Research Article

An NMR Metabolomics Approach and Detection of *Ganoderma boninense*-Infected Oil Palm Leaves Using MWCNT-Based Electrochemical Sensor

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Ganoderma boninense (*G. boninense*) has been identified as a major problem in oil palm industry which caused basal stem rot disease. Identification of metabolite variation of healthy and *G. boninense*-infected oil palm leaves at 14 days postinfection using NMR metabolomics approach followed by characterization of an electrochemical sensor based on a functionalized multiwalled carbon nanotube (MWCNT) layer-by-layer framework on modified screen-printed carbon electrode has been successfully determined. Significant differences from the ¹H NMR data were observed between healthy and *G. boninense*-infected oil palm leaves, according to principal component analysis. Gold nanoparticle-functionalized MWCNT and chitosan-functionalized MWCNT were deposited on a screen-printed carbon electrode and were applied for the electrochemical detection of healthy and *G. boninense*-infected oil palm leaves. The electrocatalytic activities of a modified electrode towards oxidation of healthy and *G. boninense*-infected oil palm leaves at a concentration of 100 mg/L were evaluated using cyclic voltammetry and linear sweep voltammetry. The limits of detection of healthy and *G. boninense*-infected oil palm leaves were calculated to 0.0765 mg/L and 0.0414 mg/L, respectively. The modified electrode shows a good sensitivity and reproducibility due to the unique characteristics of gold nanoparticles, chitosan, MWCNTs, and synergistic interaction between them.

1. Introduction

Basal stem rot is a major disease in oil palm industry which is caused by a soil-borne pathogen called *Ganoderma boninense* (*G. boninense*). This disease can cause direct loss of stand, reduced yield of diseased palms, and raised replant recurrence [1]. *G. boninense* infects oil palm at all stages, from seedlings to old plants through root to root contact. Infected young palms normally die within one or two years, whereas mature trees can survive up to three years [2]. Most of the affected trees die within six to 12 months after the sign

of symptoms if not detected earlier, and some affected trees may live for several more years [3].

Despite *G. boninense* being apparently the main problem of the disease in oil palms, strategies and approaches for the early detection and control of *G. boninense* are still unfledged. A variety of studies on diagnostic methods has been applied to overcome the disease but is not sufficient to achieve satisfactory results. At the early stage of infection, the affected trees did not show any signs or symptoms which impose challenges in detecting the disease. The physical appearance of an initial infection symptom such as the

existence of fungal mass or fruiting bodies, yellowing or browning of leaf, and stunted growth has been reported as early as two months after inoculation [4]. It has been suggested that the fungus may grow internally before the physical appearance of infection symptoms. Previous works done by other researchers have been reported on the identification of proteins of altered abundance in oil palm infected with *G. boninense* at 14 days postinfection using 2-dimensional gel electrophoresis [5]. Therefore, 14-day postinfection has been selected for the early detection of *G. boninense* in this study.

Application of ^1H NMR metabolomics approach in identifying metabolite variation of *G. boninense*-infected leaves has not been reported so far. Compared to other metabolomics platforms, NMR metabolomics platforms provide several benefits. ^1H -NMR analysis is less complex as the sample does not need prior derivatization, extraction, and separation as required by mass spectrometry. In addition, ^1H -NMR functions as an independent instrument compared to mass spectrometry which requires separation instrument like liquid chromatography or gas chromatography [6]. Thus, we applied a simple and reliable method, i.e., ^1H -NMR analysis-based metabolomics for the identification of metabolite variations in *G. boninense* in oil palm leaves at an early stage, i.e., 14 days postinfection, and continued with an alternative electrochemical sensor.

The use of carbon nanotubes (CNTs) as a surface modifier of electrode has been increased in sensor technology. It provides several benefits such as excellent carrier mobility of a crystal lattice and lower thermal and electric noises. It also gives high mechanical strength, high thermal conduction, and optical properties which are possible to use in different type of sensors [7, 8]. Referring to the structural properties, carbon nanotubes provide high aspect ratios to adsorb chemical species. The selection of immobilization chemistry and functionalization of a carbon surface with a chemical or biomarker are crucial steps in the preparation of a transducer substrate. The transducer substrate should be resistant to degradation and allow maximal retained activity of receptor and transduction at the same time. Meanwhile, the transducer surface must adhere irreversible to the biosensor and could not interfere with the transduction signal [9].

Multiwalled carbon nanotubes (MWCNTs) are polymers of pure carbon which provides opportunity to modify the structure, optimize solubility and dispersion, and allow innovative applications in sensor. Due to the large specific surface area and the combination of strong π - π interactions and hydrophobic interactions, the CNTs are capable to adsorb various chemical species such as aromatic compounds and natural organic matter [10–13]. To date, electrochemical MWCNT-based sensors have been applied widely in agriculture, food industry, occupational safety, medicine, and forensic chemistry. Liang et al. studied a carbon paste electrode modified by the poly(sulfosalicylic acid) and MWCNT composite for the detection of catechol [14]. Sadeghi and Gamroodi reported a screen-printed carbon electrode modified with quercetin/MWCNTs fabricated for the determination of Cr(VI) in the presence of the excess of Cr(III) without any pretreatment [15]. The two works above obtained high sensitivity and good anti-interference ability.

In this study, activated multiwalled carbon nanotube-chitosan and activated multiwalled carbon nanotube-(MWCNT-) gold nanoparticles (AuNPs) were developed to modify the surface of a disposable screen-printed carbon electrode (SPCE), applying a layer-by-layer (LBL) assembly method. The nanocomposites were produced by grafting the nanomaterials to the surface of the activated multiwalled carbon nanotube-chitosan (MWCNT-CTS). The modified electrode gave better sensing and provided a new option for constructing an effective electrochemical sensor for the determination of *G. boninense*-infected oil palm at 14 days postinfection.

2. Materials and Methods

2.1. Materials. All chemicals were used as received without further purification. Methanol, deuterated methanol- d_4 (CD_3OD , 99.8%), nondeuterated potassium dihydrogen phosphate (KH_2PO_4 , pH 6.0), deuterium oxide (D_2O , 99.9%), and trimethylsilylpropionic acid- d_4 sodium salt (TSP) were purchased from Merck (Darmstadt, Germany). Gold (III) chloride trihydrate ($\geq 99.9\%$) and sodium citrate dehydrate ($\geq 99\%$) used for the synthesis of gold nanoparticles of different sizes, multiwalled carbon nanotubes ($\pm 90\%$, 110–170 nm in diameter and 5–9 μm in length), and chitosan were all obtained from Sigma-Aldrich (St. Louis, USA). Iron (III) trioxonitrate (V) nonahydrate was purchased from Fluka (Durban, South Africa). Ethanol (99.8%), methanol, and sulphuric acid (95–97%) were bought from Friendemann Schmidt (Parkwood, Australia). Citrate buffer solutions (CBS) with a pH range of 3.0–6.2 at 25°C were prepared by mixing stock solutions of 0.11 M citric acid and 0.10 M sodium citrate dehydrate, while adjusting the pH with HCl (1.0 M) and NaOH (2.0 M) solutions.

2.2. Preparation of Rubber Wood Blocks for Artificial Inoculation. The rubber wood block (RWB) was prepared according to the method previously described [16]. Cultures of *G. boninense* PER 71 were obtained from the stock collection of the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. RWBs in a size of 6 cm $\times$ 6 cm $\times$ 6 cm were purchased from Wah Heng Rubber Wood Factory Sdn Bhd, Semenyih, Selangor. Small pieces of *G. boninense*-infected oil palm leaves which have been cut into 1 cm $\times$ 1 cm were inoculated into the RWBs and incubated in the dark for 30 days until the blocks were fully colonized.

2.3. Plant Cultivation. Commercial DxP GH500 germinated seedlings which have been used as planting materials were bought from Sime Darby Seeds & Agricultural Services Sdn Bhd, Banting. Five germinated seedlings were treated with *G. boninense*-inoculated RWBs whereas five germinated seedlings without any treatments of RWBs were used as a control. Treatment was performed for up to 14 days. Leaves samples were harvested at 14 days postinfection. Liquid nitrogen was used to quench each sample and ground into a powder form before being stored at -80°C . A freeze-dry

system (Scanvac Labogene) was employed to remove the water content in the samples.

2.4. Sample Preparation. The freeze-dried leaves powder of healthy and *G. boninense*-infected oil palm (150 mg) was extracted by sonication in 80% methanol (30 min, 40°C). The extraction step was repeated twice, and the total combined supernatant was filtered through a filter paper (Whatman, 125 mm) and evaporated using a rotary evaporator. The crude extracts were then kept at -80°C until analysis.

2.5. NMR Measurement and Multivariate Data Analysis. A 500 MHz Varian INOVA NMR spectrometer (Varian Inc., Palo Alto, CA, USA), functioning at a frequency of 499.887 MHz at room temperature (25°C), was employed to determine ^1H -NMR and J -resolved experiments. Samples were prepared according to the method previously described [17]. A 50 mg sample was weighed in a 2.0 mL Eppendorf tube. A total of 0.75 mL of a 1:1 mixture of methanol- d_4 and potassium dihydrogen phosphate buffer (pH 6.0) in deuterium oxide containing 0.1% trimethylsilylpropionic acid- d_4 sodium salt was added. The mixture was vortexed (1 min), ultrasonicated (20 min), and centrifuged (10,000 rpm, 10 min) at room temperature. The supernatant (0.6 mL) was transferred to an NMR tube to run NMR analysis subjected to ^1H -NMR measurement. A presaturation sequence was applied to eliminate the residual water signal. The resulting spectra were manually phased and baseline corrected using the Chenomx software (v.5.1, Alberta, Canada) with a consistent setting for all spectra. Spectral intensities were binned by equal width (δ 0.04) corresponding to the region of δ 0.50–10.00. The regions of δ 4.70–4.90 representing water and δ 3.23–3.36 representing residual methanol were excluded. A 2D J -resolved experiment was determined for additional support in the metabolite identification. Multivariate data analysis by principal component analysis (PCA) was performed with the SIMCA-P software (v. 13.0, Umetrics, Umeå, Sweden) using scaling based on Pareto. Metabolite identification of the oil palm leaves was determined by comparing the identified metabolite peaks with the Chenomx NMR Suite 7.7 library and standard NMR metabolite databases, i.e., the Human Metabolome Database (HMDB; <http://www.hmdb.ca/>).

2.6. Preparation of Modified Electrode. The modified electrode was prepared according to the method described previously [18–22]. Layer-by-layer (LBL) assembly was employed to modify the electrode. The bare screen-printed carbon electrode (SPCE) was rinsed several times with deionized water and immersed into gold nanoparticle-carbon nanotube (AuNP-CNT) dispersion for 5 min. Then, it was removed and immersed in deionized water for 2 min. After that, it was removed. These steps were repeated twice to complete a cycle, thus coating a bilayer of the nanocomposite, AuNP-CNT, and CTS-CNT on the electrode surface, namely, AuNP-aCNT/CTS-aCNT/SPE. This process was continued until four bilayers are successfully deposited on the substrate and labelled as BL4/SPCE. The developed electrodes were

dried in an oven at 500°C for two hours and stored in desiccators until further analysis.

2.7. Characterization of Modified Electrode in Healthy and *G. boninense*-Infected Leaves. The characterization of the developed electrode was characterized in 100 mg/L healthy and *G. boninense*-infected oil palm leaves under the optimized conditions (-0.52 V , 180 s, and 0.06 V/s). The cyclic voltammetry (CV) and the linear sweep voltammetry (LSV) measurements were performed via a DropSens potentiostat μStat 8000 electrochemical workstation (Asturias, Spain), for reproducibility, sensitivity, and interference study of the developed electrode. The scanning potential was swept between -0.6 V and $+0.6\text{ V}$.

3. Results and Discussion

3.1. ^1H NMR Spectra and Metabolite Identification. Discrimination of the metabolite variation between healthy and *G. boninense*-infected oil palm leaves was employed using ^1H NMR metabolomics analysis. Figure 1 shows the ^1H -NMR spectra of oil palm leaves extracts with identified metabolites. These metabolites including sugars, amino acids, and phenolics were identified based on the 2D J -resolved experiment (Figure 2) and comparison with the NMR spectra of the reference compounds measured under the same conditions as extracts. All of the spectra showed signals in the aliphatic (δ 0.5–3.0), carbohydrate (δ 3.0–5.5), and aromatic regions (δ 5.5–8.0).

The identified metabolites from oil palm leaves extracts and their characteristic signals are shown in Table 1. A total of 22 metabolites were identified, including primary and secondary metabolites. Isobutyric acid, 3-aminoisobutyric acid, β -cryptoxanthin, and alanine were present in the aliphatic region between δ 1.00 and δ 1.92 whereas *N*-acetylcysteine, homocysteine, and tyramine appeared between δ 2.04 and δ 2.92.

Alpha-glucose, arabinose, fructose, sucrose, and xylose were ascribed based on the signals displayed in the carbohydrate region of δ 3.0– δ 5.5. The presence of α -glucose was confirmed by the peaks at δ 5.16 (*d*, $J = 5.0\text{ Hz}$) whereas peaks of arabinose were detectable at δ 4.00 (*m*) and δ 3.90 (*m*). Fructose and xylose were detectable at δ 4.08 (*d*, $J = 5.0\text{ Hz}$) and δ 4.56 (*d*, $J = 10.0\text{ Hz}$), respectively. Several peaks observed at δ 5.40 (*d*, $J = 5.0\text{ Hz}$), δ 4.16 (*d*, $J = 9.0\text{ Hz}$), and δ 3.44 (*t*, $J = 10.0\text{ Hz}$) suggested the presence of sucrose. Other metabolites detectable in the carbohydrate region were choline and indole-3-acetic acid which were detectable at δ 3.20 (*s*) and δ 3.64 (*s*), respectively.

Several phenolics and other metabolites were present in the aromatic region (δ 5.5–8.0). Biotin, 2,3,4-trihydroxybenzoic acid, *trans*-aconitic acid, and kaempferol were determined between δ 6.40 and δ 6.76. The characteristic peaks of gallic acid, caffeic acid, and *p*-hydroxybenzoic acid were detectable at δ 7.04, δ 7.16, and δ 8.01, respectively.

3.2. Discrimination of Healthy and *G. boninense*-Infected Oil Palm Leaves at 14 Days Postinfection. Identification of metabolite variations of healthy and *G. boninense*-infected

(a)

(b)

(c)

FIGURE 2: Continued.

FIGURE 2: 2D J -resolved experiments of oil palm leaves: (a) in the region from δ -0.50 to δ 8.50; (b) in the region from δ 0.80 to δ 2.93; (c) in the region from δ 3.10 to δ 5.50; and (d) in the region from δ 6.30 to δ 8.15. For the interpretation of the numbers assigned to the metabolites in the 2D J -resolved experiments, refer to Table 1.

TABLE 1: Assignments of NMR signals for metabolites identified in ^1H and 2D NMR spectra of oil palm leaves extracts with corresponding multiplicity (s : singlet; d : doublet; t : triplet; m : multiplet) and scalar coupling constant (J (Hz)) values.

Peak no.	Metabolites	Chemical shift (multiplicity, J)
1	Xylose	δ 4.56 (d , J = 10.0 Hz)
2	Sucrose	δ 5.40 (d , J = 5.0 Hz), δ 4.16 (d , J = 9.0 Hz), δ 3.44 (t , J = 10.0 Hz)
3	Arabinose	δ 4.00 (m), δ 3.92 (m)
4	Fructose	δ 4.08 (d , J = 5.0 Hz)
5	α -Glucose	δ 5.16 (d , J = 5.0 Hz)
6	Alanine	δ 1.48 (d , J = 10.0 Hz)
7	Caffeic acid	δ 7.16 (d , J = 10.0 Hz)
8	Gallic acid	δ 7.04 (s)
9	Indole-3-acetic acid	δ 3.64 (s)
10	Choline	δ 3.20 (s)
11	<i>trans</i> -Aconitic acid	δ 6.60 (s)
12	Isobutyric acid	δ 1.00 (d , J = 20.0 Hz)
13	3-Aminoisobutyric acid	δ 1.19 (d , J = 10.0 Hz)
14	<i>N</i> -Acetyltyrosine	δ 1.92 (s)
15	<i>N</i> -Acetylcysteine	δ 2.04 (s)
16	Homocysteine	δ 2.84 (t , J = 10.0 Hz)
17	Tyramine	δ 2.92 (t , J = 5.0 Hz)
18	Biotin	δ 6.40 (s)
19	2,3,4-Trihydroxybenzoic acid	δ 6.52 (d , J = 15.0 Hz)
20	<i>p</i> -Hydroxybenzoic acid	δ 8.01 (d , J = 5.0 Hz)
21	β -Cryptoxanthin	δ 1.76 (s), δ 1.68 (s), δ 1.12 (s)
22	Kaempferol	δ 6.76 (d , J = 2.5 Hz)

oil palm leaves was done by principal component analysis (PCA). PCA was used to cluster the features of the samples and analyze the metabolites that contributed to the variation.

Healthy and *G. boninense*-infected oil palm leaves at 14 days postinfection could be discriminated clearly as shown

in Figure 3(a). The first two principal components (PC1 and PC2) cumulatively accounted for 87.5% of the total variation. Separation of the healthy and *G. boninense* infected oil palm leaves in score plots was achieved by combining PC1 and PC2.

FIGURE 3: (a) PCA score plot (component 1 vs. component 2) and (b) loading line plot of component 1 of ^1H NMR data for comparing healthy and *G. boninense*-infected oil palm leaves at 14 days of infection. For the interpretation of the numbers assigned to the metabolites in the loading column plot, refer to Table 1.

The metabolites in the healthy and *G. boninense*-infected oil palm leaves which could be illustrious using PCA is shown in the loading line plot for PC1 (Figure 3(b)). The PCA and loading line plots correlate with each other. The position of an object in a given direction within the score plot is influenced by a variable which lies in the same direction in the loading plot [23].

From the PCA analysis, the levels of xylose, α -glucose, gallic acid, and *N*-acetylcysteine were more abundant in healthy leaves whereas sucrose, arabinose, fructose, alanine, caffeic acid, indole-3-acetic acid, choline, *trans*-aconitic acid, isobutyric acid, 3-aminoisobutyric acid, *N*-acetyltyrosine, homocysteine, tyramine, biotin, 2,3,4-trihydroxybenzoic acid, *p*-hydroxybenzoic acid, β -cryptoxanthin, and kaempferol levels were higher in *G. boninense*-infected leaves.

Primary metabolites which are produced by plant cells are directly involved in growth, development, and reproduction, whereas the secondary metabolites such as alkaloids, terpenoids, compounds containing sulphur, and phenolics are involved in host defense response [24].

Sugars play important roles as carbon and energy sources [25]. Also, they have been recognized as signaling molecules of plant defense under biotic and abiotic stresses [26–30]. Accumulation of sucrose and fructose in *G. boninense*-infected leaves should be related to their role as energy sources for pathogen replication [31].

In contrast, α -glucose was decreased in infected leaves which indicated a part of the available carbon gathered in carbohydrates is conceivably diverted towards secondary metabolism. The carbohydrates also provide the necessary energy to support increased secondary metabolite biosynthesis [32].

The high level of alanine in *G. boninense*-infected leaves was observed in the present study. Similarly, *Vitis vinifera* showed increased levels of alanine when infected with *Botrytis cinerea*, demonstrating its defensive role against biotic stresses [33]. It has been suggested that alanine synthesis is increased to regulate cellular osmosis which is decreased by high cellular carbohydrate levels under stress

conditions in infected plants wherein γ -aminobutyric acid (GABA) catabolism by GABA transaminase leads to alanine accumulation [33–36].

Meanwhile, accumulation of choline amount in infected leaves is associated to an increase in the synthesis of membrane components after pathogen infection. It has been reported that infected leaves induce choline synthesis through the jasmonic acid signal transduction pathway [37].

Phenolics such as kaempferol, 2,3,4-trihydroxybenzoic acid, *p*-hydroxybenzoic acids, and caffeic acid level were more prominent in *G. boninense*-infected leaves. These metabolites were produced by plants to help in cell wall lignifications [38], antimicrobial activity [39, 40], modulation of plant hormones involved in defense signaling pathways, and scavenging of reactive oxygen species [41].

Indole-3-acetic acid (IAA) level was found higher in infected leaves which coincided with previous studies on metabolic changes in *Agrobacterium tumefaciens*-infected *Brassica rapa*. IAA acts as an inhibitor of virulence gene expression in plants. It also functions as a chemical agent metabolite in plant defense against pathogen [42].

3.3. Electrochemical Characterization of Modified Electrode for Healthy and *G. boninense*-Infected Oil Palm Leaves at 14 Days Postinfection. Our coauthor has characterized previously the morphological structures of MWCNT and AuNP-aMWCNT composites using an ultrahigh resolution field emission scanning electron microscope (FESEM) [22]. The pristine-MWCNT shows a uniform and equivalent tubular arrangement with some voids. The tubes were not shortened on activation even the spaces are closed up to an extent [22, 43]. Unevenness of the surface was observed in their finding after activation due to the attachment of carboxylic functional groups to the surface. The surface of aMWCNT was craggier with the incorporation of AuNPs which confirm successful loading of AuNPs on the aMWCNT [22].

The modification of the surface of the bare SPCE via LBL self-assembly approach was characterized using FESEM by Akanbi et al. [22]. They reported that the gradual coverage

FIGURE 4: LSVs of five replicates independently produced BL4/SPCEs in 100 mg/L (a) (i) healthy leaves and (b) (i) *G. boninense*-infected leaves. A plot of anodic peak current versus regenerated BL4/SPCEs in (a) (ii) healthy leaves and (b) (ii) *G. boninense*-infected leaves.

of the surface of the SPCE as the numbers of bilayers of nano-hybrid materials increases. The presence of AuNPs assisted the catalytic properties of the MWCNT, while chitosan nanoparticles affect proper binding tendency towards the analyte via its functional groups. These beneficial effects largely improve the overall performance of the developed electrode, which is apparent in the electronic transition enhancement. When the surface of the SPCE is almost covered, the further deposition of nanohybrid materials may lead to excessive deposition, which may retard electron movement to the site of chemical reaction on the electrode platform.

Akanbi et al. also studied the functional group presence in the MWCNTs, a-MWCNTs, and CTSNP-aMWCNT using Fourier-transform infrared spectroscopy [22]. Functional groups of ($-N-H$, $-O-H$), ($C=O$), and ($C-N$) were observed in CTSNP-aMWCNT composites whereas $C-H$ bond which is a dominating bond was present in pristine-MWCNTs. Other functional groups determined were ($C=O$), ($-O-H$), and ($-C-O$), indicating the carboxylation of the pristine-MWCNTs [22].

The characterization of the modified electrode was conducted utilizing the linear sweep voltammetric method under optimized conditions of -0.52 V accumulation potential, 180 s accumulation time, and a scan rate of 0.06 V/s. The potential was scanned from -0.6 V to $+0.6$ V with a potential step of 0.002 V and an equilibrium time of 10 s.

3.3.1. Reproducibility Study. Figure 4 shows LS voltammograms of 100 mg/L healthy and *G. boninense*-infected leaves at the platform of five independently prepared BL4/SPCEs (Figures 4(a) (i) and 4(b) (i)). Five replicates of BL4/SPCEs were prepared independently to determine the oxidation anodic peak current and I_{pa} response of 100 mg/L healthy and *G. boninense*-infected leaves in the absence of supporting electrolyte (Figures 4(a) (ii) and 4(b) (ii)). The peak potential at the five BL4/SPCEs for each of healthy and *G. boninense*-infected leaves approximates in the range of 0.10 - 0.20 V. The calculated relative standard deviation (RSD) from the plot of healthy and *G. boninense*-infected leaves was 0.73% and 5.08% , respectively. The RSD value for healthy leaves may suggest that all the five differently modified electrodes gave approximately the same oxidation anodic current response towards the oxidation of healthy leaves. Consequently, there is divergent response at the surface of the other five modified electrodes towards the oxidation of *G. boninense*-infected leaves at 14 days postinfection. This value implies that the electrode is reproducible.

3.3.2. Sensitivity Study. The linear-sweep voltammograms shown in Figures 5(a) (i and ii) and 5(b) (i and ii) demonstrate the excellent response of the BL4/SPCE sensor provided as a function of the healthy and *G. boninense*-infected leaves concentration in the absence of a supporting

FIGURE 5: Linear sweep voltammograms of BL4/SPCE in different concentrations of (a) (i) healthy leaves and (b) (i) *G. boninense*-infected leaves, and linear relationship of anodic peak current, I_{pa} , and concentrations of (a) (ii) healthy leaves and (b) (ii) *G. boninense*-infected leaves.

TABLE 2: Comparison of electroanalytical data of various modified electrodes.

Modifier	Detection	Limit of detection (mg/L)	References
ZnO-rGO-GCE	Hydrogen peroxide	0.068	[43]
Poly(vinylpyrrolidone)-modified carbon-paste electrode	Quercetin	0.051	[44]
ZnO-rGO-GCE	Ascorbic acid	0.055	[45]

electrolyte. It is evident that as the anodic peak current increases, there was a slight shift in the peak potential as the concentration of the samples is elevated. This may be attributed to the catalytic effect of the nanocomposite hybrid surface modifiers on the chemical composition of the samples.

The corresponding calibration curves obtained from Figures 5(a) (i) and 5(b) (i) are adjacently shown in Figures 5(a) (ii) and 5(b) (ii). The calibration curves obtained for the oxidation of healthy and *G. boninense*-infected leaves are linear in the concentration range of 0.1 to 0.5 mg/L. The linear regression equation for healthy leaves can be expressed according to the function $I_{pa} (\mu\text{A}) = 0.1901 + 0.0754$ (correlation coefficient (R^2) = 0.9732) whereas the linear regression equation for *G. boninense*-infected leaves was $I_{pa} (\mu\text{A}) = 0.2502 + 0.1913$ (correlation coefficient (R^2) = 0.9716).

The difference observed between the anodic peak current values of healthy and *G. boninense*-infected leaves crude extract solution is resulted by the abundance of the higher level of secondary metabolites such as phenolic compounds (kaempferol, 2,3,4-trihydroxybenzoic acid, *p*-hydroxyben-

zoic acids, and caffeic acid) and auxin (indole-3-acetic acid) in the *G. boninense*-infected leaves crude extract. In addition, the primary metabolites such as sugars and amino acids were also contributed to the difference.

The limit of detection (LOD) was calculated according to the following equation: $\text{LOD} = 3.3 \text{ Sb}/B$, where Sb is the standard deviation of the linear coefficient and B is the slope of the calibration curve. The LOD value obtained for healthy leaves crude extracts was calculated to 0.0765 mg/L whereas the LOD value for *G. boninense*-infected leaves crude extracts was 0.0414 mg/L. As shown in Table 2, the LOD of the *G. boninense*-infected leaves crude extract electrode has been compared with other reported previously [44–46]. It can be suggested that the low LODs of the pair AuNP-aMWCNT and ChTSNP-aMWCNT nanohybrid materials could be suitable for the modification of screen-printed carbon electrodes for the detection of healthy and *G. boninense*-infected leaves.

3.3.3. Interference Study. The changes in the response of the anodic peak current (I_{pa}) of the healthy and *G. boninense*-

FIGURE 6: A plot of percent interference in the anodic peak current (I_{pa}) response of (a) healthy leaves and (b) *G. boninense*-infected leaves against interfering species at modified electrode under optimized conditions (-0.53 V, 180 s, 0.06 V/s). (-) campherochinon: (-) Camp; (+) campherochinon: (+) Camp; 4-(dimethyl amino)pyridine: 4-(DAP); 2,4,6-trimethylpyridine: 2,4,6-TMP; 2,4-dinitrophenol: 2,4-DNP; 1-chloro-2,4-dinitrobenzene: 1-Cl-2,4-DNB.

infected leaves at the developed electrode platform were measured in the presence of different potential interferences (Figure 6). These interferences were predominantly organic due to the nature of the sample being considered. They include (-) campherochinon, (+) campherochinon, glucose, palmitic acid, pyridine, maltodextrin, cholestane, phenol, 4-(dimethyl amino)pyridine, 2,4,6-trimethylpyridine, 1-chloro-2,4-dinitrobenzene, and 2,4-dinitrophenol. 500-fold excess concentration of each interference was prepared for this determination. The obtained signal for a fixed concentration of each leaves was compared with the signal values obtained in the presence of known concentrations of the employed interfering species. The tolerance limit was considered the maximum concentration of interfering species that cause an error less than or greater than 10% for the detection of healthy and *G. boninense*-infected leaves. As displayed in Figures 6(a) and 6(b), a closer look at Figure 6(a) revealed that (+) campherochinon, pyridine, and 4-(dimethyl amino)pyridine interfered with the detection of healthy leaves. However, it is 4-(dimethyl amino)pyridine that is said to be the strongest in interference with a percent interference greater than -10%. In the case of *G. boninense*-infected leaves (Figure 6(b)), the effects of the interference is negligible, though 4-(dimethyl amino)pyridine still possesses the highest percent interference of almost -8%.

4. Conclusion

It can be concluded that NMR spectra of healthy and *G. boninense*-infected leaves at 14 days postinfection exhibited differences which were discriminated and clustered into groups through multivariate data analysis of PCA. AuNP-aCNT and CTS-aCNT nanocomposite were successfully prepared via a simple method and used to fabricate a novel electrode for the electrochemical detection of

healthy and *G. boninense*-infected leaves. The AuNP-aMWCNT/ChTSNP-aMWCNT film was found to enhance the electrooxidation activity of healthy and *G. boninense*-infected leaves, due to the catalysis of AuNPs, binding potential of CTSNPs, and wide surface area provided by aMWCNTs. The results show good signal enhancement of healthy and *G. boninense*-infected leaves on AuNP-aMWCNT/ChTSNP-aMWCNT ultrathin films which offer a new sensitive, selective, cost-effective, and environmentally friendly electrode.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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