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TRANSFORMING PADDY HUSK INTO COMPOST USING Bacillus spp. FROM TERMITE GUT TO ENHANCE Zea mays L. GROWTH

CARLINA FREDDIE SIMOL @ RACHELLYNNA

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By

CARLINA FREDDIE SIMOL @ RACHELLYNNA

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Chair : John Keen Anak Chubo, PhD
Faculty : Agricultural Science and Forestry (Bintulu)

Domestic consumption of rice generated vast amount of paddy husk (PH) as waste. Burning of PH is an approach to instantly eliminate the accumulation of PH but burning can contribute to environmental pollution. Therefore, it is suggested that PH be transformed into a valuable product such as compost that can be used to improved soil fertility and plant productivity. Paddy husk decomposition is difficult due to its high lignin content. Composting of PH with microbes is an alternative method to manage such waste and the termite gut is known for having microbes particularly bacteria that can digest lignin. The objectives of this study were to: (i) screen and identify potential bacteria from termite's gut as a lignin degrader during PH composting, (ii) determine the nutrient content in PH compost and assess its suitability as a soil amendment, (iii) determine the ability of PH compost to retain and release nutrients into soils, and (iv) determine the effects of PH compost on soil nutrient uptake and maize (*Zea mays* L.) biomass increment. Composting was carried out for 60 days at Universiti Putra Malaysia Bintulu Sarawak Campus. Three most promising *Bacillus* spp. were added to polystyrene boxes containing compost mixtures consisting of air-dried PH, chicken feeds, molasses, and leguminous leaves. A control consisting of all raw materials without the inclusion of any additional microbes was also included. *Bacillus toyonensis* (*Bto*), *B. cereus* (*Bce*), and *B. thuringiensis* (*Bt*) was added to T2, T3, and T4 respectively. T5 to T7 were inoculated with a combination of two microbes (*Bto* + *Bce*) for T5, (*Bce* + *Bt*) for T6, and (*Bto* + *Bt*) for T7. T8 was supplied with *Bto* + *Bce* + *Bt*, whereas microbes for T9 were sourced from chicken manure. The decomposition experiment was arranged in a completely randomized design (CRD) with three replications. A soil leaching experiment was conducted for a period of 30 days, whereas the incubation experiment was conducted for a total of 90 days and samples were collected at every 30 days interval to determine the ability of composted PH in retaining nutrients when added to soil. Destructive method was adopted in the sampling of soil for the incubation experiment. The composts were tested in a pot study arranged in a randomized complete block design (RCBD) with three blocks

under a controlled environment and maize as a test crop. RCBD was adopted as there were differences in light intensity under the shade house throughout the day due to its surrounding. Maize plants were harvested during tasselling for dry matter determination. Composts with *Bacillus* spp. gave significant amount of P, Ca, Mg but not total N. Electrical conductivity (EC) was high at 60-day for most treatments and pH was slightly acidic in composts with *Bacillus* spp. Germination index (GI) in composted PH with *Bacillus* spp. ranged from 82.51 to 95.83%, indicating non-toxicity. *Bacillus* spp. showed the ability to degrade wastes with high lignin. Soil pH decreased but total soil P and Mg increased with the incubation period. Plant growth parameters such as height and leaf number significantly increased with composted organic amendments. Organic amendments led to significant increase in plant leaves, stem, and root dry matter yield. Plants treated with compost also showed significantly higher uptake of total N, P, K, Ca, and Mg while composted organic amendments indicated higher pH, total C, P, K, and Mg. The composition of total N, P, and K in composts with *Bacillus* spp. (T5, T6 and T7) suggested that these composts can be used as soil organic amendments similar to T9 (unidentified microbes from chicken manure) and are recommended for their ability in retaining plant nutrients. These findings indicated that PH composted using microbes extracted from termite gut not only increased the value of composted PH but also increased soil chemical properties, nutrient availability and uptake, and also aided in improving the overall plant growth parameters.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TRANSFORMASI SEKAM PADI KEPADA KOMPOS MENGGUNAKAN SPESIES *Bacillus* DARI USUS ANAI-ANAI UNTUK MENINGKATKAN PERTUMBUHAN *Zea mays* L.

Oleh

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Januari 2021

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Penggunaan beras domestik menghasilkan banyak sekam padi (PH) sebagai sisa. Pembakaran PH adalah salah satu pendekatan segera untuk mengelakkan pengumpulan PH tetapi pembakaran boleh menyumbang kepada pencemaran persekitaran. Oleh itu, adalah dicadangkan agar PH diubah menjadi produk berharga seperti kompos yang dapat digunakan untuk meningkatkan kesuburan tanah dan produktiviti tanaman. Penguraian PH adalah sukar kerana kandungan lignin yang tinggi. Pengkomposan PH menggunakan mikrob adalah kaedah alternatif untuk mengurus sisa tersebut dan usus anai-anai diketahui mengandungi mikrob terutamanya bakteria yang mampu mencerna lignin. Objektif kajian ini adalah untuk: (i) menyaring dan mengenal pasti bakteria berpotensi dari usus anai-anai sebagai pengurai lignin semasa pengkomposan PH, (ii) menentukan kandungan nutrien dalam kompos PH dan menilai kesesuaiannya sebagai bahan untuk pembetulan tanah, (iii) menentukan kemampuan kompos PH untuk mengekalkan dan membebaskan nutrien ke dalam tanah, dan (iv) menentukan kesan kompos PH terhadap pengambilan nutrien tanah dan peningkatan biojisim *Zea mays* L. Pengkomposan dijalankan selama 60 hari di Universiti Putra Malaysia Kampus Bintulu Sarawak. Tiga spesies *Bacillus* paling berpotensi ditambahkan ke dalam kotak polistirena yang mengandungi campuran kompos yang terdiri daripada PH kering udara, makanan ayam, gula perang, dan daun kekacang. Rawatan kawalan yang terdiri dari semua bahan mentah tanpa sebarang mikrob tambahan juga disertakan. *Bacillus toyonensis* (*Bto*), *B. cereus* (*Bce*), dan *B. thuringiensis* (*Bt*) masing-masing ditambahkan ke dalam rawatan T2, T3, dan T4. T5 hingga T7 diinokulasi dengan kombinasi dua mikrob (*Bto* + *Bce*) untuk T5, (*Bce* + *Bt*) untuk T6, dan (*Bto* + *Bt*) untuk T7. T8 dibekalkan dengan *Bto* + *Bce* + *Bt*, manakala mikrob untuk T9 diperolehi dari najis ayam. Eksperimen penguraian disusun dalam reka bentuk rawak sepenuhnya (CRD) dengan tiga replikasi. Eksperimen larut-resap tanah dilakukan selama 30 hari, manakala eksperimen inkubasi tanah dilakukan selama 90 hari dan pensampelan dikumpul setiap 30 hari untuk menentukan

kemampuan kompos PH bagi mengekalkan nutrien apabila ditambah kepada tanah. Ujian musnah digunakan dalam pensampelan tanah untuk eksperimen pengeraman tanah. Kompos diuji dalam satu kajian dalam pasu yang disusun dalam reka bentuk blok rawak penuh (RCBD) dalam persekitaran terkawal dan menggunakan jagung sebagai tanaman kajian. RCBD digunakan kerana terdapat perbezaan dalam intensiti cahaya di bawah naungan sepanjang hari akibat persekitarannya. Tanaman jagung dituai semasa tahap pembentukan jambak bunga untuk penentuan bahan kering. Kompos dengan spesies *Bacillus* memberikan jumlah P, Ca, Mg yang bererti tetapi tidak untuk jumlah N. Kekonduksian elektrik (EC) adalah tinggi pada 60 hari untuk kebanyakan rawatan dan pH didapati sedikit berasid pada kompos dengan spesies *Bacillus*. Indeks percambahan (GI) dalam kompos PH dengan spesies *Bacillus* berjulat 82.51 hingga 95.83%, menunjukkan tiada ketoksikan. Spesies *Bacillus* menunjukkan keupayaan untuk mendegradasi sisa dengan lignin yang tinggi. pH tanah didapati menurun tetapi jumlah P dan Mg tanah meningkat dengan masa inkubasi. Pembolehubah pertumbuhan tanaman seperti ketinggian dan jumlah daun meningkat secara bererti dengan bahan pembaik pulih kompos organik. Bahan pembaik pulih kompos organik mengakibatkan peningkatan bererti dalam hasil bahan kering daun, batang, dan akar tumbuhan. Tanaman yang dirawat dengan kompos juga mempunyai penyerapan jumlah N, P, K, Ca, dan Mg yang jauh lebih tinggi manakala bahan pembaik pulih kompos organik memberikan pH, jumlah C, P, K, dan Mg yang lebih tinggi. Komposisi jumlah N, P, dan K dalam kompos dengan *Bacillus* spp. (T5, T6 dan T7) mencadangkan kompos ini boleh digunakan sebagai bahan pembaik pulih organik tanah seperti T9 (mikrob tidak dicam dari najis ayam) dan disarankan kerana keupayaannya untuk mengekalkan nutrien tanah. Penemuan ini menunjukkan PH yang dikompos menggunakan mikrob yang diekstrak dari usus anai-anai tidak hanya meningkatkan nilai kompos PH tetapi turut meningkatkan sifat kimia tanah, ketersediaan dan pengambilan nutrien, serta membantu meningkatkan keseluruhan parameter pertumbuhan tanaman.

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I certify that a Thesis Examination Committee has met on 15 January 2021 to conduct the final examination of Carlina Freddie Simol @ Rachellynna on her thesis entitled "Transforming Paddy Husk into Compost using *Bacillus* spp. from Termite Gut to Enhance *Zea mays* L. Growth" 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.

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LIST OF ABBREVIATIONS

PH	Paddy Husk
AAS	Atomic Absorption Spectrophotometer
CRD	Complete Randomized Design
RCBD	Randomized Complete Block Design
ppm	Part Per Million
rpm	Revolutions Per Minute
DNA	Deoxyribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
CFU	Colony Form Per Unit
PRC	Polymerase Chain Reaction
BLAST	Basic Local Alignment Search Tools
NCBI	National Centre for Biotechnology Information
RCC	Relative Chlorophyll Content
DOI	Days of Incubation
ATP	Adenosine Triphosphate
TSP	Triple Superphosphate
KH_2PO_4	Potassium Dihydrogen Phosphate
$(NH_4)_6M_{o7}O_{27}$	Ammonium Molybdate
H_2SO_4	Sulfuric Acid
K_2SO_4	Potassium Sulfate
$KSbOC_4H_4O_4$	Potassium Antimony Tartrate
GI	Germination Index
RRG	Relative Root Growth

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Paddy husk (PH) is one type of agricultural wastes that could contribute to environmental pollution (Kawai and Tasaki, 2016). Annually, approximately 998 million tons of agricultural wastes are produced globally yearly and Malaysia contributed about 1.2 million tons to this figure (Tahir and Hamid, 2012; Kadir *et al*., 2016). The high lignin content in PH slowed down the degradation processes necessary to transform it into a rich organic amendment (Shak *et al*., 2014). Proper decomposition of PH could turn the waste into a high quality organic amendment which can improve soil physical and chemical properties, plant nutrient retention, nutrient availability, and nutrient use efficiency (Diacono and Montemurro, 2011). Approaches to turn ligninolytic biomass, including PH, into soil organic amendments has been developed in many studies (Latifah *et al.,* 2017; 2018; 2019).

Compost can be applied as a soil amendment to improve soil fertility and productivity. Organic soil amendments such as composts can be used to enhance soil productivity by providing soil organic matter (SOM) and improving the biological cycle of nutrients that are essential for the successful management of acid soils. Trujillo (2002) opined that the use of composts in agriculture did not only enhance the retention of nutrients in chemical fertilizers, but, supplied nutrients to crops while supporting rapid nutrient cycling (through microbial activities). Increasing soil microbial activities increased the decomposition of SOM thereby increasing nutrient mineralization and nutrient availability (Fageria and Baligar, 2008).

Most of studies carried out on the management of PH, are related to composting and charcoal production. However, PH is resistant to most microbial decomposition because of its high carbon to nitrogen ratio (85:1) and high content of lignocellulose (Pode, 2016; Thiyageshwari *et al*., 2018). There are a wide variety of microbes that has the ability to enhance the decomposition of lignocellulose biomass (Saritha and Arora, 2012; Rabemanolontsoa and Saka, 2016). In addition, for a more efficient decomposition of lignocellulose in PH, microbes residing in termite gut were hypothesized to be most suitable (Tsegaye *et al.*, 2018).Termites are xylophage insects with the ability to digest a wide variety of lignocellulose including high lignin wood with assistance from the termite-specific gut microbes. The feeding and digestion regimes of different termite species vary, with some species having the ability to digest crystalline cellulose and overcome the lignin barrier (Scharf, 2008; Lee *et al*., 2013). For these reasons, microbes from termites' gut are deemed to be the most promising source of plant cell wall-degrading microorganisms for the decomposition of PH into compost.

Termites are a group of eusocial insects that harbour a consortium of aerobic, anaerobic, and microaerophilic bacteria which are collectively responsible for the degradation of cellulose and hemicellulose benefitting their host organism (Scharf, 2008; Bignell, 2011). The bacterial groups from the intestinal systems are mainly affiliated to the proteobacteria dominated by the gram-positive groups. *Bacillus* spp. are among the bacteria groups known for their ability to degrade lignin and cellulose (König *et al*., 2013).

The use of *Bacillus* spp. in composting promotes decomposition of organic matter by increasing the supply or availability of primary nutrients for plant uptake (Li *et al*., 2013; Saxena *et al*., 2019). *Bacillus* spp. have abilities to transform nutritionally important elements from non-usable to highly assimilable forms without deleterious effects on the natural environment (Alley and Vanlauwe, 2009). The application of *Bacillus* spp. is a key element in maintaining soil fertility and crop productivity on the sufficiently high level, which is indispensable to achieve sustainability of farming. Addition of *Bacillus* spp. in compost production may also help to mitigate pitfalls arising from the growing demand of global population for food and the widespread of chemicalization in agroecosystems (Li *et al*., 2013). Increase interest towards organic farming cause a surge in demand on biological organic fertilizers which are based on microbial activities and become vital part of modern-day crop production which emphasizes the significance of biological inoculants. A number of rhizosphere microorganisms is known to show growth promoting activities, but very few have been extracted for the degrade lignin.

A solution for effective degradation of PH into compost with improved nutrient and mineral content using promising microbial consortium must be sourced. Combinations of *Bacillus* spp. and fungi have been reported to effectively degrade lignin and cellulose from PH (Lynd *et al*., 2002; Azizi-Shotorkhoft *et al*., 2016). However, most *Bacillus* spp. used in PH composting was isolated from soil and food sources.

1.2 Justification of Study

Based on studies on termites, it is known that the presence of different groups of bacteria and fungi in the termite gut can assist in the degradation of lignin and cellulose. These microbes, particularly bacteria, from the termite gut may also have the potential of assisting the decomposition of PH which are high in lignin and cellulose and thus should be screened and isolated. In addition, information available on the use of bacteria isolated from termite gut to degrade high lignin in PH is scarce. To the best of our knowledge, there is also limited published report on the composting of PH using ligninolytic microbial consortium.

1.3 Research Questions

i. Can bacteria in termite's gut degrade lignin and cellulose in PH and produce compost of good quality and nutrients content?
ii. Can the product of composted PH improve soil physico-chemical properties of planting media?
iii. Does different composted PH produced using different microbes affect the quality of *Zea mays* L. growth?

1.4 Hypothesis

Compost produced from PH using bacteria from termite gut will enhance soil nutrients availability and improve *Zea mays* L. growth

1.5 Objectives

The general objective of the study was to produce a compost from PH waste using lignolytic bacteria from termite gut to enhance pH decomposition and to test the compost product for suitability as a soil amendment.

Therefore, this specific objectives of the study were to:

i. screen and identify potential bacteria from termite's gut as a lignin and cellulose degrader during PH composting,
ii. determine the nutrient content in PH compost and assess its suitability as a soil amendment,
iii. determine the ability of PH compost to retain and release nutrients into soils, and
iv. determine the effects of PH compost on soil nutrient uptake and *Zea mays* L. biomass increment.

1.6 Assumptions and Limitations

All experiments in this study were conducted under controlled environment i.e., plant nursery and laboratory. The compost mix used in the study was obtained from a single source and was mixed thoroughly prior to the experiment. Meanwhile, soil used in the leaching experiment was also obtained from the same site and was mixed thoroughly to form a homogenous soil mix before being used in the experiment. Thus, it was assumed that all basic compost and soil mixes were similar physically, chemically, and even biologically in all treatments prior to the experiment, and the differences came only from the microbe added to the compost mix in the composting experiment or the compost mixed to the

soil in the leaching experiment. It is however noted here that soil in the open field can be variable physically, chemically, and biologically although they come from the same site. Therefore, the limitation of this study is that it may produce a different outcome when conducted in the open field due to soil variability factors.

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BIODATA OF STUDENT

Carlina Freddie Simol @ Rachellynna was born on 1 November 1978 at Queen Elizabeth Hospital, Kota Kinabalu, Sabah. She received her primary education at SRJK (C) Yue Min, Penampang, Sabah. She continued her secondary education at SMK Datuk Peter Mojuntin, Penampang, Sabah. She obtained her Diploma in Animal Health and Science Production in 1999 and Bachelor of Science (Honours) in Bioindustry (Animal Science) in 2002 from Universiti Putra Malaysia, Serdang. After completing her studies, she worked at the University Agriculture Park of Universiti Putra Malaysia Bintulu Campus Sarawak as an Agriculture Officer since 2002, and was appointed as the Head of Department in 2010 until 2015, she was promoted to a Senior Agriculture Officer in 2016. In 2009, she enrolled as a part time master's student at Universiti Malaysia Sarawak. She was awarded a Master of Science in Animal Nutrition in 2012. She presented an oral paper at the 33rd Malaysian Society of Animal Production Annual Conference, Langkawi in 2012. She pursued her Doctor of Philosophy (Agronomy) as a full time student in Universiti Putra Malaysia Bintulu Campus Sarawak beginning 2016. Among her research interests are Animal nutrition and Agricultural Waste management.

LIST OF PUBLICATIONS

Papers Published

Carlina, F. S., John, K. C., Patricia, J. H. K., Ong, K. H., Cindy, C., and Khalid N. (2021). Qualitative and Molecular Screening of Potential Ligninolytic Microbes from Termite (*Coptotermes curvignathus*) Gut. *Borneo Journal of Resource Science and Technology*, 11(1): 35–42. DOI: https:doi.org/10.33736/bjrst.2879.2021

Carlina, F. S., John, K. C., Patricia, J. H. K., Ong, K. H., Cindy, C., Ira, C. S., and and Khalid, N. (2021). Nutrient availability in acidic soil added with composted paddy husk amendment. *International Journal of Scientific Engineering and Applied Science*, 7(7): 37–52.

Papers Submitted/Accepted

Carlina, F. S., John, K. C., Patricia, J. H. K., Ong, K. H., Cindy, C., Lirong, Y. A., and Khalid, N. Enhancing plant nutrient availability in composted paddy husk using *Bacillus* spp. isolated from termite (*Coptotermes curvignathus*) gut. [Manuscript Reference No. MS 2021-0008 Science Asia]

Poster Published

Carlina, F. S., John, K. C., Cindy, C., Patricia, J. H. K., Ong, K. H., Ira, C. S., and Khalid, N. (2020). Phytotoxicity assessment and essential nutrients content of paddy husk compost following application of ligninolytic bacteria. Soil Management towards Plant Productivity & Environmental Sustainability. *Proceedings of the Soil Science Conference of Malaysia* 2020, p. 98–101.