UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ORGANIC AMENDMENTS AND FERTILIZERS ON BIOMASS YIELD, BIOACTIVE COMPOUNDS AND HEAVY METALS CONTENT OF Phyllanthus niruri L.

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By

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

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April 2017

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Soil amendment using organic materials such as chicken manure and/or biochar may potentially improve the soil physico-chemical properties, and crop production. Both biochar and chicken manure are commonly used for alleviating the infertility of highly weathered soil. The general aim of this study was to determine optimum rate of soil amendment and investigate effects of rice husk biochar and chicken manure as a soil amendment on biomass yield, bioactive compounds, nutrient uptake and heavy metals concentration of *Phyllanthus niruri*. A polybag study was conducted with treatments of three rates of biochar application (0, 5 and 10 t ha^{-1}) and three rates of chicken manure (0, 2.5 and 5 t ha^{-1}) laid out in Randomized Complete Block Design (RCBD) with four replications. Results showed that co-application of 10 t ha^{-1} biochar and 2.5 t ha^{-1} chicken manure produced optimum yield of *Phyllanthus niruri* which are 1720.80 mg $plant^{-1}$ and higher phyllanthin and hypophyllanthin contents (11.85 mg $plant^{-1}$ and 12.77 mg $plant^{-1}$). Application of biochar and chicken manure increased N, P and K uptake by 37% and 42% compared to control treatment. Application of chicken manure and biochar also able to reduce the heavy metal concentrations (cadmium and arsenic) in plant tissue. Application of chicken manure without biochar has produced about 5.25 to 8.89 mg kg^{-1} arsenic concentration (above Maximum Permissible Levels) in plant tissue compared to combination treatment between biochar and chicken manure which range between 3.06 to 5.06 mg kg^{-1}. Cadmium and arsenic uptake by *Phyllanthus niruri* were below the Maximum Permissible Levels. There are about 40% increases in phyllanthin content and 34% increase in hypophyllanthin content with the selected rate of amendment. However, phyllantin and hypophyllanthin concentration in *P. niruri* was not affected both biochar and chicken manure amendment.

Excessive use of chemical fertilizer has resulted in negative effects to the environment. Thus, organic waste can be converted to valuable source of fertilizer. Organic fertilizers can help to improve the soil chemical properties, enhance crop performance and increase the uptake of nutrient to the plants. The optimum rate of biochar and chicken manure treatment (10 t ha^{-1} and 2.5 t ha^{-1} respectively) was selected as amendment for second study. A field study was conducted to investigate effects of different types and rates of compost as an organic fertilizer for improvement of biomass yield, bioactive compounds concentration and yield, nutrients uptake and in reducing the heavy metals concentration in *Phyllanthus niruri.* The treatments comprised of repetition different types of compost (animal-based and plant-based) with five rates of application (0, 100, 200, 300 and 400 kg N ha^{-1}) laid out in split-plot design with four replications. Shoot dry weight of *Phyllanthus niruri* increased by 43% and 41% with application of plant-based and animal-based fertilizer at rate 400 kg N ha^{-1} compared to control treatment. Application of plant-based fertilizer at rate 400 kg N ha^{-1} increased the N, P and K uptake by 43%, 31% and 29% compared to animal-based fertilizer at same rate. Concentrations of cadmium and lead also decreased with increasing rate of organic fertilizer and were below the Maximum Permissible Levels (MPLs). Result shows that application of 400 kg N ha^{-1} animal-based fertilizer reduced 43% cadmium concentration and 42% lead concentration compared to control treatment, while there were 20% decreased in lead and 24% decreased in cadmium compared to application of 400 kg N ha^{-1} plant-based fertilizer. However, phyllanthin and hypophyllanthin concentrations were not affected by types of organic fertilizer and application rate. This study also suggested that the optimum rate of 400 kg N ha^{-1} animal-based fertilizer revealed the best biomass yield, phyllanthin and hypophyllanthin contents, and low heavy metal concentration of *Phyllanthus niruri*. Overall, this study demonstrates the potential of biochar and chicken manure as organic amendment along with animal-based organic fertilizer in producing organically *Phyllanthus niruri* with better yield, higher phyllanthin and hypophyllanthin contents, high nutrient uptake and low in heavy metal concentration compared to control treatment which is the critical factor for medicinal herbs production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PERAPI TANAH ORGANIK DAN BAJA TERHADAP HASIL BIOJISIM, BAHAN BIOAKTIF DAN KANDUNGAN LOGAM BERAT OLEH *Phyllanthus niruri* L.

Oleh

ZAHIDAH BINTI AB RAZAK

April 2017

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Perapi tanah menggunakan bahan organik seperti tahi ayam dan biochar berpotensi untuk meningkatkan sifat fizik dan kimia tanah dan pengeluaran tanaman. Kedua-dua biochar dan tahi ayam kebiasaannya digunakan untuk mengurangkan ketidaksuburan tanah yang terluluhawa. Oleh itu, matlamat utama kajian ini adalah untuk menentukan kadar optimum bagi perapi tanah dan menyiasat kesan sekam padi biochar dan tahi ayam sebagai perapi tanah ke atas hasil tanaman, bahan bioaktif, pengambilan nutrien dan kepekatan logam berat oleh dukung anak. Kajian polibeg telah dijalankan dengan menggunakan rawatan tiga kadar biochar (0, 5 dan 10 t ha^{-1}) dan tiga kadar tahi ayam (0, 2.5 dan 5 t ha^{-1}) menggunakan rekabentuk blok lengkap secara rawak dengan empat replikasi. Keputusan menunjukkan kadar 10 t ha^{-1} biochar dan 2.5 t ha^{-1} tahi ayam telah menunjukkan hasil yang optimum untuk dukung anak dimana 1720 mg per pokok terhasil dan kandungan phyllanthin dan hypophyllanthin yang tinggi (11.85 dan 12.77 mg per pokok). Penggunaan sekam padi biochar dan tahi ayam meningkatkan kadar pengambilan N, P dan K sebanyak 37% dan 42% berbanding rawatan kawalan. Penggunaan sekam padi biochar dan tahi ayam juga mampu menurunkan kandungan logam berat (kadmium dan arsenik) di dalam tisu tumbuhan. Penggunaan tahi ayam tanpa sekam padi biochar menghasilkan kira-kira 5.25 ke 8.89 mg kg^{-1} kepekatan arsenik di dalam tisu tumbuhan berbanding dengan gabungan rawatan diantara sekam padi biochar dan tahi ayam yang berada dalam julat 3.06 ke 5.06 mg kg^{-1}. Pengambilan kadmium dan arsenik oleh dukung anak adalah di bawah tahap maksimum yang dibenarkan. Terdapat peningkatan sebanyak 40% dalam kandungan phyllanthin dan 34% dalam hypophyllanthin dengan kadar perapi tanah yang dipilih. Walaubagaimanapun, kepekatan phyllanthin dan hypophyllanthin tidak terjejas dengan penggunaan sekam padi biochar dan tahi ayam.

Penggunaan baja kimia yang berlebihan telah menyebabkan kesan negatif terhadap alam sekitar. Jesteru itu, sisa bahan organik boleh diproses menjadi sumber baja. Baja organik boleh membantu untuk meningkatkan sifat kimia tanah, meningkatkan prestasi tanaman dan meningkatkan pengambilan nutrien di dalam tumbuhan. Kadar optimum bagi rawatan menggunakan biochar dan tahi ayam (10 t ha^{-1} dan 2.5 t ha^{-1}) telah digunakan sebagai perapi tanah dalam kajian kedua. Kajian di ladang telah dijalankan untuk menyiasat kesan jenis dan kadar baja organik yang berbeza dalam meningkatkan hasil tanaman, kepekatan dan hasil bahan bioaktif, pengambilan nutrien dan mengurangkan kepekatan logam berat di dalam tisu dukung anak. Rawatan mengandungi dua jenis baja organik (baja berasaskan sisa haiwan dan baja berasaskan sisa tumbuhan) dengan menggunakan lima kadar baja organik (0, 100, 200, 300 dan 400 kg N ha^{-1}) menggunakan rekabentuk split-plot dengan 4 replikasi. Berat pucuk kering dukung anak meningkat sebanyak 43 % dan 41% dengan penggunaan baja berasaskan sisa tumbuhan dan haiwan pada kadar 400 kg N per hektar berbanding rawatan terkawal. Penggunaan baja berasaskan sisa tumbuhan pada kadar 400 kg N per hektar telah meningkatkan pengambilan N, P dan K sebanyak 43%, 31% dan 29% berbanding penggunaan baja berasaskan sisa haiwan. Kepekatan kadmium dan plumbum juga menurun dengan peningkatan kadar baja organik dan berada di bawah tahap maksimum yang dibenarkan. Keputusan menunjukkan penggunaan 400 kg N per hektar baja berasaskan sisa haiwan mengurangkan kepekatan kadmium dan plumbum sebanyak 43% dan 42% berbanding rawatan kawalan, manakala terdapat pengurangan sebanyak 20% dan 24% di dalam kepekatan plumbum dan kadmium berbanding dengan penggunaan baja berasaskan sisa tumbuhan pada kadar 400 kg N per hektar. Walaubagaimanapun, kepekatan phyllanthin dan hypophyllanthin tidak terjejas dengan jenis dan kadar baja organik yang berbeza. Kajian ini juga mendapati baja berasaskan sisa haiwan pada kadar 400 kg N per hektar menunjukkan hasil dukung anak dan kandungan phyllanthin dan hypophyllanthin yang tinggi, dan kepekatan logam berat yang rendah di dalam dukung anak. Secara keseluruhan, kajian ini menunjukkan potensi biochar dan tahi ayam sebagai perapi tanah organik berserta baja berasaskan sisa haiwan dalam menghasilkan dukung anak secara organik dengan hasil yang tinggi, hasil phyllanthin dan hypophyllanthin yang tinggi, pengambilan nutrien yang tinggi dan rendah kepekatan logam berat berbanding rawatan kawalan yang mana ia merupakan faktor penting dalam penghasilan herba.

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I certify that a Thesis Examination Committee has met on 13 April 2017 to conduct the final examination of Zahidah binti Ab Razak on her thesis entitled "Effects of Organic Amendments and Fertilizers on Biomass Yield, Bioactive Compounds and Heavy Metals Content of *Phyllanthus niruri* L." 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

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

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This is to confirm that:

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Name of Member of Supervisory Committee: Radziah Binti Othman

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

w/v	weight per volume
g	gram
mL	milliliter
M	molar
°C	degree Celsius
v/v	volume per volume
mL/min	milliliter per minute
µL	microliter
µg/ml	microgram per milliliter
%	percent
m	meter
t ha^{-1}	tonnes per hectares
cm	centimeter
mg kg^{-1}	milligram per kilogram
µg kg^{-1}	microgram per kilogram
kg ha^{-1}	kilogram per hectare
ANOVA	analysis of variance
LSD	Least Significant Difference
MPLs	Maximum Permissible Limits

CHAPTER 1

INTRODUCTION

Malaysian rainforest is considered as one of the most diverse in the world. This biodiversity support a lot of species especially medicinal plants. Medicinal plants are getting more attention over the past few years, especially in Malaysia, due to increasing demand for alternative medication and natural health products. In 2007, 351 hectares of land in Malaysia has produced 1317 metric tonnes of herbs, however, the production of herbs has increase to 2800 metric tonnes with total land of 578 hectares (DOA, 2015). The Malaysian Economic Transformation Program, through National Key Economic Areas (NKEA) Agriculture sector, has identified several potential herbs as one of the high value herbal crop that need to be commercially exploited for a new source of economic growth in the herbal industry and one of these herbs is *Phyllanthus niruri*, locally known as dukung anak.

Phyllanthus niruri L. (Euphorbiaceae) has been traditionally used as a remedy against fever, diarrhea, jaundice, syphilis, gonorhoea and kidney disorder (Rai and Mehrotra, 2007; Bharat and Khotari, 2011; Prashanth Kumar et al., 2012). Increasing demand of *P. niruri* has led to adulteration; thus, there is global need to develop quality plant materials. Besides as a potential hepatitis B and viral infection remedy, the anti-tumor and anti-carcinogenic activities of this plant have drawn great attention from researcher (Rajeshkumar et al., 2002). Recently, this plant is focused for its antioxidant and antibacterial activities (Harish and Shivanandappa, 2006; Shonkunbi and Odetola, 2008; Yerra et al., 2008). Lignans and tannin were present in extracts of *P. niruri* (Rajeshkumar et al., 2002).

Due to shortage of raw materials and lacking of expertise in related herb, the herbal industry faced a lot of constraints such as low in production and limited supply of herbs. Furthermore, Malaysian soils are mainly Ultisols and Oxisols, which are highly weathered acidic soils known acid sulphate soil produce low crop yield due to the low pH, which results in aluminium and manganese toxicity. Thus, organic amendment needs to be incorporated into the soil in order to improve soil fertility and crop productivity.

Application of poultry manure as a soil amendment is traditionally used for vegetable crops in Malaysia. Chicken manure can be a good soil amendment which can contribute to plant nutrient availability for rapid growth of seedlings (Duncan, 2005; Agbede et al., 2008). Application of manure into the soil improves soil physical and biological properties and at the same time increased crop performance and dry weight (Kaplan et al., 2009, Valiki and Ghanbari, 2015). However, usage of chicken manure can also contributed to higher heavy metals contents in plants and soils if applied at high dosage. So, application of chicken manure should not exceed the recommended rate.

Recently, application of biochar as a soil amendment has been given serious attention by researchers since it has potentials to sequester carbon and increase soil fertility and crop yield. Biochar refers to materials that are produced from combustion of biomass or agricultural wastes under pyrolysis process in the absence or limited oxygen (Frederik et al., 2013). Biochar are able to improve soil chemical properties by increasing pH, available nitrogen and phosphorous, cation exchange capacity, exchangeable cations and base saturation (Liang et al., 2006; Ogawa and Okimori, 2010; Van Zwieten et al., 2010). Biochar can also improve soil water-holding capacity (Laird et al., 2010) and improve the biomass yield (Kammann et al., 2011). According to Schmidt and Noack (2000), biochar can fixed and store the carbon longer in the soil, thus mitigates climate change and reduce greenhouse gases. The pore and surface structures of biochars potentially provide suitable habitats for soil microorganisms (Warnock et al., 2007; Downie et al., 2009).

However, usage of organic amendment without the addition of fertilizer is not sufficient to supply nutrient for plant uptake. In an organic system, application of organic fertilizers from plant or animal based is required to increase biomass yield.

Nowadays, wastes generated from poultry farm industry and other agricultural activities are increasing. These wastes can be converted into valuable fertilizer to supply adequate amount of nutrient for plant growth. Application of organic materials as fertilizers for crop production has gaining people's interest for sustainable crop productivity (Dong et al., 2012; Arif et al., 2014). Chicken manure is commonly used as a fertilizer by vegetable growers as it contains plant nutrients (N, P, and K) that are vital for plant growth (Ariffin et al., 2006; Agbede et al., 2008). It provides source of all necessary macro and micronutrients in available forms during mineralization and thereby improving physical and chemical properties of soils (Chaterjee et al., 2005). Akande et al. (2003) proved that application of organic fertilizers, mostly produced by composting process could increase soil pH and ameliorate slightly acidic tropical soils to improve crop production. Oka et al. (2000) indicated that organic addition have constantly produced beneficial effects on soil nutrients, soil physical conditions, and soil biological activities thereby improving the health of plants.

Currently, there is an increasing demand for organically grown herbs especially for medicinal purposes. This research was conducted with the aim of producing quality and safe *Phyllanthus niruri* for human consumption as well as increasing soil fertility and low heavy metal contents. Therefore, the following objectives were established:

1. To determine effects of organic soil amendments on biomass yield, nutrient uptake, heavy metals and bioactive compounds (phyllanthin and hypophyllanthin) of organically grown *Phyllanthus niruri.*

2. To evaluate effects of two different types and application rates of organic fertilizers (animal-based and plant-based) on biomass yield, nutrient uptake, heavy metals and bioactive compounds of *Phyllanthus niruri*, using selected soil amendments (rice husk biochar and chicken manure).

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