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ZnO-NPs/rGO/SPCE-MODIFIED ELECTRODE FOR EARLY DETECTION OF *Ganoderma boninense* IN OIL PALM TREES

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By

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Ganoderma boninense is a major disease that infects oil palm trees. This problem has caught researchers' attention all around the world, especially those from Asian countries as *Ganoderma boninense* is difficult to trace in its early stage. Undoubtedly, many researchers have lent a hand by suggesting ways to diagnose the disease through the introduction of many methods to detect symptoms related to the *Ganoderma* disease, including tomography, selective media, Indirect Enzyme-linked Immunosorbent Assay (ELISA) and polymerase chain reaction (PCR). However, these diagnostic techniques are less accurate, time-consuming and lead to cost increments. Therefore, another method to detect *Ganoderma boninense* at a faster, lower cost and higher accuracy was introduced which is through the use of electrochemical sensors. Initially, electrode modification was done by reducing graphene oxide (GO) to reduced graphene oxide (rGO). Subsequently, the addition of zinc oxide nanoparticle (ZnO-NPs) was electrodeposited on top of it using the electrodeposition method. Both modifiers were chosen as they have been acknowledged to have large surface areas to interact with analytes, and have great electroconductivity. In this study, this disposable modified SPCE was used as a sensing material in order to detect secondary metabolites acting as possible biomarkers for the *Ganoderma boninense* disease in healthy and infected leaves at the 14 and 30 day periods. It is because secondary metabolites are produced once oil palm trees are infected by *Ganoderma boninense*. The combination of physical and electrochemical characterisation of graphene oxide (GO) before and after reduction together with the deposition of zinc oxide nanoparticles (ZnO-NPs) were observed using field emission scanning electron microscopy (FESEM), energy dispersive x-ray (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy and cyclic voltammetry (CV) to reveal the successful modification of ZnO-NPs/rGO/SPCE.

As a consequence, the electrochemical analysis presented a current increment in each modification higher than the bare SPCE due to electrocatalytic activity. Afterwards, the electrochemical signals from secondary metabolites were indicated from differential pulse voltammetry techniques (DPV) under optimum conditions. ZnO-NPs/rGO/SPCE established a good correlation coefficient (R^2) of both healthy and infected oil palm after 14 days and 30 days (R^2 = 0.9691, R^2 = 0.9652, R^2 = 0.9905 and R^2 = 0.9710) with detection limits of 1.52 ppm, 1.58 ppm, 3.20 ppm and 3.02 ppm respectively. Consequently, the outstanding sensing performance of ZnO-NPs/rGO/SPCE in the detection of secondary metabolites can be applied to control the *Ganoderma boninense* disease at the earlier stage before spreading out.

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

ELEKTROD MODIFIKASI ZnO-NPs/rGO/SPCE UNTUK PENGESANAN AWAL *Ganoderma boninense* BAGI POKOK KELAPA SAWIT

Oleh

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Ganoderma boninense adalah penyakit utama yang menyerang pokok kelapa sawit. Hal ini menjadi tarikan kepada ramai penyelidik di seluruh dunia terutama penyelidik dari negara Asia kerana penyakit *Ganoderma boninense* sukar untuk dikesan pada peringkat awal jangkitan. Tidak dinafikan, ramai penyelidik cuba membantu untuk mengawal penyakit *Ganoderma* ini termasuklah memperkenalkan imbasan tomografi, kultur sel, penetapan kadar imunosorben taut-enzim (ELISA) dan reaksi berantai polimerase (PCR). Akan tetapi, teknik diagnostik tersebut kurang tepat, mengambil masa yang lama untuk bertindak balas dan kos yang agak tinggi. Maka, sebagai satu cara yang lain untuk mengesan penyakit *Ganoderma boninense* dengan tindak balas yang lebih cepat, lebih tepat dan kos yang lebih rendah adalah dengan memperkenalkan pengesan elektrokimia. Sebagai pemulaan, modifikasi elektrod direncanakan dengan proses penurunan dari grafin oksida (GO) kepada grafin oksida teturun (rGO). Seterusnya, nanozarah zink oksida (ZnO-NPs) terenap dengan kaedah elektroenapan di atasnya. Kedua-dua pengubah digunakan untuk modifikasi elektrod kerana terkenal dengan keluasan permukaan yang besar untuk bertindak balas dengan analit dan aktiviti elektrokatalitik yang tinggi. Elektrod modifikasi serbaguna SPCE ini dihasilkan bagi mengesan metabolit sekunder sebagai penanda bio dalam penyakit *Ganoderma boninense* diuji keatas daun yang sihat dan yang telah dijangkiti setelah 14 dan 30 hari. Ini kerana, metabolit sekunder akan terhasil secara spontan apabila diserang oleh *Ganoderma boninense*. Gabungan pencirian fizikal dan elektrokimia untuk GO sebelum dan selepas proses penurunan dan terenapan nanozarah zink oksida telah diuji menggunakan mikroskop elektron pengimbas pancaran medan (FE-SEM), spektroskopi tenaga serakan (EDX), fourier spektroskopi inframerah (FTIR), spektroskopi Raman, voltammetri berkitar bagi memastikan modifikasi elektrod ZnO-NPs/rGO/SPCE berjaya. Hasilnya, elektrokimia analisis telah menunjukkan peningkatan arus bagi

setiap modifikasi di mana ianya lebih tinggi dari SPCE disebabkan ciri aktiviti elektrokatalitik. Selanjutnya, isyarat elektrokimia dari metabolit sekunder diterjemahkan melalui tindakbalas voltammetri denyut dalam keadaan optimum. ZnO-NPs/rGO/SPCE memberi keputusan yang memberangsangkan untuk pekali kolerasi bagi daun sihat dan yang telah dijangkiti selepas 14 dan 30 hari iaitu (R^2 = 0.9691, R^2 = 0.9652, R^2 = 0.9905 and R^2 = 0.9710), manakala masing-masing menunjukkan had pengesanan 1.52 ppm, 1.58 ppm, 3.20 ppm and 3.02 ppm. Sebagai hasilnya, ZnO-NPs/rGO/SPCE memberi impak yang bagus dalam prestasi pengesanan berdasarkan metabolit sekunder dalam mengawal *Ganoderma boninense* pada peringkat awal daripada terus merebak.

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LIST OF ABBREVIATIONS

8-OHdG	8-Hydroxy-2′-deoxyguanosine
A	Effective surface area
ANN	Artificial Neural Network
BSR	Basal stem rot
C/O	Carbon-to-oxygen ratio
CE	Counter electrode
CV	Cyclic voltammetry
CVD	Chemical vapour deposition
DIBA	Dot immunobinding assay
DLC	Double layer charging
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
EDTA	Ethylenediamine-tetraacetic acid
EDX	Energy Dispersive X-Ray
ELISA	Indirect enzyme-linked immunosorbent assay
e-nose	Electronic nose
FE-SEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
GCE	Glassy carbon electrode
GC-MS	Gas chromatography
GDP	Gross Domestic Product
GO	Graphene oxide
GRAS	Generally Recognised as Safe
GSH	Glutathione
HR	Hypersensitive response
I_D/I_G	Intensity ratio of D and G band
IR	Infrared radiation
ITS	Internal transcribed spacer
LC-MS	Liquid chromatography-mass spectroscopy
LOD	Limit of detection
MEA	Malt extract agar
MWCNT	Multiwall carbon nanotube
NMR	Magnetic resonance spectroscopy
OTA	Ochratoxin A
PCR	Polymerese chain reaction
PDA	Potato dextrose agar
rDNA	Ribosomal RNA gene
RE	Reference electrode
rGO	Reduced graphene oxide
ROS	Reactive oxygen species
RWB	Rubber wood block
s	Seconds
sp.	Species
SPCE	Screen printed carbon electrode
SPGE	Silica paste glass electrode
USR	Upper stem rot
VOC	Volatile organic compounds
WE	Working electrode
ZnO-NPs	Zinc oxide nanoparticles

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia has been recognised as one of the richest countries in terms of oil palm production. Recently, Malaysia invested about 4.49 million hectares of land for oil palm cultivation which produce a rolling output of approximately 25.64 million tonnes of palm products. Consequently, Malaysia's oil palm industry has become one of the main contributors to Malaysia's Gross Domestic Product (GDP) and foreign exchange earnings, and is the source of many job opportunities. Meanwhile, the export of palm oil has tremendously increased from 1.17 million tonnes in 1975 to 16.05 million tonnes in 2016. Malaysia's exports target market is more than 200 markets worldwide including Africa, West Asia, Europe, the Indian sub-continent and Asia (Nambiappan et al., 2018).

However, a major issue that needs to be accounted when dealing with oil palm trees is the fatality of trees as it can cause a big loss to the palm oil industry. *Ganoderma boninense* is one of the diseases that can infect oil palm trees (Susanto et al., 2005). Mature trees can survive slightly longer, but young trees can live approximately one to two years only. The *Ganoderma boninense* infection commonly occurs at the bottom and upper parts of the stem, and are known as basal stem rot (BSR) and upper stem rot (USR) respectively (Durand-Gasselin et al., 2005).

Over a decade ago, electrochemical sensors have been introduced to detect many elements, including heavy metals (Zhu et al., 2009), gases, biological materials as it can give extensive response to the interested analyte. Basically, the analyte will be diluted with a supporting electrolyte to improve conductivity. Thus, the analyte will interact with the working surface of the electrode to give a current response based on the redox reaction activity.

The nanotechnology field has grown up tremendously all around the world. Nanotechnology applications including nanowire, nanoparticles, carbon nanotubes, and quantum dots are widely utilised in food, electronic devices, cosmetics, and environmental industries. In this context, the employment of nanoparticles in electrodes can enhance the current signal as it provides a high surface area to the electrode.

Graphene oxide (GO) is a nanomaterial that has been used in various applications. Decades ago, GO has been explored due to its variety of applications and advantages. GO has been said to have important qualities such as low cost, non-toxic and having a huge potential to be used as a modifier due to its large surface area (Liu et al., 2013). Along with that, zinc oxide nanoparticles have been applied as one of the famous nanomaterials used as a sensing material. With its great properties such as a versatile semiconductor material (Janotti and Van de Walle, 2009) and a good electrocatalyst (Wayu et al., 2013), a sensor of good conductivity can be made.

1.2 Problem Statement and Research Motivation

The detection of *Ganoderma boninense* at the early stage is significant to researchers as the trees may not show any reactions as soon as they are being attacked. The disease is symptomless at early stage and usually manifested when it has already reached a critical stage, thus imposing challenges on effective disease management. Several foliage symptoms can be appeared at the bottom of the stem where it undergoes decaying process due to the development of basidiocarps. The process caused limitation on water and supplements uptake from the root, making the loss of leaf pigments. At the advanced stage, more fronds shrivel and hang down to a skirt structure. In worst cases, those stems may be crack (Parisa et al, 2017).

Until now, there are many techniques and methods applied to diagnose the disease. In the early studies on BSR, tomography (Idris et al., 2010; Abdullah et al., 2013), selective media (Ariffin et al., 1995), immunoassay and PCR methods have been used to detect the *Ganoderma* disease. Unfortunately, these techniques are time consuming, costly and some of the techniques need expertise to interpret the data, thus limiting the scope of research. Meanwhile, the sensor application is a more sensitive, simple, low cost, and less time-consuming method to diagnose the *Ganoderma boninense* disease.

Therefore, an electrochemical sensor is introduced as one of the alternative ways to detect *Ganoderma boninense*. This method is specifically recommended because it is less time consuming and is low cost. Using this way, the secondary metabolites of an oil palm tree as possible biomarkers of *Ganoderma boninense* can be extracted from the tree, thus giving a more specific response to electrochemical analysis. In addition, modification of bare electrode can be made to enhance a higher surface area of electrode.

Thus, the utilisation of reduced graphene oxide and zinc oxide nanoparticles as electrode modifiers is introduced. Graphene oxide demonstrated a good capability towards electrochemical sensor application based on metal-rGO nanomaterials owing to the unique electrochemical and structural properties of rGO as well as the catalytic characteristics of transition metals. As one of the element in transition metals, zinc oxide nanoparticles can improve electrochemical performance and elevate electron transfer between the working electrode and secondary metabolites. However, this modified electrode has not been established yet as the capability of zinc oxide nanoparticles to capture secondary metabolites is still doubted. Hence, in this study zinc oxide nanoparticle was tested based on its electrochemical performance towards the behaviour of secondary metabolites in oil palm trees.

1.3 Objectives of Study

1.3.1 General Objective

The main objective of this research is to develop a sensor specifically to detect an oil palm's disease contributed from *Ganoderma boninense* using ZnO-NPs/rGO/SPCE modified electrode.

1.3.2 Specific Objective

I. To prepare and characterise reduced graphene oxide (rGO) and zinc oxide nanoparticles (ZnO-NPs) using field emission scanning electron microscopy (FESEM), energy dispersive x-ray (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy and cyclic voltammetry (CV).
II. To modify an electrode using drop cast of graphene oxide (GO), reduced graphene oxide (rGO) by CV and zinc oxide nanoparticles (ZnO-NPs) through chronoamppherometry technique.
III. To develop a ZnO-NPs/rGO/SPCE electrochemical sensor for the detection of secondary metabolites as possible biomarkers of *Ganoderma boninense*.

1.4 Scope and Limitation

In this research, crude leaf extract is extracted from oil palm trees at durations of 14 days and 30 days after infection, and 14 days and 30 days after plantation. On account of that, the crude extracts were only able to maintain their performance for about three months in cold conditions. It is because beyond these conditions, the crude extracts' performance may be affected by moist and vapour from the storage condition. In addition, oil palm trees secrete thousand groups of secondary metabolites to defend themselves from diseases. Thus, it is quite difficult to specifically detect the secondary metabolites secreted from oil palm in defence towards *Ganoderma boninense*.

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BIODATA OF STUDENT

Nurhamizah binti Rahmat was born on 2nd May 1992 at Hospital Kajang, Selangor. She received her primary education in Sekolah Kebangsaan Ulu Semenyih, Semenyih, Selangor and secondary education in SMKA Maahad Hamidiah Kajang, Kajang, Selangor. After that, she went to Universiti Sains Islam Malaysia (USIM) to further her studies. In 2015, she graduated as a bachelor student holding the certificate of Bachelor of Science with Honours, majoring in Industrial Chemistry from the Faculty of Science and Technology. In 2016, she continued her studies in Master of Science at the Institute of Advanced Technology (ITMA), Universiti Putra Malaysia under the supervision of Prof. Dr. Nor Azah Yusof and Assoc. Dr. Wong Mui Yun. During her studies, she became a laboratory demonstrator to assist and monitor undergraduate students taking Physical and Inorganic Chemistry, and Organic 1 subjects.

LIST OF PUBLICATIONS AND CONFERENCE ATTENDED

Publications

Rahmat, N., & Yusof, N. A. (2017). Modification of SPCE by Reduction of Graphene Oxide and Electrodeposition of Zinc Oxide Nanoparticles for Electrochemical Sensor. *Malaysian Journal of Catalysis*, 3(1): 32-35.

Detection of Secondary Metabolites in Oil Palm Trees using ZnO-NPs/rGO/SPCE Modified-Screen Printed Electrode (NMAS 2017 - Submitted).

Conferences

1. NanoMITe Annual Symposium (NMAS 2016) on 28th September 2016 in University Technology Malaysia (UTM KL) as oral presenter.

2. Symposium on Advanced Materials and Nanotechnology (SAMN 2017) on 18th – 19th of July 2017 at Hotel Bangi- Putrajaya, Malaysia as oral presenter.

3. NanoMITe Annual Symposium (NMAS 2017) on 14th – 15th November 2017 in Universiti Putra Malaysia (UPM) Serdang, Malaysia as oral presenter.

Project meeting and others

1. Colloquium On Nanotechnology in Detection and Control of *Ganoderma boninense* (CONGRAB 7) on 28th September 2017 in Cyberview Lodge, Cyberjaya.

2. Nanotechnology in Detection and Control of *Ganoderma boninense* (CONGRAB 8) on 14th – 15th December 2017 at Institute of Nano Electronic Engineering, Universiti Malaysia Perlis and Langkawi Research Centre, Langkawi, Kedah Darul Aman.

3. Summer School Nanosciences: Fundamental and Applications Emphasizing in Nanomedicine on 24th July to 5th August 2017 at Institut Teknologi Bandung (ITB) Bandung, Indonesia.

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