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EFFECTS OF SOIL AUGMENTATION BY Pontoscolex corethrurus ON BANANA PLANT RESPONSE TO FUSARIUM WILT

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EFFECTS OF SOIL AUGMENTATION BY *Pontoscolex corethrurus* ON BANANA PLANT RESPONSE TO FUSARIUM WILT

By

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EFFECTS OF SOIL AUGMENTATION BY *Pontoscolex corethrurus* ON BANANA PLANT RESPONSE TO FUSARIUM WILT

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Chair: Assoc. Prof. Nor Azwady Abd Aziz, PhD
Faculty: Science

Fusarium wilt is a major problem to banana cultivation worldwide and thus, effective environmental-friendly control measures for the disease is in great demand. One of the method is using endogeic earthworms of which the activities of the worm influence nutrient cycle and composition of microbes in soil. This study was conducted to assess the impact of soil augmentation by *Pontoscolex corethrurus,* a tropical endogeic earthworm on banana plant response to Fusarium wilt. Photographic record and scaling of external and internal wilt symptom, plant wet weight, chlorophyll content and disease severity index (DSI) were performed. Additionally, Salicyclic acid (SA) and Jasmonic acid (JA) levels of the plants were also analysed due to their significant role in plant defense mechanism against pathogen. The results showed that the presence of earthworms contributed to higher plant wet weight, chlorophyll content and lower DSI compared to treatment without earthworm in non-infected and infected banana plants. The plants with earthworm inoculation also showed one week delayed appearances of external and internal wilt symptoms. The concentration of SA in non-infected plants with earthworm treatment showed higher value (0.72±0.09 μg/g, $p<0.05$) compared to without earthworm. For JA, the concentration in week 1 was doubled for infected plant with earthworm (0.96±0.01 μg/g) compared to without earthworm (0.47±0.03 μg/g). Thus, earthworms were proposed to improve plant basal defense *via* intensifying the plant defense hormones. The present study suggested that soil augmentation by *P. corethrurus* could improve plant growth, delayed appearance of wilt symptom and improve phytohormone production for plant defense system.

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

KESAN PENAMBAHBAIK TANAH OLEH *Pontoscolex corethrurus* KE ATAS TINDAK BALAS POKOK PISANG TERHADAP LAYU FUSARIUM

Oleh

SITI ROHANI SULAIMAN

November 2016

Pengerusi: Prof. Madya Nor Azwady Abd Aziz, PhD
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Penyakit layu Fusarium merupakan masalah utama penanaman pisang di seluruh dunia dan langkah yang berkesan dan mesra alam untuk mengawal penyakit ini sangat diperlukan. Salah satu cara adalah dengan menggunakan cacing tanah yang mana aktiviti cacing tersebut mempengaruhi kitaran nutrien dan komposisi mikrob dalam tanah. Kajian ini dijalankan untuk menilai kesan penambahbaik tanah oleh *Pontoscolex corethrurus,* sejenis cacing tanah endogik di tropika terhadap tindak balas pokok pisang kepada layu Fusarium. Rekod fotografi dan penskalaan gejala layu luaran dan dalaman, berat basah tumbuhan, kandungan klorofil dan indeks keterukan penyakit (DSI) telah dilakukan. Disamping itu, kepekatan asid salisilik (SA) dan asid jasmonik (JA) daripada tumbuhan juga telah dianalisa kerana kedua-duanya memainkan peranan penting dalam mekanisma pertahanan tumbuhan terhadap patogen. Hasil kajian menunjukkan kehadiran cacing tanah menyumbang kepada peningkatan berat basah tumbuhan, kandungan klorofil yang tinggi dan DSI lebih rendah berbanding dengan pokok pisang tanpa cacing tanah yang tidak dijangkiti dan dijangkiti. Pokok dengan inokulasi cacing tanah juga menunjukkan kelewatan seminggu munculnya gejala layu luaran dan dalaman. Kepekatan SA dalam pokok dengan rawatan cacing yang tidak dijangkiti menunjukkan nilai yang lebih tinggi (0.72±0.09 μg/g, $p<0.05$) berbanding tanpa cacing tanah. Untuk JA, kepekatan pada minggu pertama adalah dua kali ganda untuk pokok dengan cacing tanah yang dijangkiti (0.96±0.01 μg/g) berbanding tanpa cacing tanah (0.47±0.03 μg/g). Oleh itu, cacing tanah dicadangkan boleh meningkatkan pertahanan asas pokok dengan meningkatkan hormon pertahanan tumbuhan. Hasil kajian ini menunjukkan bahawa penambahbaikkan tanah oleh *P. corethrurus* boleh meningkatkan pertumbuhan tumbuhan, melambatkan kemunculan gejala layu dan meningkatkan penghasilan hormone tumbuhan bagi system pertahanan pokok.

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I certify that a Thesis Examination Committee has met on 7 November 2016 to conduct the final examination of Siti Rohani Binti Sulaiman on her thesis entitled "Effects of soil augmentation by *Pontoscolex corethrurus* on banana plant response to Fusarium wilt" 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.

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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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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celcius
µg/g	Microgram/gram
µL	Microliter
ABA	Abscisic acid
ANOVA	Analysis of variance
Avr	Avirulence
BDB	Blood disease bacteria
C	Carbon
CH_3CN	Acetonitrile
CHOOH	Formic acid
Cm	Centimeter
CMV	Cucumber mosaic virus
CRD	Completely randomized design
DNA	Deoxyribonucleic acid
DSI	Disease severity index
Foc	*Fusarium oxysporum* f. sp. *cubense*
G	gram
GAP	Good agricultural practice
H_2O	Water
HL	Human lysozyme
HR	Hypersensitive response
ICS	Isochorismate synthase
INA	Isonicotinic acid
ISR	Induced systemic resistance
JA	Jasmonic acid
Jas	Jasmonates
Kg	Kilogram
LAR	Locally acquired resistance
M	Meter
MBC	Methyl benzimidazole carbamate
mg/cm^2	Milligram/centimeter square
mgL^{-1}	Milligram per liter
mL	Milliliter
N	Nitrogen
Nm	nanometer
NPR	Nonexpresser pathogenesis-related protein
O_2	Oxygen
PAL	Phenylalanine ammonia-lyase
PDA	Potato dextrose agar
PGPR	Plant growth-promoting bacteria
PR	Pathogenesis-related
R	Resistance
RuBisCO	Ribulose biphosphate carboxylase/oxygenase
SA	Salicylic acid
SAMT	Salicylic acid methyl transferase
SAR	Systemic acquired resistance

SE	Standard error
SR	Systemic resistance
TCV	Turnip crinkle virus
TR4	Tropical Race 4
UFLC	Ultra-fast liquid chromatography
US$	United of State Dollar

CHAPTER 1

INTRODUCTION

Banana is one of the most important fruit crops grown commercially in Malaysia. It is also one of the world most valuable agricultural commodities (Ploetz, 2015). In Malaysia, 294,000 metric tonnes of banana was produced annually, valued at US$24 million (Tengku Ab. Malik *et al*., 2011). Most of the production is for domestic consumption but it is also exported to Singapore, Indonesia, Brunei, Saudi Arabia, and Hong Kong (Husain and William, 2011). The potential of banana plantation growth is however hampered due to a myriad of diseases that greatly affects the fruit yield. One of the extremely destructive banana diseases is Fusarium wilt.

Fusarium wilt is a major disease that causes serious problem to the global banana cultivation. It is caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), a soil-borne fungus (Ploetz, 2006). In Southeast Asia especially Indonesia and Malaysia, Fusarium wilt was discovered in many banana commercial plantations in the early 1990s (Molina *et al.*, 2009). This disease affects not only the large plantation but also involves small cultivated area. A serious epidemic of Fusarium wilt has caused big losses in banana production involving millions of dollars (Molina *et al.*, 2009). Fusarium wilt affects a wide variety of bananas consisting of cooking and dessert cultivars. The high variability of this fungus which include its ability to remain for decades in soil, wide host range, easily spreadable and along with its lethal impact to the host plant makes it difficult to control. The pathogen can be transferred easily through water, soil, farming tools, machinery or through insect vector which enables this disease to spread rapidly in the plantation (Tengku Ab. Malik *et al*., 2011). Thus, disease management approaches or srategies that can control this disease is highly demanded.

Control strategies that have been implemented to overcome this disease includes chemical control, genetic improvement, use of disease-free planting materials and application of biological control (Moore *et al.,* 1999; Nel *et al*., 2007; Ploetz, 2015). However, there are various constraint in the application of the strategies such as the negatives effects on the environment, public concern on food safety, and practical limitation. Biological control approach has become an increasingly popular consideration for disease management because it is environmental friendly and highly practical. For instance, the use of cyanobacteria and *Trichoderma* based formulation to control root rot disease in cotton (Babu *et al.*, 2015). However, it is only effective for short-cycle plant such as tomato or radish, but it is not suitable for banana which is a perennial plant (Ploetz, 2007). In addition, the effectiveness depends largely on the concentration of the microbes in the soil that need to be replenished frequently. With many constraints in disease control application, focus was given more on approaches of promoting plant health to increase its defense.

Promoting plant health is of global interest at present as potential strategies for disease control against the pathogen in sustainable agriculture. Most of the existing practices have failed to tackle the importance of soil ecology that might contribute to the emergence of disease. Earthworms is a significant organism that can contribute to soil ecological improvement (Edwards and Fletcher, 1988; Doube *et al.*, 1994; Elmer, 2009; Amossé *et al.*, 2015; Pelosi *et al.*, 2015). The nutrient cycle and decomposition in soil are enhanced by their burrowing, feeding and casting activities (Brown *et al.*, 2004; Capowiez *et al.*, 2014). Thus, they give positive impacts on the overall soil function and ecosystem. *Pontoscolex corethrurus,* an endogeic earthworm is one of the most important fauna which is dominant in the tropical soil has significant effects on soil properties (Sabrina *et al.*, 2009). This earthworm gives significant changes in soil composition especially in the first 10 cm stratum. *P. corethrurus* actively consume soil and humified organic matter on the upper soil layer and form burrows within the soil (Bottinelli *et al.*, 2010). Their presence in soil help to lay out a conducive condition for plant growth. Earthworms also have the ability to regulate soil microbes which are proven to enhance the plant growth (Edward and Bohlen, 1996; Zhang *et al.*, 2014). In this perspective, earthworms may direct or indirectly affect soil-borne pathogen that causes plant diseases. Teng *et al.* (2016) reported on antimicrobial properties of earthworm excretion that could inhibit blood disease bacteria (BDB). This shows that earthworm presence can cause direct effect on plant pathogen. Besides that, plant growth and health improvement via earthworm activities in soil may indirectly improve plant defense towards pathogen.

Plant health may influence the plant defense which is linked to their metabolic system (Van Loon, 2007). Phytohormones that play a major role in plant system, have great contribution in mediating the plant responses towards biotic and abiotic stresses including pathogen infection. Salicylic acid (SA) and jasmonic acid (JA) are the phytohormones that actively involved in building resistance towards biotrophic and necrotrophic pathogens (Glazebrook, 2005). SA and JA production in plants may also induced by external factors such as abiotic and biotic factors (Puga-Freitas and Blouin, 2014). As one of the major contributors to healthy soil ecosystem, earthworms may enhance plant defense system by improving plant health. If the plant is healthy, it can produce more of this compound where the chances for successful pathogen infection and invasion can be reduced and thus decrease disease prevalence in the plant (Elmer and Ferrandino, 2009). The earthworm-pathogen relationship was earlier thought to only involve direct interaction like predation, habitat destruction, competition for organic matter, and production of antibacterial or antifungal properties that decrease the pathogen population (Brown *et al.*, 2004). However, it is found that earthworm interaction with plants actually induces some of the genes in plants which contribute to better plant growth and defense mechanisms (Puga-Freitas and Blouin, 2014). Though, it is still unclear whether earthworms can have significant effects on the level of plant defense hormone.

Currently, there is a lack of study on the earthworm role as soil engineer in inducing ecological improvement which may affect banana plant and its response to Fusarium wilt. Furthermore, there is also no report on earthworm effect on phytohormones responsible for the plant defense specifically SA and JA. Therefore, the present study was designed to look into the possibility of soil augmentation caused by *P. corethrurus* to affect the banana plant with Fusarium wilt infection and investigate the earthworm effects on SA and JA levels that reflects the plant defense. Therefore, objectives of the present study are:

- To assess the impact of soil augmentation by *Pontoscolex corethrurus* on banana plant growth and its response to fusarium wilt disease infection

- To conduct bioassay study on salicylic acid and jasmonic acid content of healthy banana plant and those infected with fusarium wilt disease grown in soil augmented by *Pontoscolex corethrurus*

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