SYNTHESIS OF FUNGICIDE-ZINC/ALUMINIUM-LAYERED DOUBLE HYDROXIDE NANODELIVERY SYSTEMS FOR CONTROLLING GANODERMA DISEASE IN OIL PALM

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By

ISSHADIBA FAIKAH BINTI MUSTAFA

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

November 2018

Dedicated to,

My beloved parents, Mustafa Yusoff and Nafisah Abd. Rahman, my siblings especially Kak Shu, Abe Iswadi, Yayah, Kak, Bang Jad and Bang Wang, who always make Adik feel alive throughout the intermediate life. Thank you for the endless support and love. Adik love all of you so much ♥

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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Chair : Professor Mohd Zobir Hussein
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Fungicide, namely hexaconazole was successfully intercalated into the intergalleries of zinc/aluminium-layered double hydroxide (ZALDH) using ion exchange method. Due to the intercalation of hexaconazole, the basal spacing of the ZALDH was expanded from 8.7 Å in ZALDH to 29.5 Å in hexaconazole-intercalated ZALDH (HZALDH). Fourier transform infrared (FTIR) study shows that the absorption band of the resulting nanocomposite is composed of both features of the hexaconazole and ZALDH which further confirmed the intercalation episode which subsequently enhanced the thermal stability of the hexaconazole. The fungicide loading was estimated to be 51.8 %. The nanodelivery system also shows better inhibition towards the Ganoderma boninense growth than the counterpart, free hexaconazole. The value of EC_{50} for hexaconazole, ZALDH and HZALDH was found to be 0.05, 2.03 and 0.03 ppm, respectively. These findings indicate that the resulting nanodelivery system of hexaconazole developed in this work is more effective in combating *G. boninense* compared to its counterpart, the free hexaconazole as indicated by the lower EC_{50} value, 0.03 compared to 0.05 ppm, respectively. Another type of fungicide, dazomet also was intercalated into the ZALDH via the ion-exchanged method and labeled as dazomet-intercalated ZALDH (DZALDH). The dazomet loading was found to be 32 % and thus increased the basal spacing to 29.65 Å. All the other characterisation studies supported that dazomet was intercalated into the ZALDH nanolayers. Further studies on oil palm seedlings had revealed that both the HZALDH and DZALDH treatments show the positive effects on all the parameters tested. Most of the treatments have almost similar seedling height of 50 % of the control. The leaf width data for the DZALDH was 52.8 compared to 51.6 cm^2 for the control. The dry weight achieved by the ZALDH, HZALDH and DZALDH were 2.05, 1.73 and 1.99 g, respectively which were significantly higher compared to the hexaconazole-based commercially available fungicide (HC) and the dazomet-based commercially available fungicide (DC), which are 0.57 and 0.69 g, respectively.

The HC treatment achieved the lowest value in all the parameters showing that the growth of the seedlings under this treatment were significantly inhibited and suppressed. The results obtained indicated that both hexaconazole-intercalated ZALDH (HZALDH) and dazomet-intercalated ZALDH (DZALDH) also promoting significant growth of oil palm seeds compared to their commercially available counterparts. Therefore, the development of HZALDH and DZALDH as fungicide nanodelivery systems has a great potential to be used as a new generation of safer and environmentally friendly agronanochemicals.

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

SINTESIS SISTEM PENYAMPAI NANO RACUN KULAT ZINK/ALUMINIUM HIDROKSIDA BERLAPIS BERGANDA UNTUK MENGAWAL PENYAKIT GANODERMA BAGI KELAPA SAWIT

Oleh

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Racun kulat iaitu heksakonazol telah berjaya disisipkan ke dalam ruang zink/aluminium- hidroksida berlapis berganda (ZALDH) dengan menggunakan kaedah pertukaran ion. Oleh kerana interkalasi heksakonazol berlaku, jarak basal ZALDH mengembang daripada 8.7 Å bagi ZALDH kepada 29.5 Å bagi heksakonazol-terinterkalasi-ZALDH (HZALDH). Kajian transformasi fourier inframerah (FTIR) menunjukkan bahawa jalur penyerapan nanokomposit yang dihasilkan terdiri daripada kedua-dua ciri heksakonazol dan ZALDH yang selanjutnya mengesahkan terjadi interkalasi yang kemudiannya meningkatkan kestabilan terma bagi heksakonazol. Kandungan racun kulat dalam nanokomposit dianggarkan sebanyak 51.8%. Sistem penyampaian nano juga menunjukkan perencatan yang lebih baik terhadap pertumbuhan *Ganoderma boninense* bagi HZALDH berbanding heksakonazol bebas. Nilai EC_{50} untuk heksakonazol, ZALDH dan HZALDH didapati masing-masing ialah 0.05, 2.03 dan 0.03 ppm. Penemuan kajian ini menunjukkan bahawa sistem heksakonazol yang dihasilkan dalam kerja ini lebih efektif bagi memerangi *G. boninense* berbanding heksakonazol bebas seperti yang ditunjukkan oleh nilai EC_{50} yang lebih rendah, masing-masing 0.03 berbanding dengan 0.05 ppm. Satu lagi racun kulat, iaitu dazomet juga diinterkalasikan ke dalam lapisan ZALDH melalui kaedah penukaran ion dan dilabelkan sebagai dazomet-interkalasi ZALDH (DZALDH). Kandungan dazomet didapati sebanyak 32% dan dengan itu meningkatkan jarak lapisan kepada 29.65 Å. Semua kajian pencirian yang lain menunjukkan bahawa dazomet diinterkalasikan ke dalam nanolapisan ZALDH. Kajian lanjut mengenai anak benih kelapa sawit telah menunjukkan bahawa kedua-dua rawatan HZALDH dan DZALDH menunjukkan kesan positif terhadap semua parameter yang diuji. Kebanyakan rawatan mempunyai ketinggian benih hampir sama dengan 50% daripada kawalan. Data lebar daun untuk DZALDH adalah 52.8 berbanding dengan 51.6 cm^2 untuk kawalan. Berat kering yang dicapai oleh ZALDH, HZALDH dan DZALDH masing-masing adalah 2.05, 1.73 dan 1.99 g yang mana ia adalah lebih tinggi berbanding dengan racun kulat (HC) yang boleh didapati secara komersial berasaskan heksakonazol dan racun kulat komersial (DC) yang berasaskan dazomet dengan nilai yang masing-masing ialah 0.57 dan 0.69 g. Rawatan dengan HC mencapai nilai terendah dalam semua parameter, yang menunjukkan bahawa pertumbuhan anak benih di bawah rawatan ini sangat terbantut. Keputusan yang diperoleh menunjukkan bahawa kedua-dua heksakonazol-terinterkalasi ZALDH (HZALDH) dan dazomet-interkalasi ZALDH (DZALDH) juga membantu pertumbuhan ketara biji kelapa sawit berbanding rakan dagangan mereka yang tersedia secara komersil. Oleh itu, pembangunan HZALDH dan DZALDH sebagai sistem racun kulat penyampaian nano mempunyai potensi yang besar untuk digunakan sebagai kimia pertanian generasi baharu yang lebih selamat dan mesra alam.

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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

ANOVA	Analysis of variance
ATR	Attenuated total reflection
BSR	Basal stem rot
BGH	Bovine growth hormone
CMNC	Ceramic matrix nanocomposites
CRF	Controlled release fertilizer
DZALDH	Dazomet intercalated zinc zluminium layered double hydroxide
DC	Dazomet - based commercially available fungicide
2D	2 dimension
3D	3 dimension
34D	3,4-Dichlorophenoxy acetic acid
DBS	Dodecyl benzene sulfonate
EC_{50}	Effective concentration
FESEM	Field emission scanning electron miscroscope
FTIR	Fourier transform infrared spectroscopy
HZALDH	Hexaconazole intercalated zinc zluminium layered double hydroxide
HZALDH	Hexaconazole - zinc aluminium layered double hydroxide
HC	Hexaconazole - based commercially available fungicide
HPLC	High performance liquid chromatography
$HTCO_3$	Hydrotalcite carbonate
LDH	Layered double hydroxide
MMNC	Metal matrix nanocomposites

MITC	Methylisothiacynate
MAP	Monoammonium phosphate
PIRG	Percentage inhibition of radical growth
PMNC	Polymer matrix nanocomposites
PDA	Potato dextrose agar
PXRD	Powder X-Ray diffraction
SRF	Slow release fertilizers
SDBS	Sodium dodecylbenzene sulfonate
SPAD	Soil plant analysis development
TGA/DTG	Thermogravimetric and differential thermogravimetric
UV/VIS	Ultraviolet-Visible Spectrophotometer
ZALDH	Zinc aluminium layered double hydroxide

LIST OF EQUATIONS

CHAPTER 1

INTRODUCTION

1.1 Research background

Nanotechnology is an interdisciplinary field, which referring to a general purpose technology. The term "*nano"* was originated from a Greek word "*nanos"* which means 'dwarft'. The nanotechnology is a creation of materials in the range of 1-100 nanometers in size and exploitation of their physical, chemical and biological properties at that length scale (Taniguchi, 1971). In 1959, nanotechnology was first introduced by a Nobel Laurate in Physic, named Richard P. Feynman in his lecture at California Institute of Technology. However, this field has been very active only in recent two decades.

The high demand that faced by a world on providing basic commodities such as food, water, energy and healthcares with minimum impact to the environment and climate had opened the scientists' mind to develop the wide array of nanotechnology applications in daily lives (Chadha, 2013). The enormous use of nanotechnology in producing functional materials, devices and systems has meet the society and world demands as the properties of products has been rapidly improved. The nano scale effect has contribute to several unique properties in various sectors.

For instance, the nanosomes used in cosmetics field has successfully improved the solubility level of ingredients while nanoemulsions has preserved the active ingredients such as vitamins and anti-oxidants. Apart from that, the use of nanomaterials in construction field are widely use where lighter, stronger materials such as cars and planes are produced. In medical or agricultural area, nanodelivery or nanoformulation systems are developed to enhance bioavailability of drug, drug solubility and reduce the consumption of drug by targeting the drug at specific site.

Methods of preparation for nanomaterials can be divided into two building strategies; "top-down" and "bottom-up". The top-down approach is a traditional method that allows breaking a large bulk to smaller structures through nanolithography, milling or precision engineering process. On the other hand, the bottom-up approach involves a combination of individual atoms or molecules to produce larger and more organized systems (García et al., 2010).

Currently, nanomaterials are widely used in formulating a new nanodelivery system as a new strategy for agricultural management. The conventional method practices especially in pest management has found plethora of side effects in living systems and environment. The advances in nanotechnology enable novel agronanochemicals to be developed with green and efficient properties, particularly controlled release formulations (CRF). The advantages of CRF are 1) provide continuous amount of pesticide at sufficient level to perform optimum activities, 2) reduce application rates by increasing the activity period through a single application, 3) cost reduction due to less applications, 4) reducing the environment pollution such as leaching and 5) reduce the phytotoxic and mammalian effects by lowering the pesticide mobility in soil (Dubey et al., 2011). CRF systems can be designed for dual-functions, for example as fungicide and fertilizer.

Nanocomposite is a multiphase solid material where one of the phases can have two or three dimensions at nanoscale. The component materials can be zero-dimensional (gold nanoparticle), one-dimensional (nanotubes and nanowires), two-dimensional (clay and metal phosphate) and three-dimensional materials (zeolites and fullerenes). Nanocomposite can be classified into three different classes, ceramic matrix nanocomposites (CMNC), metal matrix nanocomposites (MMNC) and polymer matrix nanocomposites (PMNC) (Henrique et al., 2009). Nanocomposites lead to a new and high performance material as the properties, mostly the stability and strength aspects has been improved. The application of nanoparticles and nanocomposites as smart delivery systems in controlling plant disease, nutrient storage, gene carrier for plant breeding and nanoclays as a filter for water purification has showed some promising results, thus giving an opportunity to be tested in real plantation before they are commercialized to the market.

1.2 Problem Statement

Elaeis guineensis Jacq. or known as oil palm has been nominated as “the golden crop of Malaysia” since it produces profitable export earnings for the country and are truly nature’s gifts in alleviating poverty in Malaysia (Basiron, 2007). In Malaysia, oil palm is blessed by being largely disease free, but basal stem rot (BSR) disease that caused by an ancient fungus, *Ganoderma boninense* has been a disastrous malady for oil palm plantation, which has devastated thousand hectares of plantings with an economic loss of RM 1.8 billion in oil palm industry for the past decades (Lee and Chong, 2015). BSR disease was first reported in 1931, which infected oil palm trees that older than 25 years (Latiffah and Ho, 2005). However, the disease was so widespread and become a topical issue in this century as it affects almost all plantations within first year of planting oil palm.

Hexaconazole is a fungicide, belongs to triazole group that extensively used in controlling fungi, particularly *Asomycetes, Basidiomycetes* and Ganoderma family. Its fungicidal properties have prevented the *Ganoderma boninense* mycelium growth, thus prolonging the productive life of infected palms. However, the extensive use of hexaconazole in plantation has increased the soil acidity (Maznah et al., 2015). Figure 1.1 showed the chemical structure of hexaconazole.

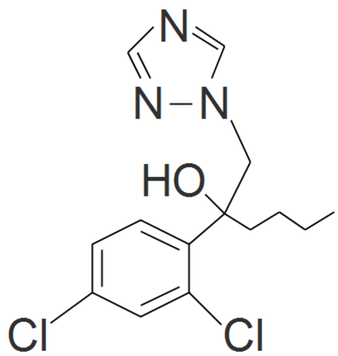

Figure 1.1: Chemical structure of hexaconazole

Dazomet is a type of fumigant used in controlling various pests including soil insects, pathogens and weed seeds. This fumigant now is actively used in controlling the basal stem rot as it could effectively eradicate *Ganoderma* inoculum within the infected stumps (Idris and Maizatul, 2012). Nevertheless, this fumigant releases methyl isothiocyanate (MITC) when in contact with water thus contribute adverse effects towards the living systems especially human and also to the environmental by destroying ozone layer. Figure 1.2 showed the chemical structure of dazomet.

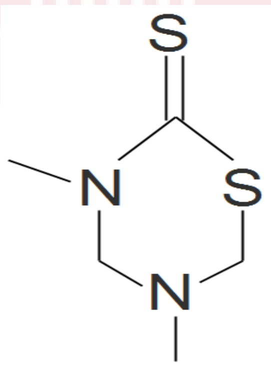

Figure 1.2: Chemical structure of dazomet

1.3 Objectives

The main objective of this work is to synthesis fungicide-zinc/aluminium layered double hydroxide nanodelivery systems for controlling ganoderma disease in oil palm.

And the specific objectives of this study are as follows:

1. To synthesis and optimize the hexaconazole- and dazomet-intercalated zinc/aluminium-layered double hydroxides nanocomposites, characterize their physico-chemical properties and study their controlled release properties.
2. To study their fungicidal activity towards the pathogenic, *Ganoderma boninense* of the fungicide-LDH nanocomposites via *in vitro.*
3. To study their phytotoxicity effect of the fungicide-LDH nanocomposites towards the oil palm seeds growth via *in vivo.*

1.4 Significance of study

The present studies were performed in order to develop new agronanochemicals based on layered double hydroxide. Two nanocomposites with high fungicide loading were prepared and their sustained release properties were examined. These nanocomposites could enhance the fungicidal activity of the actives towards the pathogen by inhibiting the pathogen at lower EC_{50} values and at the same time both nanocomposites were capable to supply a good nutrient due to their inorganic components which promoting oil palm growth. The literature indicates that no such studies were done for the palm oil upstream activities.

1.5 Scope of study

The scope of this study is to prepare and characterize fungicide-based zinc/aluminium layered double hydroxide. In this study, zinc/aluminium layered double hydroxide (ZALDH) was used as the zinc and aluminium composition could contribute to oil palm growth by supplying these elements to the shoots and roots. Two types of fungicides, hexaconazole and dazomet were used in the intercalation process using ion exchange method. Subsequently the two as-synthesized nanocomposites from this study are labeled as hexaconazole-

ZALDH (HZALDH) and dazomet-ZALDH (DZALDH) nanocomposites. Their commercial fungicides counterparts, Anvil and Basamid respectively, were also used for comparison.

In Chapter 4, the study covers HZALDH nanocomposite and its physicochemical properties. A release profile of hexaconazole from their synthesized nanocomposite was studied in phosphate buffered solution (PBS) at pH 5.5 to mimic the loam soil pH condition. The effectiveness of the nanocomposite was also proved by the antifungal study via in vitro assays with fungal culture approach, *Ganoderma boninense.* In Chapter 5, the scope is about DZALDH nanocomposite, its physicochemical properties and the comparison of HZALDH and DZALDH nanocomposites on their phytotoxic effects towards the oil palm seeds.

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