DEVELOPMENT OF CALCIUM-BASED METAL-ORGANIC FRAMEWORKS AS FUNGICIDES CARRIER AGENTS AGAINST *Ganoderma boninense*

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 Doctor of Philosophy

June 2023

FS 2023 25

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Chairman : Professor ChM Mohd Basyaruddin bin Abdul Rahman, PhD
Faculty : Science

Fungicides are crucial for defending against major plant pathogens that cause severe diseases to plants. Fungal diseases such as basal stem rot (BSR) caused by *Ganoderma* spp. fungi have affected one of the most important commodities in Malaysia. Most of the fungicide active ingredients are not stable which required them to be formulated with inert ingredients such as organic solvents. The application of these organic solvents contributed to the environmental problem as there is no control in the release rate of the fungicide active ingredients. Increasing attention to improving fungicide stability is believed to be helpful in preventing environmental degradation and ecological issues. One of the methods for reducing the amount of fungicide usage while maintaining its effectiveness is through the incorporation of carrier agents in sustaining the delivery system for an extended time. The objective of this study is to develop calcium-based metal-organic framework as slow-released fungicide carrier agents against *Ganoderma boninense*. In this study, plant acid linkers, namely, L-lactic, L-malic, and D-tartaric were used to construct calcium-based metal–organic frameworks (MOFs). L-Lactic, L-malic, and D-tartaric acids are naturally occurring compounds found in citrus fruits, making them safe and environmentally friendly for biological and environmental applications. The crystal structures of MOF-1201, UPMOF-1, and UPMOF-2 were determined by single-crystal X-ray diffraction (SCXRD) analysis and fully characterized by powder X-ray diffraction (PXRD), thermogravimetric analysis (TGA), Fourier-transform infrared (FTIR) spectroscopy, elemental analysis, field emission scanning electron microscope (FESEM), and energy dispersive X-ray (EDX) microscopy. MOF-1201, UPMOF-1 and UPMOF-2 are three-dimensional networks and crystallize in monoclinic and orthorhombic lattice systems. Both UPMOF-1 and UPMOF-2 are novel MOFs where UPMOF-1 exhibits a new topology, and UPMOF-2 possesses gra topology. Hexaconazole, a systemic fungicide from triazole group was incorporated in calcium-based metal-organic frameworks using a microwave irradiation technique. The loading content of hexaconazole in MOF-1201, UPMOF-1, and UPMOF-2 was determined using High-Performance Liquid Chromatography (HPLC). From the results, Hexa-MF, Hexa-UP1, and Hexa-UP2 were abled to maintain their crystallinity with high encapsulation efficiency (EE) values more than 80.0%. Hexa-MOFs loaded with different amount of hexaconazole were used as effective antifungal agents against *G. boninense*. Hexa-MOFs effectiveness were evaluated via artificial inoculation of oil palm seedlings with the rubber woodblock, which fully colonized with the *G. boninense* mycelium. The highest disease reduction of 100.0% was obtained from Hexa-UP1 loaded with 3.0% hexaconazole at 26 weeks after inoculation (WAI). This study provides a potential in the development of efficient carrier agents that are capable to sustain the active ingredient for a longer period.

Abstrak tesis ini dikemukankankan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

PEMBANGUNAN KERANGKA LOGAM ORGANIK BERASASKAN KALSIUM SEBAGAI EJEN PEMBAWA RACUN KULAT TERHADAP *Ganoderma boninense*

Oleh

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Racun kulat adalah penting sebagai pertahanan terhadap patogen penting yang menyebabkan penyakit-penyakit berbahaya kepada tumbuh-tumbuhan. Penyakit kulat seperti reput pangkal batang (BSR) berpunca dari *Ganoderma* spp. telah menjejaskan salah satu komoditi yang paling penting di Malaysia. Kebanyakan bahan aktif untuk racun kulat adalah tidak stabil dan memerlukannya untuk dirumus dengan bahan lengai seperti pelarut organik. Penggunaan pelarut organik menyumbang kepada masalah alam sekitar memandangkan tiada kawalan dalam kadar pelepasan bahan aktif untuk racun kulat tersebut. Meningkatkan tumpuan dalam penambahbaikan kestabilan racun kulat dianggap dapat membantu mencegah kemerosotan alam sekitar dan isu-isu ekologi. Salah satu kaedah untuk mengurangkan jumlah penggunaan racun kulat sambil mengekalkan keberkesanannya adalah melalui penggabungan ejen pembawa bagi mengekalkan sistem penghantaran untuk jangka masa yang panjang. Objektif kajian ini adalah untuk membangun kerangka logam organik berasaskan kalsium sebagai pelepasan perlahan racun kulat ejen pembawa terhadap *G. boninense*. Dalam kajian ini, penghubung asid tumbuh-tumbuhan, iaitu, L-laktik, L-malik, dan D-tartarik digunakan untuk membina kerangka logam organik (MOFs) berasaskan kalsium. Asid L-laktik, L-malik, dan D-tartarik ialah sebatian semula jadi ditemui dalam buah-buahan sitrus, menjadikannya selamat dan mesra alam untuk aplikasi biologi dan alam sekitar. Struktur kristal bagi MOF-1201, UPMOF-1, dan UPMOF-2 ditentukan melalui analisis pembelauan kristal tunggal sinar-X (SXRD) dan diperincikan sepenuhnya melalui pembelauan serbuk sinar-X (PXRD), analisis termogravimetrik (TGA), spektroskopi inframerah transformasi Fourier (FTIR), analisis unsur, mikroskop elektron pengimbasan pelepasan medan (FESEM), dan and mikroskop penyebaran tenaga sinar-X (EDX). MOF-1201, UPMOF-1 dan UPMOF-2 merupakan kerangka tiga-dimensi dan menghablur dalam sistem kekisi monoklinik dan ortorombik. Kedua-dua UPMOF-1 dan UPMOF-2 merupakan MOF baharu di mana UPMOF-1 mempamerkan topologi baharu, dan UPMOF-2 memiliki topologi gra. Hexaconazole, racun kulat sistemik dari kumpulan triazol telah disepadukan dalam kerangka logam organik berasaskan kalsium menggunakan teknik radiasi

gelombang mikro. Kandungan pemuatan hexaconazole dalam MOF-1201, UPMOF-1, dan UPMOF-2 ditentukan melalui kromatografi cecair berprestasi tinggi (HPLC). Berdasarkan hasil kajian, Hexa-MF, Hexa-UP1, dan Hexa-UP2 mampu mengekalkan kehabluran dengan kecekapan enkapsulasi (EE) yang tinggi melebihi 80.0%. Hexa-MOFs dimuatkan dengan jumlah hexaconazole yang berlainan telah digunakan sebagai ejen anti-kulat yang berkesan terhadap *G. boninense*. Keberkesanan hexa-MOFs dinilai melalui inokulasi tiruan anak pokok kelapa sawit dengan blok kayu getah, yang dipenuhi koloni miselium *G. boninense*. Pengurangan penyakit yang tertinggi dengan 100.0% telah diperolehi dari Hexa-UP1 yang dimuatkan dengan 3.0% hexaconazole pada 26 minggu selepas inokulasi. Kajian ini memberikan potensi kepada pembangunan ejen pembawa yang berkesan dalam mengekalkan bahan aktif dalam jangka masa panjang.

ACKNOWLEDGEMENTS

All praise to Allah S.W.T, for blessing me with the strength and health I was able to complete this doctor of philosophy degree project.

First and foremost, I would like to express my deepest appreciation to my supervisor, Professor ChM Dr. Mohd Basyaruddin Abdul Rahman, for his dedication, guidance, and invaluable advice throughout this study. Without his support and motivation, it might be more challenging for me to walk on this journey. I would like to thank my supervisory committee, Dr. Norhayu Asib for the advice and guidance on agriculture study, and Dr. Adila Mohamad Jaafar for the valuable insight in my study. I would like to express my sincere gratitude to our collaborators, Mr Kyle E. Cordova from Royal Scientific Society, Amman, Jordan and Dr. Ha L. Nguyen from University of California Berkeley for their continuous guidance and knowledge throughout my study.

Special thanks go to my amazing labmates, Mohammad Firdaus Ahmad, Umar Abd Aziz, Dr. Velan Raman, Dr. Nurul Akmarina Mohd Abdul Kamal, Dr. Azren Aida Asmawi, Nur Aininie Yusoh, Fatimah Afifah Alias, Tuan Nurul Azura Tuan Kob, Nazhirah Mohd Nasri, Nurul Natasya Muhamad Khirudin and others for sharing the literature, knowledge, assistance, and encouragement throughout my study. I would like to thank the staff and lab assistants of the Department of Chemistry, Faculty of Science and Department of Plant Protection, Faculty of Agriculture for their assistance. Special thanks to my lovely housemates, Nurul Nadia Masron, Intan Suraya Masron, and Rosazima Abdullah for their encouragement and help during my study.

Last but not least, my heartfelt gratitude to the most important people in my life, my beloved father Ahmad Aljafree Mohd Razalli and my beloved mother Noreha Mohd Yusof for their unconditional love, support, and encouragement throughout this journey. I would like to thank my beloved grandmothers, Rahmah Mohd Shaari and Maimumah Mat Jais for their du'a and comfort. To my wonderful family, thank you for their support and encouragement. Indeed, all good deeds and kindness will never be forgotten or uttered.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSR	Basal stem rot
BET	Brunauer–Emmett–Teller
Ca-MOF	Calcium-based metal-organic framework
CD	Cyclodextrin
CHNSO	Carbon, hydrogen, nitrogen, sulphur, oxygen
COF	Covalent organic framework
DI	Disease incidence
DMA	Dimethylacetamide
DMF	*N,N*-dimethylformamide
DMSO	Dimethyl sulfoxide
DSI	Disease severity index
EDX	Energy dispersive X-ray
EtOH	Ethanol
EE	Encapsulation efficiency
EU	European Union
FAO	Food and Agriculture Union
FESEM	Field emission scanning electron
FTIR	Fourier Transform Infrared
HPLC	High-Performance Liquid Chromatography
LC	Loading capacity
MeOH	Methanol
MPOB	Malaysian Palm Oil Board
MSPO	Malaysian Sustainable Palm Oil
MOF	Metal-organic framework
NMR	Nuclear magnetic resonance

NMP	N-Methyl-2-pyrrolidone
P	Phosphorus
PDR	Percentage disease reduction
PSM	Post-synthetic modification
PXRD	Powder X-ray diffraction
SBU	Secondary building units
SCXRD	Single crystal X-ray diffraction
TGA	Thermogravimetric Analysis
THF	Tetrahydrofuran
RWB	Rubber wood block
WAI	Week after inoculation

CHAPTER 1

INTRODUCTION

1.1 Overview

Oil palm, (*Elaeis guineensis*) is considered as the most important global oil crop owing to its products exported worldwide in variety form of oil, meal and other derivatives. Malaysia is the world's second largest palm oil producer (Kate, 2021). In 2018, Malaysia has 5.85 million hectares of planted oil palm that covered more than 60% of the agricultural land (Maluin *et al.,* 2020). Oil palms are prone to be infected by fungal pathogens which could cause an economical reduction. Infected oil palms due to disease can reduce yields between 50 to 80% (Murphy *et. al.,* 2021). In Malaysia, basal stem rot disease (BSR) was reported to infect oil palms starting from immature palms and mature oil palms (Paterson, 2019; Zulkifli *et. al.,* 2010). The basal stem rot disease is caused by the pathogenic fungi from *Ganoderma* spp.

Ganoderma spp. is a parasite that lives saprophytically on a food base obtained from stumps and roots. Several *Ganoderma* spp. species, including *Ganoderma boninense*, *Ganoderma zonatum,* and *Ganoderma miniatotinctum,* are responsible for the BSR disease of oil palm in Malaysia. The most aggressive known pathogen is *Ganoderma boninense*, which caused the infected trees dead within six to 24 months for immature palms and one to two years for mature palms after the symptoms have developed (Jazuli *et al.,* 2022). The disease is capable to cause the death of up to 80% of plantings halfway to their economic life (Paterson, 2019). It is important to address the right measures in controlling the disease.

Chemical treatment such as pesticide to control BSR disease showed a considerable reduction in BSR incidence (Jazuli *et al.,* 2022). Pesticide active ingredients in their pure state are not usually suitable and required combination with other ingredients. This is because of they are chemically unstable, low solubility with water, and difficult to handle and transport (Kalyabina *et al.,* 2021). For these reasons, manufacturers introduced inert substances, such as carriers, adjuvants, and other ingredients in order to enhance the effectiveness, safety, handling, and storage of pesticide active ingredients (Płonka *et al.,* 2016). Many liquid or solid materials have been used as carriers to aid in the delivery of pesticide active ingredients. Materials such as clay minerals, siliceous materials, polymers, and variety of organic and inorganic materials exhibited useful properties such as stiffness, permeability, crystallinity, thermal stability and biodegradability (Nuruzzaman *et al.*, 2016).

Metal-organic frameworks (MOFs) have attracted wider attention from the scientific community, owing to their compatibility in the field of material science, biology, nanotechnology and delivery. Metal-organic frameworks are constructed by joining metal-containing units [secondary building units (SBUs)] with organic linkers through strong bonds to form open crystalline frameworks with permanent porosity (Furukawa *et al.,* 2013). The practical feasibility of MOFs is possible due to their abilities for biodegradability, excellent porosity, high loading capacity, ease of surface modification, among others (Beg *et al.,* 2017). The rapid growth in the utilisation of MOFs for agriculture purposes are currently expanding. For instance, MOFs have been used as carriers, sensors and antifungal agents both in pesticides and fertilizers. The used of MOFs as slow-release carrier agents was reported by several researchers. For instance, chiral MOFs (MOF-1201 and MOF-1203) constructed from Ca^{2+} and $_{L}$-lactate ions were capable to encapsulate and provide slow-release of an agriculture fumigant, *cis*-1,3-dichloropropene gas (Yang *et al.,* 2017).

1.2 Problem Statement

The demand of pesticides is increasing in many developing countries. It is estimated that around two million tonnes of pesticides were used per year on a global basis (European Union, 2021). Heavy used of pesticides could cause collateral damage on farmers and workers, the environment, and public health. Furthermore, the unreasonable and unsystematic use of pesticides could strengthen pathogen resistance, reducing nitrogen fixation and biodiversity, and increasing bioaccumulation of pesticides in the plants, soil and water.

Heavy use of pesticides/weedicides causes pests/weeds to develop resistance, therefore making it harder to control their population. For instance, in Australia, a significant rise in the population of herbicide-tolerant ryegrass has been documented in various region while the agricultural land in Ukraine was polluted by 4% and 20% of hexachlorocyclohexane and dichlorodiphenyltrichloroethane (DDT) (Sharma *et al.,* 2019). The application of pesticides in substantial amount causing soil and water pollution and harming its microflora and microfauna by preventing them from absorbing vital mineral nutrients.

The addition of inert ingredients influence pesticides effectiveness, environmental fate, and risk profiles by reducing pesticides drift (Benbrook *et al.,* 2021). However, current United States and European Union law consents pesticide registrants the right to claim the pesticide formula as confidential information (Benbrook *et al.,* 2021). Hence, there is no means to know the exact materials used as inert ingredients. This rises a concern regarding these pesticide formulations might pose severe toxicity to non-target organism compared to the pesticide active ingredient itself.

Current carriers used for pesticides formulations such as organic solvents increase the solubility of pesticide active ingredients. However, the application of these organic solvents had contributed to the environmental problem due to its volatility, particularly on the lack of control in pesticide release rate (Purkait & Hazra, 2019). On the other hand, carriers such as clay, biochar, mesoporous silica, hydroxyapatite, and zeolite were seen beneficial as pesticide-controlled release agents. Nevertheless, some of them were associated with the tendency to deposit in the soil as they can prolong the persistence in soil (Singh *et al.,* 2020). Furthermore, some of these materials were made up from heavy metals such as aluminium, arsenic, cadmium, and lead which resulted in phosphorus immobilization and soil toxicity (Alengebawy *et al.,* 2021). The high level concentration of heavy materials impacts both soil and plants which treaten ecosystem function and structure.

Both Department of Agriculture and Department of Environment Malaysia have highlighted environmental issues arising from pesticides application. Hence, there is an urgent need to create more effective formulations that are able to increase the solubility of active ingredients and control its release rate while having minimal impact on non-target organism and environment. In particular, fungal diseases such as basal stem rot (BSR) caused by *G. boninense* fungus have affected oil palm industry in Malaysia. Furthermore, it was reported that the use of the fungicides recommended had increased soil acidity as their residue was found to be accumulated in soil at the double recommended dosage (Mustafa *et al.,* 2018).

In this case, the grand challenge is to develop an eco-friendly carrier that is capable of maintaining a high degree of selectivity, efficiency, and activity to minimize off target effects. An ideal agrichemical carrier would witness a targeting mode of operation (selectivity) that reduces reliance on its over usage by lowering the quantity of the active in-gredient while maintaining its overall beneficial properties, efficiency, and by providing positive effects to crop growth, protection, and production activity.

Metal- organic frameworks (MOFs) were seen as the cutting edge of porus materials in the agricultural field due to their diverse features that include multiple frameworks, tunable porosity, active sites,high surface areas and loading capacity. Currently, MOFs-based platforms associated with agrochemicals are primarily used for agrochemical removal in water, pesticide sensing detection, and controlled agrochemical release (Wang *et al.,* 2021).

1.3 Hypothesis

Calcium-based metal organic frameworks (Ca-MOFs) incorporated with fungicide active ingredient, hexaconazole will provide high loading efficiency and act as slow release carrier agents due to its degradation in the soil environment. Ca-MOFs will be synthesized from low-molecular plant acid linkers and their physicochemical properties will be fully characterized. The synthesized Ca-MOFs will be loaded with hexaconazole fungicide active ingredients using several encapsulation methods to obtain the highest loading amount. The slow-release of fungicide active ingredients will be confirmed through the *in-vitro* release of the fungicide-MOFs. The effective concentration and efficacy of fungicide-MOFs will be validated in bio-assay and nursery trial.

1.4 Research Objectives

The specific objectives of this study are as follows:

1. To synthesize calcium-plant acid metal-organic frameworks and characterize their physicochemical properties.
2. To optimize loading efficiency fungicide active ingredient via optimization of encapsulation strategy (post-synthetic, *in-situ*, and microwave encapsulation).
3. To determine the *in vitro* release mechanism of fungicide-MOFs via kinetic studies.
4. To evaluate the effective concentration of fungicide-MOFs through bioassay study.
5. To assess the efficacy of fungicide-MOFs in nursery trial.

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