UNIVERSITI PUTRA MALAYSIA

BIOLOGY AND DEVELOPMENT OF PROTOCOLS FOR CULTURING SOIL-DWELLING ENDOGEIC WORM Pontoscolex corethrurus MÜLLER FOR POTENTIAL USE IN SOIL REHABILITATION

KOK HO YIN

FS 2014 26
BIOLOGY AND DEVELOPMENT OF PROTOCOLS FOR CULTURING
SOIL-DWELLING ENDOGEIC WORM Pontoscolex corethrurus MÜLLER
FOR POTENTIAL USE IN SOIL REHABILITATION

By

KOK HO YIN

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

May 2014
All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

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

BIOLOGY AND DEVELOPMENT OF PROTOCOLS FOR CULTURING SOIL-DWELLING ENDOGEIC WORM Pontoscolex corethrurus MÜLLER FOR POTENTIAL USE IN SOIL REHABILITATION

By

KOK HO YIN

May 2014

Chairman : Nor Azwady Abd. Aziz, PhD
Faculty : Science

Pontoscolex corethrurus (Müller, 1857) is the dominant tropical soil-dwelling endogeic worm and has the ability to live in a variety of soils differing in pH and organic matter content. This suggested that this species may serve as a potential agent for soil rehabilitation particularly in the tropics. Unfortunately, the relevant information about P. corethrurus is very limited in Malaysia. A detailed understanding of life history of P. corethrurus is required because the biological characteristics of earthworms (e.g., growth and reproduction rates) could affect their potential in soil rehabilitation programs. The rates of growth and reproduction of P. corethrurus on different stocking densities were also evaluated to determine the optimum stocking density for culturing this species. This study discovered that the optimum stocking for culturing P. corethrurus was around 200 earthworms per m². This study also reported that P. corethrurus has a high survival rate and has the capability to reproduce via parthenogenesis. These biological characteristics enable P. corethrurus to colonize new environments hence, increasing its potential for the use in worm inoculation activities. In order to obtain a large and consistent supply of earthworms for worm inoculation activities in soil rehabilitation, indoor culturing of P. corethrurus is crucial. However, there are difficulties in using natural soil as the medium for culturing endogeic worms due to the heterogeneous nature of soils in different localities that will surely affect the culture performance. Thus, a protocol was developed using artificial soil to replace nature soil for culturing P. corethrurus. This study found that clay content can affect the growth of P. corethrurus and the manipulation of the clay content in artificial medium (consists of kaolinite clay, industrial quartz sand and decomposed cow dung) could result it as an alternative medium for culturing P. corethrurus. Furthermore, the properties of earthworm surface cast, worm-worked soil and bulk soil (soil without earthworm inoculation) were analyzed to study the influence of P. corethrurus activities on soil properties. Earthworm surface cast and worm-worked soil recorded significantly higher amounts of available nutrients than bulk soil. Soil enzymes (protease and acid phosphatase) activities, microbial (bacteria) population and humic acid content were also significantly
higher in earthworm cast than in bulk soil. A subsequent polybag-plant experiment was conducted to determine how the presence of *P. corethrurus* in soil could benefit plant productivity. With the application of organic amendment, the inoculation of *P. corethrurus* into the planting medium (+E+O treatment) significantly increased plant height (20%), root (27%) and shoot (37%) dry weights as well as crop yield (15%) compared to treatment without earthworm inoculation (–E+O treatment). The present study suggested that the tropical soil-dwelling endogeic worm *P. corethrurus* can be considered as a good soil rehabilitation agent for rehabilitating soil and increasing plant productivity.
Abstract tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOLOGI DAN PEMBANGUNAN PROTOKOL PENGKULTURAN CACING TANAH ENDOGEIK, *Pontoscolex corethrurus* MÜLLER YANG BERPOTENSI DALAM PEMULIHAN TANAH

Oleh

KOK HO YIN

Mei 2014

Pengerusi : Nor Azwady Abd. Aziz, PhD
Fakulti : Sains

(bakteria) dan kandungan asid humik dalam tinja cacing juga lebih tinggi daripada tanah tanpa cacing. Seterusnya, eksperimen polibeg-tumbuhan dijalankan untuk menentukan sama ada kehadiran *P. corethrurus* dalam tanah boleh memberi manfaat kepada produktiviti tanaman. Dengan pembajaan, inokulasi *P. corethrurus* ke dalam tanah (+E+O) meningkatkan tinggi tumbuhan (20%), berat kering akar (27%) dan pucuk (37%) serta peningkatan hasil tanaman (15%) berbanding dengan rawatan tanpa kehadiran cacing (–E+O). Kajian ini mencadangkan cacing tanah endogeik tropika *P. corethrurus* boleh berfungsi sebagai ejen pemulihan tanah yang baik untuk memulihkan tanah dan meningkatkan productiviti tanaman.
ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest appreciation to my Supervisor, Dr. Nor Azwady Abd. Aziz for his supervision, advice on research methodology, the art of scientific writing, and his valued support and friendship throughout this study. Without his guidance and persistent help, this thesis would not have been possible. Thanks should also be extended to my Co-Supervisors, Associate Professor Dr. Muskhaizli Mustafa and Dr. Syaizwan Zahmir Zulkifli, for their guidance and valuable suggestions.

Special thanks to Encik Baharuddin and Puan Zaleha from Organic and Aroma Section, Taman Pertanian Universiti (TPU), for their assistance and friendship throughout the field experiment of my study. I would also like to thank all the Laboratory Assistants in the Department of Biology for their help and encouragement.

In addition, I would like to take this opportunity to record my thanks to my fellow postgraduate students in the Department of Biology, especially Khye Er, Suk Kuan and Yi Wei from Vermitechnology Laboratory for their help and unselfish sharing of knowledge and skills in overcoming problems that arose in my study.

Lastly, I would like to express my heartfelt thanks to my beloved family, especially my dad for his patience and moral support during my days at the university.
I certify that a Thesis Examination Committee has met on 8 May 2014 to conduct the final examination of Kok Ho Yin on her thesis entitled "Biology and Development of Protocols for Culturing Soil-Dwelling Endogeic Worm Pontoscolex corethrurus Müller for Potential Use in Soil Rehabilitation" 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:

Shamarina binti Shohaimi, PhD  
Senior Lecturer  
Faculty of Science  
Univeristi Putra Malaysia  
(Chairman)

Hishamuddin bin Omar, PhD  
Senior Lecturer  
Faculty of Science  
Univeristi Putra Malaysia  
(Internal Examiner)

Mohamed Hanafi bin Musa, PhD  
Professor  
Faculty of Agriculture  
Univeristi Putra Malaysia  
(Internal Examiner)

José Adolfo Amador, PhD  
Professor  
University of Rhode Island  
United States  
(External Examiner)

NORITAH OMAR, PhD  
Associate Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 21 July 2014
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Nor Azwady Abd Aziz, PhD**  
Senior Lecturer  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Muskhazli Mustafa, PhD**  
Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

**Syaizwan Zahmir Zulkifli, PhD**  
Lecturer  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

**BUJANG KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

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: 7July 2014

Name and Matric No.: Kok Ho Yin (GS24393)
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: Dr. Nor Azwady Abd Aziz
Senior Lecturer Faculty of Science Universiti Putra Malaysia

Signature: 
Name of Member of Supervisory Committee: Assoc. Prof. Muskhazli Mustafa
Associate Professor Faculty of Science Universiti Putra Malaysia

Signature: 
Name of Member of Supervisory Committee: Dr. Syazwan Zahmir Zulkifli
Lecturer Faculty of Science Universiti Putra Malaysia
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2  LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>2.1 Soil biota</td>
<td>4</td>
</tr>
<tr>
<td>2.2 Earthworms</td>
<td>4</td>
</tr>
<tr>
<td>2.2.1 Earthworm ecological categories</td>
<td>4</td>
</tr>
<tr>
<td>2.2.2 Earthworm biological characteristics</td>
<td>7</td>
</tr>
<tr>
<td>2.3 Earthworm-related studies in Malaysia</td>
<td>7</td>
</tr>
<tr>
<td>2.4 A potential species for soil rehabilitation in the tropics:</td>
<td>8</td>
</tr>
<tr>
<td>endogeic worm <em>Pontoscolex corethrurus</em></td>
<td></td>
</tr>
<tr>
<td>2.5 Cultivation of soil-dwelling earthworms</td>
<td>10</td>
</tr>
<tr>
<td>2.5.1 Stocking density</td>
<td>10</td>
</tr>
<tr>
<td>2.5.2 Temperature</td>
<td>11</td>
</tr>
<tr>
<td>2.5.3 Moisture content</td>
<td>12</td>
</tr>
<tr>
<td>2.5.4 Organic matter</td>
<td>12</td>
</tr>
<tr>
<td>2.5.5 Soil texture</td>
<td>13</td>
</tr>
<tr>
<td>2.5.6 Soil pH</td>
<td>14</td>
</tr>
<tr>
<td>2.6 Earthworm activities on soil properties</td>
<td>14</td>
</tr>
<tr>
<td>2.6.1 Burrowing activity</td>
<td>14</td>
</tr>
<tr>
<td>2.6.2 Feeding activity and gut transition</td>
<td>16</td>
</tr>
<tr>
<td>2.6.3 Casting activity</td>
<td>17</td>
</tr>
<tr>
<td>2.7 Earthworms and soil ecosystems</td>
<td>19</td>
</tr>
<tr>
<td>2.7.1 Land improvement</td>
<td>19</td>
</tr>
<tr>
<td>2.7.2 Plant productivity</td>
<td>20</td>
</tr>
<tr>
<td>2.8 Summary</td>
<td>20</td>
</tr>
<tr>
<td>3  EFFECT OF STOCKING DENSITY ON SURVIVAL, GROWTH AND REPRODUCTION OF</td>
<td>22</td>
</tr>
<tr>
<td><em>Pontoscolex corethrurus</em></td>
<td></td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>22</td>
</tr>
<tr>
<td>------------------</td>
<td>----</td>
</tr>
<tr>
<td>3.2 Materials and methods</td>
<td>23</td>
</tr>
<tr>
<td>3.2.1 Field samplings</td>
<td>23</td>
</tr>
<tr>
<td>3.2.2 Preparation of soil and decomposed cow dung</td>
<td>23</td>
</tr>
<tr>
<td>3.2.3 Setup for offspring production</td>
<td>24</td>
</tr>
<tr>
<td>3.2.4 Growth of the first generation earthworms</td>
<td>24</td>
</tr>
<tr>
<td>3.2.5 Statistical analysis</td>
<td>27</td>
</tr>
<tr>
<td>3.3 Results</td>
<td>27</td>
</tr>
<tr>
<td>3.3.1 Survival rate</td>
<td>27</td>
</tr>
<tr>
<td>3.3.2 Earthworm biomass</td>
<td>28</td>
</tr>
<tr>
<td>3.3.3 Sexual maturity</td>
<td>29</td>
</tr>
<tr>
<td>3.3.4 Cocoon production</td>
<td>31</td>
</tr>
<tr>
<td>3.3.5 Hatching success, incubation period and number of hatchlings per cocoon</td>
<td>31</td>
</tr>
<tr>
<td>3.4 Discussion</td>
<td>33</td>
</tr>
</tbody>
</table>

4 PROTOCOLS FOR CONTROLLED CULTIVATION OF *Pontoscolex corethrurus* USING ARTIFICIAL MEDIUM

<table>
<thead>
<tr>
<th>4.1 Introduction</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2 Materials and methods</td>
<td>39</td>
</tr>
<tr>
<td>4.2.1 Preparation of culture media</td>
<td>39</td>
</tr>
<tr>
<td>4.2.2 Setup for offspring production</td>
<td>41</td>
</tr>
<tr>
<td>4.2.3 Experimental design</td>
<td>42</td>
</tr>
<tr>
<td>4.2.4 Statistical analysis</td>
<td>43</td>
</tr>
<tr>
<td>4.3 Results</td>
<td>43</td>
</tr>
<tr>
<td>4.3.1 Particle-size distribution and soil texture of culture substrates</td>
<td>43</td>
</tr>
<tr>
<td>4.3.2 Preliminary study</td>
<td>45</td>
</tr>
<tr>
<td>4.3.3 Worm culture study using modified artificial soils</td>
<td>49</td>
</tr>
<tr>
<td>4.4 Discussion</td>
<td>52</td>
</tr>
</tbody>
</table>

5 IMPROVEMENT OF SOIL NUTRIENTS AVAILABILITY, HUMIC ACID CONTENT, MICROBIAL POPULATIONS, AND ENZYME ACTIVITY IN EARTHWORM INOCULATED SOIL

<table>
<thead>
<tr>
<th>5.1 Introduction</th>
<th>57</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2 Materials and methods</td>
<td>58</td>
</tr>
<tr>
<td>5.2.1 Experimental setup</td>
<td>58</td>
</tr>
<tr>
<td>5.2.2 Bulk soil, worm-worked soil and earthworm surface cast analysis</td>
<td>60</td>
</tr>
<tr>
<td>5.2.3 Statistical analysis</td>
<td>68</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>68</td>
</tr>
<tr>
<td>5.3.1 Chemical analysis</td>
<td>68</td>
</tr>
<tr>
<td>5.3.2 Biological analysis</td>
<td>69</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>70</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Ecological categories, habitat locations, feeding strategies, and morphological characteristics of earthworms</td>
<td>6</td>
</tr>
<tr>
<td>3.1</td>
<td>Mean and standard error of percentage of adult <em>P. corethrurus</em> for four different stocking densities (n=5)</td>
<td>30</td>
</tr>
<tr>
<td>3.2</td>
<td>Mean and standard error of total number of cocoons produced per vessel and average number of cocoons produced per adult worm per week for four different stocking densities (n=5)</td>
<td>31</td>
</tr>
<tr>
<td>3.3</td>
<td>Mean and standard error of percentage of hatching success and incubation period of cocoons of <em>P. corethrurus</em> for four different stocking densities (n=5)</td>
<td>32</td>
</tr>
<tr>
<td>3.4</td>
<td>Comparison of reproductive biology of tropical endogeic worms</td>
<td>35</td>
</tr>
<tr>
<td>4.1</td>
<td>The composition of industrial quartz sand, kaolinite clay and organic material (decomposed cow dung) in four different mixtures of artificial soils: AS, AS30%clay, AS40%clay, and AS50%clay</td>
<td>40</td>
</tr>
<tr>
<td>4.2</td>
<td>Mean and standard error of percentages of clay (&lt;0.002 mm), silt (0.002–0.02 mm), fine sand (0.02–0.2 mm), and coarse sand (0.2–2 mm) and soil texture classes for field-collected soil (control) and four different mixtures of artificial soils: AS, AS30%clay, AS40%clay, and AS50%clay (n=5)</td>
<td>45</td>
</tr>
<tr>
<td>4.3</td>
<td>Mean and standard error of percentage of adult <em>P. corethrurus</em> for control (field-collected soil: cow dung; 90:10) and artificial soil (AS) (n=10)</td>
<td>48</td>
</tr>
<tr>
<td>4.4</td>
<td>Mean and standard error of average number of cocoons per adult worm per week for control (field-collected soil: cow dung; 90:10) and artificial soil (AS) (n=10)</td>
<td>48</td>
</tr>
<tr>
<td>4.5</td>
<td>Mean and standard error of percentage of adult <em>P. corethrurus</em> for the field-collected soil: cow dung (90:10), AS30%clay, AS40%clay, and AS50%clay (n=10)</td>
<td>51</td>
</tr>
<tr>
<td>4.6</td>
<td>Mean and standard error of average number of cocoons per adult worm per week for the field-collected soil: cow dung (90:10), AS30%clay, AS40%clay, and AS50%clay (n=10)</td>
<td>52</td>
</tr>
</tbody>
</table>
5.1 Microwave program setting for acid digestion of soil sample

5.2 Mean and standard error of total, available N, P, K, Ca, and Mg and humic acid content for bulk soil (control), worm-worked soil and earthworm surface cast (n=5)

5.3 Mean and standard error of soil actinomycetes, bacteria and fungi populations for bulk soil (control), worm-worked soil and earthworm surface cast (n=5)

5.4 Mean and standard error of soil protease and acid phosphatase activities for bulk soil (control), worm-worked soil and earthworm surface cast (n=5)

6.1 Nutrient contents of organic amendment (composted farmyard waste), organic amendment application time intervals and rates and method of fertilizer application

6.2 Mean and standard error of dry weights of root and shoot, numbers and fresh weight of chilies produced by each plant for four different treatments: endogeic worms and organic amendment application (+E+O); organic amendment without endogeic worms inoculation (–E+O); endogeic worms inoculation without organic amendment (+E–O); and the absence of endogeic worms and organic amendment (–E–O) (n=15)

6.3 Comparison of nutrient contents of different types of fertilizers, fertilizer application rates, quantities of nutrients added to each unit plant, and chili yield between the present research and the commercial practices by farmers
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Pictorial representation of some of the characteristics of earthworm ecological categories and their external environments as proposed by Bouché (1977) (Source: Doube and Brown, 1998)</td>
<td>5</td>
</tr>
<tr>
<td>3.1</td>
<td>Mean and standard error of percentage of survivors at different time intervals for four different stocking densities (n=5)</td>
<td>28</td>
</tr>
<tr>
<td>3.2</td>
<td>Mean and standard error of individual biomass of <em>P. corethrurus</em> at different time intervals for four different stocking densities (n=5)</td>
<td>29</td>
</tr>
<tr>
<td>4.1</td>
<td>Mean and standard error of percentage of survivors at different time intervals for control (field-collected soil: cow dung; 90:10) and artificial soil (AS) (n=10)</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>Mean and standard error of individual biomass of <em>P. corethrurus</em> at different time intervals for control (field-collected soil: cow dung; 90:10) and artificial soil (AS) (n=10)</td>
<td>47</td>
</tr>
<tr>
<td>4.3</td>
<td>Mean and standard error of percentage of survivors at different time intervals, for the field-collected soil: cow dung (90:10), AS_{30}%clay, AS_{40}%clay, and AS_{50}%clay (n=10)</td>
<td>49</td>
</tr>
<tr>
<td>4.4</td>
<td>Mean and standard error of individual biomass of <em>P. corethrurus</em> at different time intervals, for the field-collected soil: cow dung (90:10), AS_{30}%clay, AS_{40}%clay, and AS_{50}%clay (n=10)</td>
<td>50</td>
</tr>
<tr>
<td>5.1</td>
<td>Sketch of the experiment; after 4 weeks, the earthworm casts produced on the soil surface (a) were handpicked for further analysis. Earthworms were removed from the vessels to obtained worm-worked soil (b). The worm-worked soil collected was homogenized for analysis</td>
<td>59</td>
</tr>
<tr>
<td>6.1</td>
<td>Mean and standard error of height of <em>C.annuumcv</em>. Kulai for four different treatments (endogeic worms and organic amendment application, +E+O; organic amendment without endogeic worms inoculation, –E+O; endogeic worms inoculation without organic amendment, +E–O; and the absence of endogeic worms and organic amendment, –E–O) over a period of 16 weeks (n=15)</td>
<td>82</td>
</tr>
</tbody>
</table>

xvi
## LIST OF PLATES

<table>
<thead>
<tr>
<th>Plate</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>External morphology of adult endogeic worm, <em>P. corethrurus</em></td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Dorsal view of anterior part of endogeic worm, <em>P. corethrurus</em></td>
<td>9</td>
</tr>
<tr>
<td>3.1</td>
<td>The “mature” cocoon of <em>P. corethrurus</em> with almost fully developed embryo</td>
<td>24</td>
</tr>
<tr>
<td>3.2</td>
<td>Incubation of cocoon in a Petri dish filled with distilled water. (a) Cocoon and (b) Newly hatched worm</td>
<td>27</td>
</tr>
<tr>
<td>3.3</td>
<td>Development of embryos observed in cocoons of <em>P. corethrurus</em>. (a) White with visible embryo (around 1 week after deposition); (b) White with dorsal blood vessel (around 2 weeks after deposition); (c) Light pink (around 3 weeks after deposition) and (d) Dark pink (around 4 weeks after deposition) (Image taken on a Leica MZ16 dissecting microscope with magnification of 2.5)</td>
<td>32</td>
</tr>
<tr>
<td>4.1</td>
<td>Constituents for artificial soil (AS). (a) Industrial quartz sand; (b) Kaolinite clay; and (c) Decomposed cow dung</td>
<td>40</td>
</tr>
<tr>
<td>5.1</td>
<td>Earthworm surface casts that have irregular shapes and sizes</td>
<td>59</td>
</tr>
<tr>
<td>6.1</td>
<td>(a) Seed tray filled with peat moss and sown with chili seeds and (b) Nursery house with automatic sprinkler irrigation</td>
<td>78</td>
</tr>
<tr>
<td>6.2</td>
<td>(a) Seedlings of chili Kulai after 4 weeks of sowing and (b) Seedling of chili Kulai after transplanting into a polybag filled with standard growth medium</td>
<td>78</td>
</tr>
<tr>
<td>6.3</td>
<td>(a) Arrangement of chili plants in the field, Taman Pertanian Universiti (TPU), Universiti Putra Malaysia (UPM) and (b) Irrigation of chili plants by automatic sprinkler irrigation</td>
<td>81</td>
</tr>
<tr>
<td>6.4</td>
<td>Growth and development of chili plants after 16 weeks. (a) Endogeic worms and organic amendment application (+E+O); (b) Organic amendment without endogeic worms inoculation (−E+O); (c) Endogeic worms inoculation without organic amendment (+E−O); and (d) The absence of endogeic worms and organic amendment (−E−O)</td>
<td>83</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Actinomycetes Isolation Agar</td>
</tr>
<tr>
<td>Al</td>
<td>Aluminium</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AS</td>
<td>Artificial Soil with 70% Industrial Quartz Sand, 20% Kaolinite Clay and 10% Decomposed Cow Dung</td>
</tr>
<tr>
<td>AS$_{30\text{clay}}$</td>
<td>Artificial Soil with 60% Industrial Quartz Sand, 30% Kaolinite Clay and 10% Decomposed Cow Dung</td>
</tr>
<tr>
<td>AS$_{40\text{clay}}$</td>
<td>Artificial Soil with 50% Industrial Quartz Sand, 40% Kaolinite Clay and 10% Decomposed Cow Dung</td>
</tr>
<tr>
<td>AS$_{50\text{clay}}$</td>
<td>Artificial Soil with 40% Industrial Quartz Sand, 50% Kaolinite Clay and 10% Decomposed Cow Dung</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CEC</td>
<td>Cation Exchange Capacity</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>IBS</td>
<td>Institute of Bioscience</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma-Mass Spectrometry</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>K</td>
<td>Potassium</td>
</tr>
<tr>
<td>K$_2$O</td>
<td>Potash</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
</tbody>
</table>
m  Meter
m²  Square Meter (area)
MARDI  Malaysian Agricultural Research and Development Institute
Mg  Magnesium
mg  Miligram
mL  Mililiter
mm  Milimeter
n  Number of Replicates
N  Nitrogen
NA  Nutrient Agar
NH₄-N  Ammonium
NO₃-N  Nitrate
OECD  Organization for Economic Co-operation and Development
P  Phosphorous
P₂O₅  Phosphorous Pentoxide
ppm  Part per Million
RBA  Rose Bengal Chloromphenicol Agar
SPSS  Statistical Package for Social Science
rpm  Revolutions per Minute
μg  Microgram
μmole  Micromole
TPU  Tanam Pertainan Universiti
UPM  Univeristi Putra Malaysia
V  Volume
Wt  Weight
WUE Water Use Efficiency
%
Percentage
CHAPTER 1

INTRODUCTION

Malaysia with the tropical climate has a fairly high, but uniform temperature and large amounts of rain delivering in intense bursts each year. Because of the weather, local agricultural soils contain low organic matter and highly compact soil texture resulting in lower crop productivity (Sabtu, 2006; APEC, 2012). A greater amount of chemical fertilizers is used each year to maintain soil fertility and boost crop productivity. However, the use of chemical fertilizers only restores soil nutrient content and does not improve the soil’s physical structure and microbiological activities. Long-term use of chemical fertilizers also results in many negative effects on the soil such as acidification, decline in organic matter content and reduction of microbial communities (Sinha et al., 2010). Therefore, the rehabilitation of local agricultural soils is necessary and important to reduce the loss of soil nutrients as well as to promote sustainable agriculture.

One of the approaches to create a healthy agro-ecosystem is introducing soil-dwelling endogeic worms into the soil (Butt et al., 1997). Endogeic worms are known as “ecological engineers” as their burrowing and casting activities result in beneficial chemical, microbiological and physical changes in the soil that increase the fertility of the soil (Jones et al., 1994; Scheu, 2003). Efforts have been made to transplant endogeic worms into degraded pastures in New Zealand which have significantly improved pasture productivity (Stockdill, 1982). In grasslands on reclaimed industrial cutaway peat in Ireland, herbage production was significantly improved by earthworm colonization through improvement in the physical structure of the soil (Curry and Boyle, 1987).

The use of earthworms to improve soil conditions is a common practice in temperate regions like New Zealand (Stockdill, 1982), The Netherlands (Hoogerkamp et al., 1983), Ireland (Curry and Boyle, 1987), and the United Kingdom (Butt et al., 1997). However, this approach is rarely practiced in the tropics including Malaysia. Available information on tropical earthworms is limited to litter-dwelling epigeic species while knowledge about the biology and ecology of tropical endogeic worms is very scarce. The differences between temperate and tropical climates are likely to influence the activities of earthworms in soils (Kale and Karmegam, 2010). The higher temperature and humidity in tropical ecosystems may increase the overall activities of earthworms in soils. The higher burrowing and casting activities may accelerate soil rehabilitation processes by improving the physicochemical and biological properties of soil and in turn create a healthy environment for plant growth. On the other hand, intense bursts of rain in tropical ecosystems may destroy earthworm surface casts. Nutrients within earthworm casts may therefore be lost through surface runoff (Jouquet et al., 2013). The overall effects of these processes may cause the effects of earthworms on soil properties
in tropical ecosystems to differ from those in temperate regions. More experimental research is necessary to determine the roles of tropical earthworms in their ecosystems.

Obtaining a sufficient number of earthworms for soil rehabilitation is a fundamental aspect to be achieved prior to the practice. Most earthworm inoculation activities conducted in temperate regions are accomplished via the mass collection of earthworms from densely populated areas and transplanted into lower density areas (Butt et al., 1995). This technique is expensive, labor-intensive and time-consuming (Butt et al., 1992). Earthworm collection sites must be amended with a lot of organic matter over a long period before further collection in order to maximize the number of earthworms present in the area (Butt, 2011). In addition, under natural conditions, earthworm availability is strongly affected by seasonal changes, organic resource distribution and farm management practices (Ghafoor et al., 2008; Pauli et al., 2010). Thus, it may be difficult to acquire a sufficient number of earthworms for inoculation purposes. An alternative method for obtaining a large number of earthworms is indoor cultivation (Butt et al., 1992). For example, successful production of epigeic worms such as Eisenia fetida, Lumbricus rubellus, Perionyx excavatus, and Eudrilus eugeniae under indoor conditions resulted in the widespread use of these species in vermicomposting, vermiculture for protein source and ecotoxicological studies.

Unlike epigeic worms, soil-dwelling endogeic worms are fairly difficult to cultivate indoors. Endogeic worms are geophagous deriving their nutrition through the consumption of large amounts of soil. They require culturing in a mineral soil-based medium (Butt and Lowe, 2011). Butt and his co-workers used soil collected from earthworm-sampling sites as the medium to culture endogeic worms (Butt et al., 1992; Butt, 1993; Lowe and Butt, 2005). However, there are difficulties in using natural soil as the medium for culturing endogeic worms. This is because field-collected soils are highly heterogeneous. Its texture, water-holding capacity, organic matter content, nutrient availability, and microbial activities differ on the basis of sampling locations, land management and plant communities (Huggins and Uberuaga, 2010). Differences in the physiochemical and biological properties of field-collected soils could affect the survival, growth, sexual maturity, and cocoon production rates of earthworms (Klok et al., 2007). Therefore, it is difficult to reach a considerable level of standardization with field-collected soil as the earthworm culture medium. Field-collected soils may also contain harmful compounds such as heavy metals, polycyclic aromatic hydrocarbons and pesticides that could adversely affect earthworm cultures. An alternative culture medium should be established in order to achieve standardization for indoor cultivation of endogeic worms and also to produce a large and consistent supply of earthworms for soil rehabilitation programs.

The soil-dwelling endogeic worm Pontoscolex corethrurus (Müller, 1857) is one of the few persistent species capable of surviving under tropical agricultural tillage conditions (Lavelle et al., 1987; Sabrina et al., 2009; Nath and Chaudhuri, 2010). In Malaysia, P. corethrurus is the only endogeic species found in local rubber and oil palm plantations.
It is widely distributed in a variety of soil types including Serdang series, Bungor series and Jawa series (Vijayandran and Gurmit, 2009). Previous studies have shown that adaptation to tropical climates, capability to tolerate a wide range of environmental factors (e.g., soil pH and organic matter content) and the ability to enter diapause under drought conditions are the adaptive strategies that enable *P. corethrurus* to be widely distributed in tropical ecosystems (Lavelle et al., 1987; James and Hendrix, 2004; Chaudhuri et al., 2008; Nath and Chaudhuri, 2010). The fact that it is widely distributed in tropical ecosystems and shows good environmental plasticity suggests that *P. corethrurus* may serve as a potential candidate for soil rehabilitation in tropical regions.

Unfortunately, in Malaysia, other than ecological data (distribution and abundance), relevant information about *P. corethrurus* is very limited. A detailed study on the effects of *P. corethrurus* on soil chemical and biological properties as well as crop productivity is required to determine its role in local soil ecosystems. Prior to determining its effects on soil properties, fundamental aspects of earthworm biology (e.g., life history) need to be studied since the biological characteristics of earthworms will affect their potential in soil rehabilitation programs. Nevertheless, the life history of earthworms is strongly affected by earthworm stocking density (Hait and Tare, 2011; Suthar, 2012). To date, there is still no study on how stocking density will influence the life history of *P. corethrurus*. In addition, a suitable culture medium for culturing *P. corethrurus* should be established to provide a large and consistent supply of *P. corethrurus* for worm inoculation activities.

Therefore, the objectives of the present research were:

i. To determine the effect of stocking density on life history of the endogeic worm *P. corethrurus*,

ii. To establish a suitable medium for culturing the endogeic worm *P. corethrurus*,

iii. To investigate the effect of the endogeic worm *P. corethrurus* on nutrient availability, humic acid content, microbial population, and enzyme activity in soil,

iv. To evaluate the effect of inoculating the endogeic worm *P. corethrurus* on plant production under field condition.
CHAPTER 1

INTRODUCTION

Malaysia with the tropical climate has a fairly high, but uniform temperature and large amounts of rain delivering in intense bursts each year. Because of the weather, local agricultural soils contain low organic matter and highly compact soil texture resulting in lower crop productivity (Sabtu, 2006; APEC, 2012). A greater amount of chemical fertilizers is used each year to maintain soil fertility and boost crop productivity. However, the use of chemical fertilizers only restores soil nutrient content and does not improve the soil’s physical structure and microbiological activities. Long-term use of chemical fertilizers also results in many negative effects on the soil such as acidification, decline in organic matter content and reduction of microbial communities (Sinha et al., 2010). Therefore, the rehabilitation of local agricultural soils is necessary and important to reduce the loss of soil nutrients as well as to promote sustainable agriculture.

One of the approaches to create a healthy agro-ecosystem is introducing soil-dwelling endogeic worms into the soil (Butt et al., 1997). Endogeic worms are known as “ecological engineers” as their burrowing and casting activities result in beneficial chemical, microbiological and physical changes in the soil that increase the fertility of the soil (Jones et al., 1994; Scheu, 2003). Efforts have been made to transplant endogeic worms into degraded pastures in New Zealand which have significantly improved pasture productivity (Stockdill, 1982). In grasslands on reclaimed industrial cutaway peat in Ireland, herbage production was significantly improved by earthworm colonization through improvement in the physical structure of the soil (Curry and Boyle, 1987).

The use of earthworms to improve soil conditions is a common practice in temperate regions like New Zealand (Stockdill, 1982), The Netherlands (Hoogerkamp et al., 1983), Ireland (Curry and Boyle, 1987), and the United Kingdom (Butt et al., 1997). However, this approach is rarely practiced in the tropics including Malaysia. Available information on tropical earthworms is limited to litter-dwelling epigeic species while knowledge about the biology and ecology of tropical endogeic worms is very scarce. The differences between temperate and tropical climates are likely to influence the activities of earthworms in soils (Kale and Karmegam, 2010). The higher temperature and humidity in tropical ecosystems may increase the overall activities of earthworms in soils. The higher burrowing and casting activities may accelerate soil rehabilitation processes by improving the physicochemical and biological properties of soil and in turn create a healthy environment for plant growth. On the other hand, intense bursts of rain in tropical ecosystems may destroy earthworm surface casts. Nutrients within earthworm casts may therefore be lost through surface runoff (Jouquet et al., 2013). The overall effects of these processes may cause the effects of earthworms on soil properties
in tropical ecosystems to differ from those in temperate regions. More experimental research is necessary to determine the roles of tropical earthworms in their ecosystems.

Obtaining a sufficient number of earthworms for soil rehabilitation is a fundamental aspect to be achieved prior to the practice. Most earthworm inoculation activities conducted in temperate regions are accomplished via the mass collection of earthworms from densely populated areas and transplanted into lower density areas (Butt et al., 1995). This technique is expensive, labor-intensive and time-consuming (Butt et al., 1992). Earthworm collection sites must be amended with a lot of organic matter over a long period before further collection in order to maximize the number of earthworms present in the area (Butt, 2011). In addition, under natural conditions, earthworm availability is strongly affected by seasonal changes, organic resource distribution and farm management practices (Ghafoor et al., 2008; Pauli et al., 2010). Thus, it may be difficult to acquire a sufficient number of earthworms for inoculation purposes. An alternative method for obtaining a large number of earthworms is indoor cultivation (Butt et al., 1992). For example, successful production of epigeic worms such as Eisenia fetida, Lumbricus rubellus, Perionyx excavatus, and Eudrilus eugeniae under indoor conditions resulted in the widespread use of these species in vermicomposting, vermiculture for protein source and ecotoxicological studies.

Unlike epigeic worms, soil-dwelling endogeic worms are fairly difficult to cultivate indoors. Endogeic worms are geophagous deriving their nutrition through the consumption of large amounts of soil. They require culturing in a mineral soil-based medium (Butt and Lowe, 2011). Butt and his co-workers used soil collected from earthworm-sampling sites as the medium to culture endogeic worms (Butt et al., 1992; Butt, 1993; Lowe and Butt, 2005). However, there are difficulties in using natural soil as the medium for culturing endogeic worms. This is because field-collected soils are highly heterogeneous. Its texture, water-holding capacity, organic matter content, nutrient availability, and microbial activities differ on the basis of sampling locations, land management and plant communities (Huggins and Uberuaga, 2010). Differences in the physiochemical and biological properties of field-collected soils could affect the survival, growth, sexual maturity, and cocoon production rates of earthworms (Klok et al., 2007). Therefore, it is difficult to reach a considerable level of standardization with field-collected soil as the earthworm culture medium. Field-collected soils may also contain harmful compounds such as heavy metals, polycyclic aromatic hydrocarbons and pesticides that could adversely affect earthworm cultures. An alternative culture medium should be established in order to achieve standardization for indoor cultivation of endogeic worms and also to produce a large and consistent supply of earthworms for soil rehabilitation programs.

The soil-dwelling endogeic worm Pontoscolex corethrurus (Müller, 1857) is one of the few persistent species capable of surviving under tropical agricultural tillage conditions (Lavelle et al., 1987; Sabrina et al., 2009; Nath and Chaudhuri, 2010). In Malaysia, P. corethrurus is the only endogeic species found in local rubber and oil palm plantations
(Loh, 2009; Sabrina et al., 2009). It is widely distributed in a variety of soil types including Serdang series, Bungor series and Jawa series (Vijianandran and Gurmit, 2009). Previous studies have shown that adaptation to tropical climates, capability to tolerate a wide range of environmental factors (e.g., soil pH and organic matter content) and the ability to enter diapause under drought conditions are the adaptive strategies that enable *P. corethrurus* to be widely distributed in tropical ecosystems (Lavelle et al., 1987; James and Hendrix, 2004; Chaudhuri et al., 2008; Nath and Chaudhuri, 2010). The fact that it is widely distributed in tropical ecosystems and shows good environmental plasticity suggests that *P. corethrurus* may serve as a potential candidate for soil rehabilitation in tropical regions.

Unfortunately, in Malaysia, other than ecological data (distribution and abundance), relevant information about *P. corethrurus* is very limited. A detailed study on the effects of *P. corethrurus* on soil chemical and biological properties as well as crop productivity is required to determine its role in local soil ecosystems. Prior to determining its effects on soil properties, fundamental aspects of earthworm biology (e.g., life history) need to be studied since the biological characteristics of earthworms will affect their potential in soil rehabilitation programs. Nevertheless, the life history of earthworms is strongly affected by earthworm stocking density (Hait and Tare, 2011; Suthar, 2012). To date, there is still no study on how stocking density will influence the life history of *P. corethrurus*. In addition, a suitable culture medium for culturing *P. corethrurus* should be established to provide a large and consistent supply of *P. corethrurus* for worm inoculation activities.

Therefore, the objectives of the present research were:

i. To determine the effect of stocking density on life history of the endogeic worm *P. corethrurus*,

ii. To establish a suitable medium for culturing the endogeic worm *P. corethrurus*,

iii. To investigate the effect of the endogeic worm *P. corethrurus* on nutrient availability, humic acid content, microbial population, and enzyme activity in soil,

iv. To evaluate the effect of inoculating the endogeic worm *P. corethrurus* on plant production under field condition.
REFERENCES


Neilson, R., Boag, B. and Smith, M. 2000. Earthworm δ^{13}C and δ^{15}N analyses suggest that putative functional classifications of earthworms are site specific and may also indicate habitat diversity. *Soil Biology and Biochemistry* 32: 1053–1061.


