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

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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

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

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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 suggestedthat 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 m^2. This study also reported that *P. corethrurus* has a high survival rate and has the capability to reproduce *vi*a 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 determinehow 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 keperluaan untuk ijazah Doktor Falsafah

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

Oleh
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Mei 2014

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Pontoscolex corethrurus (Müller, 1857) adalah cacing tanah endogeik tropika dominan dan mempunyai keupayaan untuk hidup dalam pelbagai jenis tanah yang berbeza dalam pH and kandungan bahan organik. Ini mencadangkan bahawa spesies ini boleh bertindak sebagai ejen yang berpotensi dalam pemulihan tanah terutamanya di kawasan tropika.Malangnya, maklumat berkaitan dengan *P. corethrurus* adalah sangat terhad di Malaysia.Satu pemahaman yang terperinci tentang kitaran hidup *P. corethrurus* adalah diperlukan kerana ciri biologi cacing tanah (contohnya, kadar pertumbuhan dan pembiakan) boleh mempengaruhi potensi cacing dalam program pemulihan tanah.Kadar pertumbuhan dan pembiakan *P. corethrurus* pada kepadatan yang berbeza juga dinilai untuk menentukan kepadatan yang optimum untuk pengkulturan spesies ini.Kajian ini menunjukkan bahawa kepadatan optimum untuk pengkulturan *P. corethrurus* adalah kira-kira 200 cacing tanah per m^2. Kajian ini juga menunjukkan bahawa *P. corethrurus* mempunyai kadar kemandirian yang tinggi dan bersifat parthenogenetik. Ciri biologi ini membolehkan *P. corethrurus* menguasai persekitaran baru dan meningkatkan potensinya dalam aktiviti inokulasi cacing. Pengkulturan *P. corethrurus*adalah pentinguntuk menghasilkan bekalan cacing tanah yang banyak dan konsisten untuk aktiviti inokulasi cacing. Penggunaan tanah semulajadi sebagai media kultur cacing kurang sesuai disebabkan sifat tanah yang berbeza di sesuatu kawasan akan mempengaruhi keputusan pengkulturan. Maka, protokol telah dibangunkan dengan menggunakan media buatan bagi menggantikan tanah semulajadi dalam pengkulturan *P. corethrurus*. Kajian ini mendapati bahawa kandungan tanah liat boleh menjejaskan pertumbuhan *P. corethrurus* dan memanipulasi kandungan tanah liat dalam media buatan (terdiri daripada tanah liat kaolinit, pasir dan tahi lembu reput) boleh dijadikan media alternatif dalam pengkulturan *P. corethrurus*.Sifat tinja cacing, tanah dengan kehadiran cacing dan tanah tanpa cacing juga telah dianalisa untuk mengkaji pengaruh aktiviti *P. corethrurus* ke atas tanah. Kajian ini mendapati tinja cacing dan tanah dengan kehadiran cacing mengandungi kandungan nutrien yang lebih tinggi berbanding dengan tanah tanpa cacing. Aktiviti enzim (protease dan asid fosfatase), populasi mikrob (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.

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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:

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LIST OF ABBREVIATIONS

AA	Actinomycetes Isolation Agar
Al	Aluminium
ANOVA	Analysis of Variance
AS	Artificial Soil with 70% Industrial Quartz Sand, 20% Kaolinite Clay and 10% Decomposed Cow Dung
$AS_{30\%clay}$	Artificial Soil with 60% Industrial Quartz Sand, 30% Kaolinite Clay and 10% Decomposed Cow Dung
$AS_{40\%clay}$	Artificial Soil with 50% Industrial Quartz Sand, 40% Kaolinite Clay and 10% Decomposed Cow Dung
$AS_{50\%clay}$	Artificial Soil with 40% Industrial Quartz Sand, 50% Kaolinite Clay and 10% Decomposed Cow Dung
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
cm	Centimeter
Fe	Iron
g	Gram
IBS	Institute of Bioscience
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
h	Hour
ha	Hectare
K	Potassium
K_2O	Potash
kg	Kilogram
L	Liter

m	Meter
m^2	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
NH_4-N	Ammonium
NO_3-N	Nitrate
OECD	Organization for Economic Co-operation and Development
P	Phosphorous
P_2O_5	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

(Loh, 2009; Sabrina *et al.*, 2009). It is widely distributed in a variety of soil types including Serdang series, Bungor series and Jawa series (Vijiandran 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.

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