IMPROVEMENT OF SELECTED CHEMICAL AND BIOLOGICAL PROPERTIES OF ACID SOIL AMENDED WITH FORTIFIED EFFECTIVE MICROORGANISMS

By

DARSHINI A/P RAWICHANDRAN

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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DEDICATION

This is dedicated to my parents who gave up themselves to see us succeed

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Soil acidification, a major cause of soil deterioration, severely influences soil production and the ecosystem. The contemporary agriculture industry dominates most of Malaysia's croplands, with intense cultivation and heavy chemical fertilizer input. Soil acidity has the potential to alter the structure and diversity of soil microbial communities. Organic additions in agriculture have the ability to boost crop output and improve soil health. In that context, soil amendments linked with beneficial microbes have been chosen as more advantageous in terms of gaining access to input and adding value to land holdings. Hence, this study was conducted to improve soil productivity through soil amendments by impacting the microbial population's assemblies and to identify the effective ratio of formulated soil amendments in controlled environment. Furthermore, this study was also conducted to determine the effect of formulated soil amendments on the soil's physical, chemical, and biological properties. Firstly, the EM was prepared using the standard protocol. Then, formulated soil amendments were done by mixing Effective Microorganisms (EM) with "X" materials, Perlite, Coconut

Shell Ash (CSA), and "Y" sources before they can be incubated in 250 g of soil in a round disposal container for 30 days. Two different rates were applied during this application, which were 50% and 100% based on the recommended rate from the literature review. The incubation study demonstrated the effectiveness of soil amendments in ameliorating acidity by increasing the soil pH, EC, ECEC, and macronutrients with the decrease of exchangeable acidity performed at 50% application than 100%. At 50 % soil amendment application, the colony forming units were highest from T0 to T4 than 100% soil amendment application. The isolates from the culturing medium, T0 to T4 were yeasts, and T2 bacteria were successfully sequenced and identified to classify the closely related strains respectively. Most of the isolated treatments are gram-negative. However, treatment 2 found in the soil amendment is gram-positive. This study found that the application of soil amendments at 50% increased the soil microbial diversity, which significantly enhanced the soil biological properties. This interaction demonstrating that fungi generally exhibit wider pH ranges for optimal growth of the genus *Ascomycota.* Our findings revealed that observed fungi is better equipped to withstand environmental stress and utilize many resources, increasing its dominance in acidic soils. Further correlation analysis indicated that exchangeable Mg was positively correlated with the relative abundance of microbe. This observation can potentially occur when Mg ions promote nitrification activity in yeasts by altering the enzyme activities, and this will explains the highly positive relationship between Mg concentration and fungal population. The exchangeable Na and Ca were negatively correlated with the relative abundance. In the present study, the K content increased in response to the decreasing pH value, and based on this, the relative abundance of Actinobacteria was highest in high acidity soils. As known, actinobacteria is a type of saprophytic bacteria which are present in

the soil that has the ability to decompose complex substrates. Generally, higher number of beneficial yeast strains were successfully isolated from the formulated soil amendments that has been treated with EM from T0 to T4 except for T2 that contain a bacterial strain. The isolated fungi from this study can be utilized in promoting soil fertility with an appropriate application rate. Utilization of waste in formulating soil amendments can help to reduce waste generation and environmental pollution. In general, this study indicates the importance of soil amendments in promoting beneficial microbes in acidic soil.

Keywords: Microbial inoculants, Organic matter, Sustainable agriculture, Soilhealth, Soil fertility

SDG: GOAL 2: Zero Hunger, GOAL 12: Responsible Consumption and Production, GOAL 13: Climate Action, GOAL 15: Life on Land

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

PENAMBAHBAIKAN SIFAT-SIFAT KIMIA DAN BIOLOGI TERPILIH TANAH ASID DIPINDA DENGAN MIKROORGANISMA BERKESAN YANG DIPERTETAPKAN

Oleh

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Pengasidan tanah, punca utama kemerosotan tanah, mempunyai pengaruh yang teruk terhadap pengeluaran tanah dan ekosistem. Industri pertanian kontemporari mendominasi kebanyakan tanah pertanian Malaysia, dengan penanaman intensif dan input baja kimia yang berat. Keasidan tanah berpotensi untuk mengubah struktur dan kepelbagaian komuniti mikrob tanah. Penambahan organik dalam pertanian mempunyai keupayaan untuk meningkatkan hasil tanaman dan meningkatkan kesihatan tanah. Dalam konteks itu, pindaan tanah yang dikaitkan dengan mikrob berfaedah telah dipilih sebagai lebih berfaedah dari segi mendapatkan akses kepada input dan menambah nilai kepada pegangan tanah. Oleh itu, kajian ini dijalankan untuk meningkatkan produktiviti tanah melalui penggunaan pindaan tanah dengan memberi kesan kepada himpunan populasi mikrob serta mengenal pasti nisbah berkesan pindaan tanah yang dirumuskan dalam persekitaran terkawal. Selain itu, kajian ini juga dijalankan untuk menentukan kesan pindaan tanah yang dirumuskan terhadap sifat fizikal, kimia dan biologi tanah. Pertama, EM disediakan menggunakan protokol.

Kemudian, pindaan tanah yang dirumuskan dilakukan dengan mencampurkan Mikroorganisma Berkesan dengan bahan “X”, Perlite, Abu Tempurung Kelapa, dan sumber “Y” sebelum ia boleh diinkubasi dalam 250 g tanah dalam bekas pelupusan bulat untuk 30 hari. Dua tahap berbeza telah digunakan semasa aplikasi ini, iaitu 50% dan 100%. Kajian inkubasi menunjukkan bahawa keberkesanan pindaan tanah dalam memperbaiki keasidan dengan meningkatkan pH tanah, EC, ECEC dan makronutrien dengan penurunan keasidan boleh tukar telah dilakukan pada penggunaan 50% daripada 100%. Pada permohonan pindaan tanah 50%, unit pembentuk koloni adalah tertinggi dari T0 hingga T4 daripada permohonan pindaan tanah 100%. Pengasingan daripada medium pengkulturan, T0 hingga T4 ialah yis dan T2 ialah bakteria berjaya dijujukan dan dikenal pasti untuk mengelaskan strain yang berkait rapat masing-masing. Kebanyakan rawatan terpencil adalah gram-negatif. Walau bagaimanapun, T2 yang terdapat dalam pindaan tanah adalah gram positif. Kajian ini mendapati bahawa penggunaan pindaan tanah pada 50% meningkatkan kepelbagaian mikrob tanah, yang secara signifikan meningkatkan sifat biologi tanah. Komposisi komuniti kulat kurang terjejas oleh pH, yang konsisten dengan kajian budaya tulen. Interaksi inimenunjukkan bahawa kulat secara amnya mempamerkan julat pH yang lebih luas untuk pertumbuhan optimum genus Ascomycota. Penemuan kami mendedahkan bahawa kulat yang diperhatikan lebih bersedia untuk menahan tekanan persekitaran dan menggunakan banyak sumber, meningkatkan penguasaannya dalam tanah berasid. Analisis korelasi lanjut menunjukkan bahawa Mg yang boleh ditukar berkorelasi positif dengan kelimpahan relatif mikrob. Pemerhatian ini berpotensi berlaku apabila ion Mg menggalakkan aktiviti nitrifikasi dalam yis dengan mengubah aktiviti enzim, dan ini akan menerangkan hubungan yang sangat positif antara kepekatan Mg dan populasi kulat. Na dan Ca yang boleh ditukar berkorelasi negatif dengan kelimpahan relatif. Dalam kajian ini, kandungan K meningkat sebagai tindak balas kepada penurunan nilai pH, dan berdasarkan ini, kelimpahan relatif Actinobacteria adalah tertinggi dalam tanah keasidan tinggi. Seperti yang diketahui, actinobacteria adalah sejenis bakteria saprofit yang terdapat di dalam tanah yang mempunyai keupayaan untuk menguraikan substrat kompleks. Secara amnya, bilangan strain yis berfaedah yang lebih tinggi telah berjaya diasingkan daripada pindaan tanah yang dirumuskan yang telah dirawat dengan EM daripada T0 hingga T4 kecuali T2 yang mengandungi strain bakteria. Kulat terpencil daripada kajian ini boleh digunakan dalam menggalakkan kesuburan tanah dengan kadar penggunaan yang sesuai. Penggunaan sisa dalam merumuskan pindaan tanah boleh membantu mengurangkan penjanaan sisa dan pencemaran alam sekitar. Secara umumnya, kajian ini menunjukkan kepentingan pindaan tanah dalam menggalakkan mikrob berfaedah dalam tanah berasid.

Kata Kunci: Bahan organik, Inokulan mikroba, Kesihatan tanah, Kesuburan tanah, Pertanian lestari

SDG: MATLAMAT 2: Sifar Kelaparan, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab, MATLAMAT 13: Tindakan Iklim, MATLAMAT 15: Kehidupan di Darat

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LIST OF ABBREVIATIONS

%	Percentage
H^+	Hydrogen
nm	Nanometer
kg	Kilogram
KCl	Potassium chloride
ppm	Parts per million
mg	Microgram
^{0}C	Degree Celsius
t/ha	Ton per hectare
L	Liters
mL	Milliliters
N	Normality

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Soil acidification, a major cause of soil deterioration, has a severe influence on soil production and the ecosystem. Soil acidification is a widespread issue that affects around 30-50% of the world's total land area (Kochian et al., 2004). Soil acidification has become a serious issue in soil ecosystems, attracting considerable attention from experts (Guo et al., 2010; Tian & Niu, 2015; Yan et al., 2020). Under natural settings, the pace of soil acidification is relatively gradual and happens over hundreds to millions of years (Guo et al., 2010), but with the advent of the contemporary agriculture system, where artificial fertilizer has been used excessively, this problem has grown more serious.

Anthropogenic activities such as fertilization can contribute to soil acidity (Fujii, 2014; Fundulea et al., 2015; Lani et al., 2018; Mahmud & Chong, 2022). The contemporary agriculture industry dominates most of Malaysia's croplands, with intense cultivation and heavy chemical fertilizer input (Anisuzzaman et al., 2021; S. Y. Ho et al., 2019). These methods, however, have a negative influence on soil health. They would, for example, cause soil physicochemical deterioration and disruptions to soil microbiomes (Shen et al., 2019; Zhao et al., 2020).

According to Barak et al. (1997), the rate of soil acidification produced by excessive fertilization was 25-fold greater than that caused by natural acid deposition, especially in response to nitrogen fertilizer, which releases protons and increases soil acidification (Guo et al., 2010; Huang et al., 2015).Soil fertility and productivity will drop when soil acidity rises, according to a prior analysis (Hartmann & Schikora, 2012; Matsuyama et al., 2005). Furthermore, increasing soil acidity mobilizes potentially hazardous metals such as Cu, Pb, and Zn (Itelima et al., 2018; Kaufman & Blake, 1973; L. Wang & Qu, 2009; Watmough, 1999), increasing their translocation and bioavailability (Ahmad et al., 2014; Susha Lekshmi et al., 2014; Wang & Qu, 2009).

Soil microbes are vital in facilitating soil nutrient transformation and maintenance, material circulation, and energy movement (Prescott & Grayston, 2013; Urbanová et al., 2015). Bacteria, for example, play a crucial part in the carbon and inorganic salt cycles (Dressel & Farid, 2018). Fungi are also the principal microorganisms that thrive on foreign carbon, and they function in the ecosystem as decomposers, mycorrhizal symbionts, and pathogens (Broeckling et al., 2008; O'Brien et al., 2005).

Soil acidity has the potential to alter the structure and diversity of soil microbial communities. Because soil microbial populations are sensitive to soil environmental changes, they can be employed as an essential early warning indication of agricultural soil quality changes throughout the planting process (Zhang et al., 2019). As previously documented, the application of organic amendments to agricultural soil improved soil microbial structural and functional diversity, as well as bacterial richness and evenness (Siebielec et al., 2018). This might significantly reduce their dependency on chemical fertilizers and the associated environmental concerns caused by either excessive chemical fertilizer usage or improper disposal or non-recycling of agro waste (Altieri & Esposito, 2010; Sayara et al., 2020; Urra et al., 2019).

Organic additions in agriculture have the ability to boost crop output and improve soil health. Agricultural wastes, or agro-wastes, of various origins and compositions, can be used as non-toxic organic amendments. Organic soil amendments made from agro-waste have been found to perform a variety of critical functions in soil health by giving important nutrients and boosting organic matter content. Organic amendments, according to studies, increase soil microbial activity and biomass, as well as changes in microbial community composition (with potentially concomitant effects on soil functioning) and, to a lesser extent, microbial diversity (Das et al., 2017; Hernandez-Almanza et al., 2014; Marschner et al., 2003; Pershina et al., 2015; Reardon & Wuest, 2016).

The positive benefits of organic additions can also be connected to an improvement in soil chemical properties (Li et al., 2016). Soil amendments comprising high levels of calcium and/or magnesium may produce a "liming effect." These considerable variations in soil pH have enhanced soil microbial activity indirectly (Mijangos et al., 2010).

1.2 Problem Statement

Improvement in fertility management through soil amendments inputs can enable efficient use of the inputs applied and increase overall soil system productivity. Currently, only a few studies have been related to the effectiveness of soil amendments in acidified soil. Discover promoting microbial mechanisms in acidified soils is also less researched. Soil acidification mainly affected the soil bacterial community due to narrow pH ranges for the optimal growth of bacteria (Rousk et al., 2010; Sulok et al., 2021; Tripathi et al., 2018). As a result of soil fertility reduction, declining land productivity in Malaysia is a major problem facing today.

1.3 The Hypotheses of This Study Are as Follows:

Ho: The application of soil amendments and effective microorganisms affects soil chemical properties and biological properties.

Ha: The application of soil amendments and effective microorganisms does not affect soil chemical properties and biological properties.

1.4 Objectives

The general objective of this study was to improve soil productivity through the use of soil amendments by enhancing microbial populations. The specific objectives of the study were:

i. To identify the effective rate of formulated soil amendments under a controlled environment to improve soil microbial populations.

ii. To determine the effect of formulated soil amendments on the soil's chemical, and biological properties.

1.5 Significance of this Study

Soil productivity and livelihoods might be increased by combining soil amendments, hence improving soil health. Malaysia's objective is to boost soil fertility by reducing the use of chemical fertilizers and replacing them with ecologically friendly soil amendments (Lani et al., 2018). In that context, soil amendments linked with beneficial bacteria have been chosen as more advantageous in terms of gaining access to input and adding value to land holdings (Sahid et al., 2015).

Another finding of this study is that comprehensive new types of soil amendments with added beneficial microbes in acidic soil that can be used to support ‘green technology’ for food safety and societal well-being will encourage commitment to the production and commercialization of these products (Sulaiman et al., 2021).

1.6 Conceptual Framework

The low fertility of acidic soils is manifested through the exploration of chemical and biological properties of soil to represent low soil health. The addition of soil amendments with effective microorganisms is expected to improve soil’s chemical and biological properties which consequently, increase soil health and fertility. Figure 1.1 shows the steps to be taken to improve soil productivity and the outcomes expected.

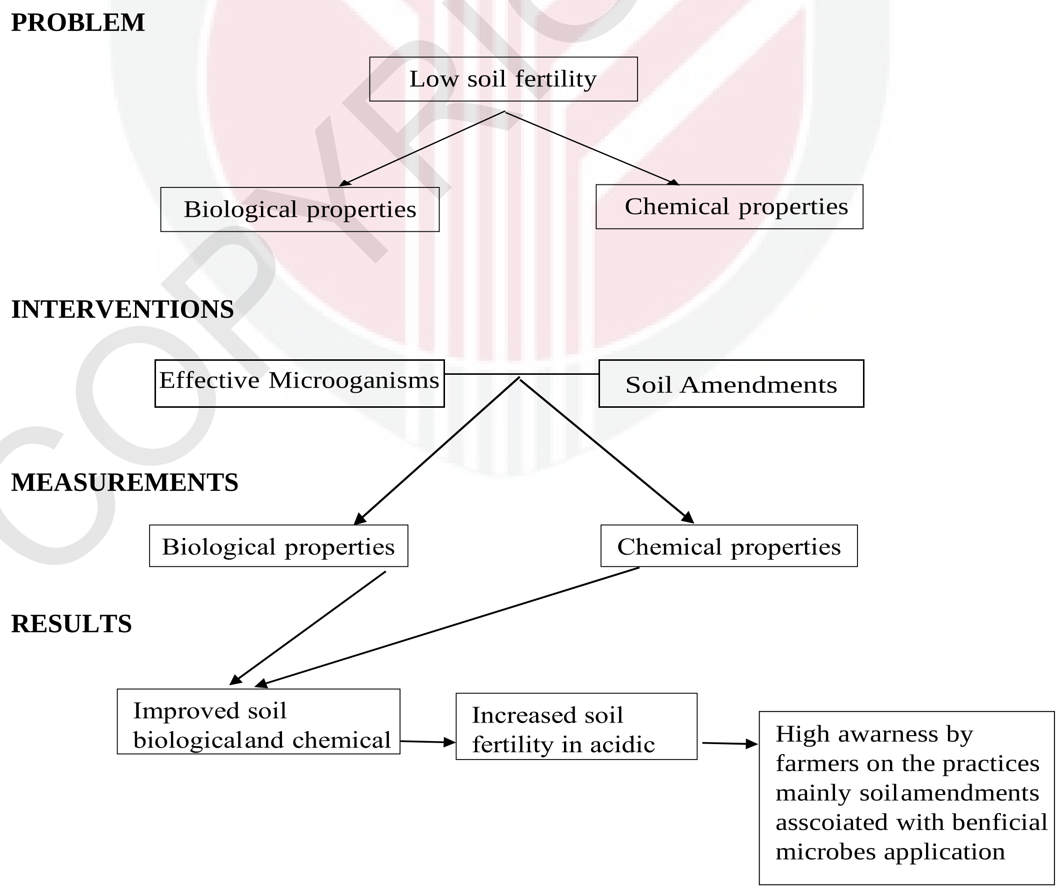

Figure 1.1 : Conceptual Framework

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