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DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR FOR DETECTION OF SECONDARY METABOLITE QUINOLINE IN Ganoderma boninense INFECTED OIL PALMS

FOWOTADE SULAYMAN AKANBI

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By

FOWOTADE SULAYMAN AKANBI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR FOR DETECTION OF SECONDARY METABOLITE QUINOLINE IN *Ganoderma boninense* INFECTED OIL PALMS

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May 2018

Chairma : Professor Nor Azah Binti Yusof. PhD
Faculty : Science

The devastating effect of Ganoderma boninense (G. b) infections in oil palms, which leads to low-income revenues, due to the low yield of diseased palms, has driven researchers to look for early diagnostic techniques. The secondary metabolite, quinoline which was reported to be excreted from the oil palms when attacked by G. b can be used to detect the pathogenic fungus. In order to facilitate an indirect early detection of G. b, a new electrode based on functionalized multi-walled carbon nanotube was developed in this study. The development of the new electrode is based on layer-by-layer self-assembly method using activated multi-walled carbon nanotubes (aMWCNTs) as a backbone, for the attachment of other nanomaterials such as gold nanoparticles (AuNPs) and low molecular chitosan nanoparticle (ChTSNPs). The synthesized gold nanoparticles dispersion was characterized using Zetasizer nano series, UV-visible spectroscopy and cyclic voltammetric (CV) technique. The aMWCNTs and prepared nanohybrid materials (AuNPs-aMWCNTs), were characterized with the aid of field emission scanning electron microscope (FESEM), energy dispersive X-Ray (EDX), while ChTSNPs-aMWCNTs and aMWCNTs were characterized utilizing Fourier-transform infrared (FTIR) spectroscopy. The electrode modification process was monitored by FESEM and voltammetric techniques. Secondary metabolites were extracted from healthy and infected oil palm extracts, using ultrasound-assisted extraction (UAE) method. The performance of the developed electrode was optimized and characterized in quinoline using CV and linear sweep voltammetric (LSV) methods. The developed electrode was characterized in the leaves and root extract secondary metabolites using LSV technique under optimized conditions. The results showed that AuNPs of size 49.27nm and polydispersity index (PDI) of approximately 46% was chosen for electrode modification. This is because the PDI is below 50%. The FESEM micrographs show distinction among the pristine MWCNTs, aMWCNTs AuNPs-

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aMWCNTs, bare electrode and the modified electrode. Also, the attachment of the carboxylic group (-COOH) to the walls of MWCNTs and the loading of the ChTSNPs onto the aMWCNTs were confirmed by the FT-IR spectral. The optimized conditions are as follow: 0.20 M citrate buffer, pH 5.5, accumulation potential, -0.52 V, accumulation time, 180 s and scan rate, 0.06 V/s. Under the measured optimal conditions, the anodic peak current (Ipa) is directly proportional to concentration of quinoline, giving rise to the linear regression equation, Ipa (μA) = 0.7684 + 43.197 [Quinoline]/ (μM), coefficient of correlation, R^2 = 0.9949, with linear range 0.0004 and 0.10 μM, limit of detection (LOD) 3.75 nM and limit of quantification (LOQ) 12.5 nM. The relative standard deviation (RSD) of Ipa of quinoline with single repeatedly used developed electrode was 2.33%, while it retained 91.7% of the current after being kept for twenty days. The evaluated reproducibility RSD for the between developed electrode anodic peak current response to quinoline oxidation was 3.52%. In addition, no apparent interference was observed in the presence of 1000-fold excess inorganic ions and 500-fold excess organics in 10.0 μM quinoline as all the percentage interferences are below ±10%. Furthermore, the newly developed electrode revealed satisfactory Ipa extract secondary metabolite response over the concentration range of 0.1 to 0.5 ppm with the limit of detection (3 S/N) ranging from 7.87 ppb to 18.54 ppb. The RSD value for reproducibility of Ipa across all the secondary metabolites ranges from 0.73% to 29.35%. The 500-fold excess of interfering organic species in 100 ppm extract secondary metabolite averagely exhibited insignificant interference in the detection process. The proposed sensor stands a brighter future in providing a point of care service in the management of BSR disease of oil palms in South East Asia, especially in Malaysia.

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

PEMBANGUNAN SENSOR ELEKTROKIMIA UNTUK PENGESANAN METABOLIT SEKUNDER DALAM *Ganoderma boninense* YANG MENJANGKITI KELAPA SAWIT

Oleh

FOWOTADE SULAYMAN AKANBI

Mei 2018

Pengerusi : Profesor Nor Azah Binti Yusof, PhD
Fakulti : Sains

Kesan buruk jangkitan Ganoderma boninense (G.b) pada kelapa sawit menyebabkan pulangan yang rendah disebabkan oleh hasil yang berkurangan daripada sawit yang dijangkiti, ini menyebabkan para pengkaji mencari kaedah diagnostik awalan. Metabolit sekunder, kuinolina yang telah dilaporkan dirembes dari pokok sawit apabila diserang oleh G.b boleh digunakan untuk mengesan kulat patogenik ini. Untuk menjalankan pengesanan awal G.b, sebuah elektrod baharu yang difungsi dengan karbon nanotiub multidinding telah dihasilkan dalam kajian ini. Penghasilan elektrod baharu ini berasaskan kaedah swa pasang lapisan demi lapisan menggunakan karbon nanotiub multidinding teraktif (aMWCNTs) sebagai tulang belakang, untuk pelekatan bahan nano lain seperti nanopartikel emas (AuNPs) dan nanopartikel kitosan rendah molekular (ChTSNPs). Ampaian nanopartikel emas yang disintesis telah dicirikan menggunakan siri Zetasizer nano, spektroskopi UV - tampak dan teknik voltammetrik kitaran (CV). aMWCNTs dan bahan nanohibrid yang disediakan (AuNPs-aMWCNT, dicirikan dengan bantuan mikroskop elektron pengimbasan pengaruh medan (FESEM), dan X-Ray penyebaran tenaga (EDX). Manakala ChTSNPs-aMWCNTs dan aMWCNTs dicirikan menggunakan spektroskopi inframerah Fourier-transform (FTIR). Proses pengubahsuaian elektrod dipantau menggunakan FESEM dan kaedah voltametrik. Metabolit sekunder diekstrak daripada pokok kelapa sawit yang sihat dan terjangkit, menggunakan kaedah pengekstrakan berbantu ultrabunyi. Keupayaan elektrod yang dibina dioptimumkan dan dicirikan dalam quinoline menggunakan CV dan kaedah volumetrik sapuan linear (LSV). Elektrod yang dibina dicirikan pada ekstrak metabolit sekunder dedaun dan akar menggunakan teknik LSV dibawah persekitaraan yang optimum. Hasilnya menunjukan AuNP bersaiz 49.27 nm dan indeks politaburan (PDI) hampir 46% dipilih untuk pengubahsuaian elektrod. Ini kerana PDI hendaklah di bawah 50%. Mikrograf FESEM menunjukkan perbezaaan

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ketara diantara MCWCNTs, aMWCNTs AuNPs-aMWCNTs elektrod terdedah dan elektrod diubahsuai. Pelekatan kumpulan karboksil (-COOH) pada permukaan MWCNTs dan pemuatan MWCNTs pada aMWCNTs dikenal pasti menggunakan FTIR. Keadaan optimum adalah seperti berikut : 0.20 M penimbal sitrat, pH 5.5, pengumpulan keupayaan, -0.52 V, masa pengumpulan, 180 s dan kadar imbas, (0.06 V/s. di bawah keadaan optimum, puncak arus anodic (I_{pa}) adalah berkadar langsung kepada kepekatan kuinolina, meningkatkan persamaan regresi linear, I_{pa} (μA) = 0.7684 + 43.197 [kuinolina]/ (μM), pekali korelasi, R^2 = 0.9949, dengan julat linear 0.0004 dan 0.10 μM, had pengesanan (LOD) 3.75 dan had kuantifikasi (LOQ) 12.5 nM. Sisihan piawai relative (RSD) I_{pa} kuinolina dengan satu elektrod ulang pakai adalah 2.33%, dan mengekalkan 91.7% arus selepas disimpan untuk 20 hari. Nilai penghasilan-semula (RSD) untuk diantara tindakbalas arus puncak elektrod bertindak balas kepada pengoksidaan kuinolina adalah 3.52 %. Tambahan, tiada gangguan yang jelas diperhatikan dengan kehadiran lebihan ion tidak organik 1000 ganda dan lebihan ion organik 500 ganda, pada 10.0 μM kuinolina, kerana peratusan gangguan keduanya adalah di bawah ±10%. Adapun, elektrod yang baharu dibangunkan ini menunjukkan tindakbalas I_{pa} ekstrak metabolit sekunder yang memuaskan pada julat kepekatan 0.1 hingga 0.5 ppm dengan had pengesanan (3 S/N) berjulat dari 7.87 ppb to 18.54 ppb. Nilai RSD untuk penghasilan semula I_{pa} diantara kesemua metabolit sekunder berjulat dari 0.73% hingga 29.35%. Lebihan spesies organik gangguan di dalam ekstrak metabolit sekunder 100 ppm secara purata mempamerkan gangguan yang tidak signifikan dalam proses pengesanan. Sensor yang dicadangkan memiliki masa depan yang cerah dalam menangani penyakit BSR miyak sawit di Asia Tenggara, terutama di Malaysia.

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I certify that a Thesis Examination Committee has met on 7 May 2018 to conduct the final examination of Fowotade Sulayman Akanbi on his thesis entitled "Development of an Electrochemical Sensor for Detection of Secondary Metabolite Quinoline in *Ganoderma boninense* Infected Oil Palms" 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

6MHLSMs	6 months healthy leaf secondary metabolites
6MILSMs	6 months infected leaf secondary metabolites
14DHLSMs	14 days healthy leaf secondary metabolites
14DHRSMs	14 days healthy root secondary metabolites
14DILSMs	14 days infected leaf secondary metabolites
14DIRSMs	14 days infected root secondary metabolites
30DHLSMs	30 days healthy leaf secondary metabolites
30DHRSMs	30 days healthy root secondary metabolites
30DILSMs	30 days infected leaf secondary metabolites
30DIRSMs	30 days infected root secondary metabolites
ABS	Acetate buffer solution
AdSV	Adsorptive stripping voltammetry
AMP	Amperometry
aMWCNTs	Activated multi-walled carbon nanotubes
Au	Gold
AuNPs	Gold nanoparticles
AuNPs- aMWCNTs	Gold nanoparticles- activated multi-walled carbon nanotubes
BBS	Borate buffer solution
BL	Bilayer
BL1/SPCE	Bilayer one screen printed carbon electrode
BSR	Basal stem rot
CaBS	Carbonate buffer solution
CBS	Citrate buffer solution

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ChTSN	Chitosan
ChTSNP-aMWCNTs	Chitosan nanoparticles- activated multi-walled carbon nanotubes
cMWCNT	Carboxylated multi-walled carbon nanotubes
CNTs	Carbon nanotubes
CSSTM	Chemically selective scanning tunneling microscopy
CV	Cyclic voltammogram / Cyclic voltammetry
DLS	Dynamic light scattering
DVP	Differential pulse voltammetry
ECS	Electrochemical sensors
EDXS	Energy Dispersive X-Ray spectroscopy
ELISA	Enzyme-linked immunosorbent assay
Epa	Anodic peak potential
Epc	Cathodic peak potential
FDL	Functional Devices Laboratory
FESEM	Field emission electron microscopy
FPs	Fallen palms
G.b	*Ganoderma boninense*
GCE	Glassy carbon electrode
GSM	Ganoderma selective medium
IC	Indirect competitive assay
Ipa	Anodic peak current
Ipc	Cathodic peak current
issAP:-Ver.A:	Single stranded DNA
ITMA	Institute of Advanced Technology

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ITO	Indium tin oxide electrode
Lac	Laccase
LbL	Layer-by-layer
LOD	Limit of detection
LOQ	Limit of quantification
LSV	Linear sweep voltammetry/ voltammogram
MBs	Magnetic beads
MOFs	Metal organic frameworks
mRNA	Molecular ribonucleic acid
NPs	Nanoparticles
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
PCS	Photon correlation spectroscopy
PDDA	Poly(diallyldimethylammonium chloride)
PEDOT	Poly (3,4-ethylene dioxythiophene)
PMs	Primary metabolites
pMWCNTs	Pristine Multi-walled carbon nanotubes
PSMs	Plant secondary metabolites
PSS	Poly(sodium 4-styrenesulfonate)
QELS	Quasi elastic light scattering
QY	Quinoline yellow
RSD	Relative standard deviation
SECM	Scanning electrochemical microscopy
SEM	Scanning electron microscopy
SMs	Secondary metabolites

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SOPs	Standing oil palms
SPCEs	Screen printed carbon electrodes
SPEs	Screen printed electrodes
SWV	Square wave voltammetry
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
UPM	Universiti Putra Malaysia
USR	Upper stem rot
UV-Vis	Ultraviolet-visible
β-CD	β-cyclodextrin

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

The incapability of plants to successfully undergo translational motion has indeed limit their escaping potentials from invading living and non-living components of the ecosystem. Despite this fact, they are able to checkmate a number of attacks by brewing up highly functional biological defense weaponry, termed plant secondary metabolites (PSMs) or simply secondary metabolites (SMs). Examples of some selected SMs that have been studied include phenylpropanoid (López-Gresa *et al.,* 2011), glucosinolates (Pedras & Hossain, 2011), phytosterols (Ekade & Manik, 2014), eugenol and 1,8-cineole (Pavarini *et al.*, 2012), indole (Ahuja *et al.*, 2012). SMs are also referred to as volatile organics or volatile organic compounds (Pavarini *et al.*, 2012; Sankaran *et al.*, 2010), bioactive compounds (Ekade & Manik, 2014; Pavarini *et al.,* 2012), antimicrobial compounds (Ahuja *et al.,* 2012). They are, therefore, biochemical molecules capable of restraining the adverse environmental effects on the standing plants (López-Gresa *et al.*, 2011). SMs are low-molecular-mass biochemical species endowed with the natural propensity to guard the plants from all kinds of stressor factors emanating from the ecosystem (Ahuja *et al.*, 2012; Ekade & Manik, 2014; López-Gresa *et al.*, 2011; Pavarini *et al.*, 2012). These groups of compounds are known to significantly exhibit two opposing traits, which include being beneficial as well as being destructive (Pedras & Hossain, 2011).

However, the concern of this present study in on their protective responsibilities for the plant kingdom. SMs protect and guard the plants against devastatingly destructive agents like insects, pests, microorganisms (Ekade & Manik, 2014; López-Gresa *et al.*, 2011; Pedras & Hossain, 2011) and drought, salinity, erosion, humidity (Ekade & Manik, 2014; López-Gresa *et al.*, 2011). SMs employed in plant defense strategies are of two classes, phytoanticipins (constitutive) and phytoalexins (inducible) (Pedras & Hossain, 2011). The focal point of this study is phytoalexins. Phytoalexins are a combination of inter-related substances displaying efficient biochemical workforce against different disease-causing microbes, whose idea was conceived seventy years ago. They are also low molecular mass secondary metabolites bearing antimicrobial property and stress induced. Based on their effective check and impedance to the spread of pathogenic invasion, they are tagged disease resistance molecular markers or simply disease resistance markers (Ahuja *et al.*, 2012). Quite unfortunate, lots of crops are lost to these devastating, non-compromising pathogens (viral, bacterial or fungal) either on the fields or off the fields (storage). The economic importance of these pathogens, no doubt has precipitated a universal headache, the world over (Ahuja *et al.*, 2012). Researchers, scientists and other stakeholders are on their toes looking for the right remedy. In this regard, the oil palms in Malaysia and Indonesia have suffered many losses from the pathogenic fungus, *Ganoderma boninense* (G.b) trading a disease called basal

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stem rot (BSR) disease. According to Nusaibah and co-workers (2016), quinoline is SMs produced in oil palms at the site of the attack by G.b within 24hours.

This tree plant species belong to the genus *Elaeis* and family *Palmae* (Bivi *et al.,* 2010). Its scientific nomenclature is *Elaeis guineensis Jacq*. The genesis of this plant is from West African sub-continent, the Gulf of Guinea to be precise.

The Portuguese transported oil palm to Brazil in the 15th century, while the Dutch catapulted it to Indonesia in the 18th century and in 1878, it was embraced as a decorative plant in Malaya (Naher *et al.*, 2013). It was also reported that the British introduced the oil palm to south-east Asia in the early part of the 1870s (Hushiarian *et al.*, 2013). The fruit of the oil palm is a drupe, with three-layered pericarp comprising of the outer skin (exocarp), outer pulp bearing palm oil (mesocarp) and inner hardcore circumscribing the kernel bearing the kernel oil (endocarp) (Naher *et al.*, 2013). The oil palm trees commence fruit procreation after thirty months of cultivation and are blessed with a proactive lifespan of twenty to thirty years (Hushiarian *et al.*, 2013). A quarter of a hectare of land is required to yield a tone of palm oil. They are topmost edible oil generating crop globally (Hushiarian *et al.*, 2013; Naher *et al.*, 2013; Tee *et al.*, 2013) Other products obtainable from oil palms include biofuels (Naher *et al.*, 2013) , kernel oil, kernel cake, oleochemicals, biodiesel and other up-stream products (Ho & Tan, 2015). Reference to the foreign exchange earnings, Indonesia and Malaysia are the leading nations in palm oil exportation around the globe (Ho & Tan, 2015; Hushiarian *et al.*, 2013) as over several billions of US dollars were pocketed by both countries in the year 2012. According to Bivi and co-workers (2010), oil palm is known as the Golden crop of Malaysia as it stands out as the only crop used as means of poverty alleviation in the country. However, this economic crop is faced with a great threat of drastic low yield which may result to proportionate low foreign exchange income on the part of exporting countries and subsequently low-quality products by the importing countries.

The oil palm is challenged by a pathogenic fungus, called *Ganoderma boninense*, causative organism for basal stem rot (BSR) disease (Bivi *et al.*, 2010; Ho & Tan, 2015; Hushiarian *et al.*, 2013; Naher *et al.*, 2013; Zain *et al.*, 2013; Nusaibah *et al.*, 2016; Tee *et al.*, 2013).

This destructively devastating disease had killed and still killing lots of oil palms, yet to be remedied. Economic losses to the tune of millions of US dollars have largely been reported as result of the lethal BSR disease (Hushiarian *et al.*, 2013; Nusaibah *et al.*, 2016; Zain *et al.*, 2013).

The uncompromising pathogenic fungus, launching an assault on oil palms specifically in Malaysia and Indonesia and other tropical regions of Thailand, Papua New Guinea and Africa have been uncovered to be *G. boninense* (Kok *et al.,* 2013).

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It is of the genus Ganoderma Karst and classified as a higher fungus. It is a member of the large family of *Ganodermataceae*, in the order *Aphyllophorales*, in subclass *Hymenomycetes* and in the class *Basidiomycetes* (Idris *et al,.* 2000). It has been affirmed that it is the causative organism for Upper stem rot (USR) and basal stem rot (BSR) diseases of oil palms (Rakib *et al.*, 2014a, 2014b). Out of the 15 Ganoderma species responsible for stem rot disease world over, 7 are documented in Malaysia, as follows *G. boninense*, *G. zonatum, G.miniatocinctum and G. tornatum, G. applanatum, G.chalceum, G. lucidum* and *G. pseudoferreum*. The first three are highly pathogenic, while *G. tornatum* is non-pathogenic. The format of disease spread is precipitated on two pathways, namely, root contact and basidiospore (Rakib *et al.*, 2014b). BSR is propagated in a very latent mode. The exploit of *G boninense* being necrotrophic is not easily deciphered at the on-set of its attack on oil palms. In the process, the fungi destroyed cell wall barrier of the oil palms through the activity of the destructive enzymes released by the fungal pathogens, thus enabling the formation of regular infection hyphae within the oil palms. Mostly, the basidiomata are revealed after the demise of the cell of the oil palms.

The mycelia of these fungi often sensitize the protective defense response in oil palms (Nusaibah *et al.*, 2016).

Both youthful and aged standing palms are prone to BSR with high severity documented in old palms (Bivi *et al.*, 2010; Kok *et al.*, 2013). Some of the symptoms include deterioration of bole, closed spear leaves, fractured frond petioles, and appearance of basidiomata around the stem, root or bole regions (Kok *et al.*, 2013), eventuality paving a way to the killing the oil palm in the process. Eighty percent of palm stands can be laid to rest by BSR, usually midway through their productive lifespan (Bivi *et al.*, 2010; Chong *et al.*, 2012; Priwiratama & Susanto, 2014). A variety of control measures have been undertaken, such as, agrophysical methods like soil drenching, crop rotation, clean clearing, chemical methods like use of fungicides, biological methods like use of endophytic bacteria (Bivi *et al.*, 2010; Chong *et al.*, 2012) or saprophytic fungi (Priwiratama & Susanto, 2014), series of molecular approaches, lignin content alteration in oil palm root. Summarily, till date, no certified cure has been adduced to *G.boninense* as most of the controls are limited in their efficacies.

Based on the foregoing, the sustainability of oil palms is greatly at stake, as no known technique has been discovered to tackle the devastating arsenal of *G. boninense* till date (Chong *et al.*, 2011). The urgent need for a proactive detective method is therefore non-negotiable. Methods employed so far include the use of Ganoderma selective medium (GSM) and polymerase chain reaction (Chong *et al.*, 2011). Some of these methods are not efficient enough or are technically deficient in the application. Santoso *et al.*, (2011) discovered and mapped oil palms with BSR disease using strongly resolving QuickBird satellite imagery in oil palm plantation located in North Sumatra, Indonesia. This imaging technique is a kind of remote sensing method. GanoSken Tomography Technology comprising of sound sensors

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and tomography software has also been utilized to monitor deterioration in tree plants.

Though effective, it is not time-friendly. Considering the large expanse of oil palms plantation a relatively simple, easy to handle and cost-effective detection equipment will be a better option. On this premise the combination of the multispectral and thermal camera had been successfully implemented by Khairunniza-bejo *et al.*, (2015) to investigate BSR diseased and BSR disease free oil palms. These technologies are still deficient in that they only detect the BSR at an advanced stage, which left the planter no option of any curative approach. Anyway, the search for early diagnostic method continues. Kandan *et al.*, (2010) applied a serological method based on enzyme-linked immunosorbent assay (ELISA) with polyclonal and /or monoclonal antibodies to detect Ganoderma disease. The shortcoming of the ELISA technique is cross-reactivity among different Ganoderma spp and other saprophytic fungi, give rise to low sensitivity and specificity. Lelong *et al.,* (2010) made use of hyperspectral reflectance spectroscopy to uncover BSR disease and monitor different stages of the pathogen infection on oil palms. However, the method is limited in that canopy optical properties arising from the white rot fungus is yet to be ratified. Rakib *et al.*, (2014b) employed geographical information system and geostatistics to monitor the pattern of distribution of the disease in the field. Nasim *et al.*, (2010) used PCR method to detect *Ganoderma* disease, utilizing powerful genetic marker, internal transcribed spacers region of the ribosomal-DNA. A number of issues arose in this approach ranging from Inefficiencies of different DNA polymerases, the presence of PCR inhibitors in the sample matrix and variation in the performance of PCR thermal cyclers.

Unarguably, the search for quick, simple, sensitive, portable, cheap and point of care detective method is on-going. A look at electrochemical approaches may herald the much-needed antidote. The only limiting factor is the absolute dearth of studies on the electrochemical detection of *G. boninense* either laboratory wise or field wise.

However, Dutse and co-workers, (2012) had eventfully detected *G. boninense* using electrochemical-based DNA approach. The advent of nanotechnology aided the team in mixing relevant nanomaterials to obtain nanocomposites of salient properties like improved biocompactibility, stability, penetration capacity, area to volume ratio, electrical conductance, catalytic ability, biocatalyst loading potential and structural feature. The team employed PEDOT-PSS blended with metallic silver nanoparticles to modify the electrode and applied a novel ruthenium complex as intercalating material for interaction and detection of *G. boninense* (Dutse & Yusof, 2011). Based on the success recorded by this team, it is hoped that pure electrochemical sensor could as well provide a functional approach for early detection of *G. boninense*.

It is a truism that many of the chemical and related research laboratories cannot do without the tool called electrochemical methods. A typical example of this method is the voltammetric method. These approaches have been successfully employed in

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basic studies of redox processes to analyze reaction mechanisms. Studies involving the kinetics and thermodynamics of ion and electron transfer processes have equally been conducted via these essential tools. Courses on adsorption and crystallization events at the surfaces of an electrode are inclusive. The broad utilization of these techniques is ascribed to the following; inexpensive instrumentation, the excellent sensitivity with a lengthy linear range of concentration, quick response period and concurrent detection and determination of many analytes (Gulaboski & Pereira, 2008).

1.2 Problem statement

Ganoderma boninense is the principal pathogenic fungus responsible for the spread of BSR in every developmental stage of the oil palms, commencing from the nursery seedlings to adults on the plantation fields. BSR can be transmitted through airborne basidiospores and mycelia of *G. boninense* during root to root contact in oil palms fields. The dreadful arsenals from the fungi easily disintegrate cell wall components, the strongly protective lignin inclusive. BSR not only promulgates retarded oil yield, it also causes the stands to collapse thus causing severe economic loss to the oil palm industry. 1928 was the year BSR came to be recognized in the country (Bivi *et al.*, 2010). The ensuing economic loss accrued to the tune of millions US dollars, thereby increasing the anxiety of stakeholders in the oil palm sector (Hushiarian *et al.*, 2013). The worry of the stakeholders is precipitated by the fact that the oil palm, is a globally acclaimed economic tree, being portrayed as one of the world's major sources of edible oil and a momentous precursor of biodiesel fuel. Therefore, there is dire need to protect the palms from extinction by detecting the devastating fungus at the onset of infection in oil palms. Thus, this study aimed at early detection of *G. boninense*.

Although *G. boninense* had been successfully identified as the pathogenic fungi causing BSR in oil palms for the past few decades, it remained to be the most serious problem in many areas in Malaysia and Indonesia. Unfortunately, there is no single reliable procedure for curtailing the spread of this disease. Unarguably, a limiting factor in controlling the BSR disease is the lack of reliable diagnostic technique(s) for early diagnosis. This implies the severity of BSR detection at its earliest stage. The findings from previous methods produced little success.

Molecular method like polymerase chain reaction (PCR) is used to detect the presence of bacteria in a sample that might be negative in a routine culture and serology due to the trace amount. In the process, DNA fragments are isolated from a specific region of a genomic DNA. However, many uncertainties from PCR results were observed with regard to whether or not bacterial DNA detected is alive or dead. Inclusive are inefficiencies of different DNA polymerases, the presence of PCR inhibitors in the sample matrix and variation in the performance of PCR thermal cyclers (Chong *et al.*, 2011). The use of blotting hybridization requires coupling with a PCR procedure that makes it laborious and requires being operated by an expert.

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On the other hand, enzyme-linked immunosorbent assay (ELISA) is another promising approach faced with cross-reactivity among different *Ganoderma spp* and other saprophytic fungi resulting in low sensitivity and specificity (Kandan *et al.*, 2010). Imagery techniques have equally been advanced such as GammaScorpion: mobile gamma-ray tomography system by Hamidon & Mukhlisin (2014). The challenges being Cumbersomeness, non-portability, long scanning period and harmful radiation effect of gamma rays emitted by the instrument. In the same vein, hyperspectral reflectance technique was employed by Khairunniza-bejo *et al.*, (2015) in oil palm plantation in Malaysia. The modus operandi of HSI can be summarized as follows, 50 – 300 images are acquirable in a given hyperspectral image cube at different wavelengths with the resolution of 1 – 10nm from a specific wavelength region. The problem encountered is the inability to image thick foliage. Anyway, research is on-going in various areas to factor out the much-needed technique to combat the menace of this pathogenic fungus on standing palms.

Despite the fact that the electrochemical pathway is convenient, simple, selective, speedy and environmentally friendly the challenge remains the development and production of a smart and efficient sensing system for the early detection of *G. boninense*, with the potential of providing a point of care service.

The current miniaturization of electrochemical detection with the advent of screen printing technology for environmental analysis may be effective in this regard. The current advancement in the technology of electrochemical sensor may be the answer to this problem. Thus, the development of a novel sensor is hereby embarked upon to facilitate the early detection of *G. boninense* in infected oil palms.

However, the present study hopes to advance an indirect approach in conciliating early detection of *G. boninense* infections in oil palms, by considering secondary metabolites (SMs) excreted during the attack of the lethargic fungi. The detection of these SMs is crucial to the early detection of *G. boninense*, the causative fungal of basal stem rot (BSR) disease in oil palms. These metabolites are often excreted by the oil palms as defensive soldiers whenever the pathogenic fungi launch a morbid attack on the economic trees. One of the SMs produced by the plants during such invasion within 24 hours of attack is quinoline.

1.3 Objective of the study

The focal point of this study is to develop a simple, efficient and cheap electrode system for the detection of secondary metabolites in *G.boninense* infected oil palms. This implies an indirect early detection of the troublesome fungus. The detection system consists of screen printed carbon electrode modified with nanohybrid materials, namely gold nanoparticles activated multi-walled carbon nanotubes (AuNPs-aMWCNTs) and chitosan activated multi-walled carbon nanotubes (ChTSNPs-aMWCNTs). The analytical performance of the developed system in the

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detection of quinoline and secondary metabolites in both healthy and *G.boninense* infected leaf and root extracts of oil palm will be investigated.

However, the general objective of this study is to develop an electrochemical sensor for the detection of secondary metabolite quinoline in *G. boninense* infected oil palms. The specific objectives of the present study are:

i. To prepare and characterize surface modifiers such as the gold nanoparticle, nanocomposites (AuNPs-aMWCNTs and ChTSNPs-aMWCNTs) and to modify the electrode and optimize the operating conditions of the modified electrode

ii. To carry out the electrochemical characterization of the developed electrode for quinoline detection using cyclic voltammetry

iii. To carry out the electrochemical characterization of the sensor for secondary metabolite detection in roots and leaves extracts from healthy and infected oil palms using linear sweep voltammetry

iv. To verify the on-site efficacy of the sensor by carrying out the characterized detection using developed electrode in the main plantation 6-months old oil palms

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