the support and love from him. Our son, Joshua Liang, was born during the time. It’s the love and responsibility to this new life which then become my most important strength to carry on.

Moreover, I want to thank the members in my research group for their support in my research; A/Prof. Mahaveer Kurkuri, Dr. Abel Santos, Dr. Diana Tran, Mrs. Shervin Kabiri, Dr. Moom Sinn Aw, Mr. John Hayles, Dr. Krishna Kant, Mr. Tushar Kumeria, Mr. Karan Gulati, Mr. Ye Wang and all other team members.

Last but not least, I want to express my earnest appreciation to the ECMS Faculty for the Divisional Scholarship, which has financially supported my living costs during this period.
LIST OF PUBLICATIONS

Journal


Conference Presentation


LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Common insect pests of stored grain</td>
<td>2</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Stored grain pest control strategies used in IPM</td>
<td>7</td>
</tr>
<tr>
<td>Table 1.3</td>
<td>Classification of major group of chemical insecticides and their modes of action</td>
<td>9</td>
</tr>
<tr>
<td>Table 1.4</td>
<td>Factors determining diatomaceous earth insecticide efficacy</td>
<td>15</td>
</tr>
<tr>
<td>Table 1.5</td>
<td>HLB values and the related functional properties</td>
<td>20</td>
</tr>
<tr>
<td>Table 1.6</td>
<td>The effect of synthetic sugar esters against insects of various species</td>
<td>36</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Products retained as to total weight of starting chemicals (w/w) after repeated washing</td>
<td>70</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Functional group characterisation using FTIR</td>
<td>71</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Ratio of reacted to unreacted fatty acids remaining in the purified sugar esters</td>
<td>75</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Fatty acid to sugar ratio for synthesized sugar esters</td>
<td>75</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Contact angles of grains with different concentrations of sorbitol octanoate</td>
<td>79</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Contact angles of grains treated with sugar esters at 1000 ppm concentration</td>
<td>80</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Toxicity of sugar ester on LGB prepared with polyol and fatty acids (4000ppm) having different chain length in 7, 14 and 60 days</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 4.2 Effect of sugar ester purification (4000ppm) on LGB mortality in 7 and 14 days and progeny formation in 60 days.

Table 4.3 Contact angles and their relative morality and progeny suppression of Katana grains coated with sugar esters at varied concentration (100 to 4000ppm)

Table 4.4 HLB value of sugar esters

Table 4.5 Morality of insect feeding on wheat granule with and without sugar ester coating (4000ppm) in 18 days

Table 5.1 Toxicity of sorbitol octanoate, DE and their combinations against LGB expressed in mortality (7 and 14 day) and progeny suppression (60th day)

Table 5.2 Lethal doses (LD50 / LD90) and relative potency (vs. Dryacide) of the optimal formulation and its component ingredients in 14 days
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Images of adult (left), larvae, pupae and eggs (right) of <em>R. dominica</em>.</td>
<td>3</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Damaged grains by <em>R. dominica</em></td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Microstructure of arthropod cuticle</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Development of insecticide resistance</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Examples of different morphology and microstructures of diatom frustules that comprise diatomaceous earth</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Examples of mono, di and poly-form of sugar ester</td>
<td>19</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Schematic representation of a cross section of tracheoles with liquid inside</td>
<td>22</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Molecular structures of naturally produced sugar esters</td>
<td>23</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Transesterification reaction</td>
<td>24</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>Transesterification reaction of sucrose forming sugar ester</td>
<td>25</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>Acyl chloride esterification of sucrose forming sugar ester</td>
<td>27</td>
</tr>
<tr>
<td>Figure 1.12</td>
<td>Molecular structure of sucrose</td>
<td>28</td>
</tr>
<tr>
<td>Figure 1.13</td>
<td>Mitsunobu esterification process</td>
<td>29</td>
</tr>
<tr>
<td>Figure 1.14</td>
<td>Selective acylation of sucrose using 3-acyl-5-methyl-1,3,4- thiazole-2(3H)-thiones</td>
<td>30</td>
</tr>
<tr>
<td>Figure 1.15</td>
<td>In-situ isomerization of sucrose with DBU or Et$_3$N.</td>
<td>30</td>
</tr>
</tbody>
</table>
Figure 1.16 Process of dibutylstannylene-acetal condensation forming dibutylstannylene-acetal

Figure 1.17 Process of sucrose ester formation by dibutylstannylene-acetal condensation

Figure 1.18 Synthesis of sugar esters using enzymes as catalysts

Figure 1.19 Lipase catalysed synthesis of sugar ester using vinyl ester as starting chemicals

Figure 1.20 Protease catalysed formation of sucrose

Figure 2.1 FTIR spectrophotometer equipment with attenuated total reflection system

Figure 2.2 Schematic setup of NMR

Figure 2.3 Photo of the jar for the bioassay

Figure 2.4 Setup of BK Plus Imaging System

Figure 2.5 Schematic illustration of insect body parts that are prompted to attacks by surface active insecticides such as DE

Figure 3.1 FTIR spectra of the raw materials and synthesized esters (a) fatty acids and (b) polyols, and end products (c) sorbitol esters and (d) xylitol esters

Figure 3.2 FTIR spectra of products obtained from each purification step (a) sorbitol octanoate; (b) sorbitol decanoate; (c) sorbitol laurate; (d) xylitol octanoate; (e) xylitol decanoate; (f) xylitol laurate

Figure 3.3 Normalized NMR spectra of sorbitol octanoate

Figure 3.4 HLB values and associated water solubility
Figure 4.1 SEM images showing the antenna, sternites, and pronotum and elytra of *Rhyzopertha dominica*, before (a, c, e) and after (b, d, f) exposure to grains treated with 4000ppm sorbitol octanoate.

Figure 5.1 Insect mortality of LGB placed in DE treated grains in 14 days.

Figure 5.2 Insect mortality of LGB placed in sorbitol octanoate treated grains in 14 days.

Figure 5.3 Insect mortality of LGB placed in Dryacide® treated grains in 14 days.

Figure 5.4 Insect mortality of LGB placed in DE / sorbitol octanoate treated grains in 14 days.

Figure 5.5 Images of insects treated with DE dust at different concentration.

Figure 5.6 Images of insects treated with sC8 at different concentration.

Figure 5.7 Images of insects treated with DE (varied in concentration) combined with 4000ppm sC8.

Figure 5.8 Images of insects treated with 2000ppm and 4000ppm sC8 combined with DE at varied levels.

Figure 5.9 Pronotum of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification.

Figure 5.10 Elytra of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification.

Figure 5.11 Images of Katana grains dusted with DE at varied concentration.
Figure 5.12 Bulk density (kg/hL) of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm)

Figure 5.13 Angle of repose of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm)