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Some Interpretations on FTIR Results for the Detection of *Ganoderma Boninense* in Oil Palm Tissue

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ABSTRACT

Background: The use of Fourier Transform Infrared (FTIR) spectroscopy has been employed for the past three decades in the study of biological samples and characterization of biomolecules. **Objective:** In this paper, the author have conducted the feasibility study of detecting *Ganoderma boninense* in infected oil palm tree using Fourier transform infrared spectroscopy (FTIR). **Results:** It was found that result from FTIR plot is capable of showing the presence of *Ganoderma boninense* with good sensitivity. **Conclusion:** In this paper, the mechanisms behind this detection are discussed. This is due to the unique functional groups that exist in the *Ganoderma boninense* which cannot be found in healthy oil palm tissue.

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INTRODUCTION

Ganoderma boninense is the causal pathogen of Basal Stem Rot (BSR) disease in oil palm. This disease is one of the most significant constraints to oil palm industry in the Southeast Asian countries. BSR was usually found on mature stands, however, diseased palms as young as 12-24 months after planting have been observed [12]. Infection of this disease can cause numerous yield losses and ultimately will result in the destruction of basal tissues hence death of diseases palms. Many efforts have been attempted to eliminate this disease, but none have given promising result [3]. In most cases, it failed due to late detection of the disease. This is because development BSR always symptomless and visible symptoms only appear at the very late stage of the infection when more than half of the root tissues have decayed, leaving no chance for the infected palms to be cured. Therefore, a viable early detection method for this disease has yet to be found. Currently, few methods have been reported to be useful to detect diseased palms even before the expression of symptoms. These include work on enzyme-linked immunosorbent assay (ELISA) [9] and Polymerase Chain Reaction (PCR) and sequence homology with specific primers for *Ganoderma boninense* [4], [8], [13]. However, the primers were reported to cross react with other saprophytic fungi and non *boninense* spp. Detection of *Ganoderma*-infected tissues using *Ganoderma* Selective Media (GSM) [2] was also reported, however, this method are time-consuming and not economic for large-scale application. In addition to these techniques, the development of PODITOO^{TM} tomography to detect decayed tissues due to *G. boninense* infection based on sound lines also have been attempted [10]. Another work conducted, on utilising an electronic nose (E-nose) also had been reported in detecting BSR disease based on odour profiles [1]. This method provides fast result on field, however, it is not specifically designed to detect infection by *G. boninense* alone, as it also generates odour profiles when palms are infected by other pathogens. Therefore, a fast, reliable and accurate detection and quantification of *G. boninense* is still on demand. In the present study, Fourier-transform infrared (FTIR) spectroscopy was used for the identification and discrimination of functional group in *G. boninense* which could possibly used as an

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indicator in BSR early detection. The detection and identification of microorganisms using FTIR spectroscopy techniques is promising as valuable tool because of its sensitivity, rapidity, low cost and simplicity [11].

Methodology:

Pure culture of *Ganoderma boninense* was obtained from Genetic Laboratory of School of Science and Technology, Universiti Malaysia Sabah. The identity of *G. boninense* had been identified and confirmed using molecular technique [4]. The pure culture was then subcultured and maintained at 25ºC on Potato Dextrose Agar (PDA). For comparison, healthy oil palm trunk tissues were taken from oil palm plantation in Sandakan, Sabah, Malaysia. Collection of trunk tissues was done according to the procedure described by [5]. The healthy tissues were confirmed free from *Ganoderma*-infection based on ergosterol analysis [6] and *Ganoderma* Selective Media (GSM) [2].

The FTIR spectrum was acquired using a Perkin Elmer 2000 Series Instrument. The spectrum resolution was set at 4 cm^{-1} and the scanning range was selected from 650 to 4000 cm^{-1}. A small amount (~ 100 mg) of dried and powdered sample was placed onto the FTIR sample holder and spectra were collected. Three independent replicate samples of either *G. boninense* or healthy trunk tissues were measured.

RESULT AND DISCUSSION

In this study, the pure cultured of mycelium *G. boninense* was examined using FTIR to find spectroscopic biomarkers for rapid detection and identification of its colonization in oil palm. The result presented in Figure 1 shows there is a unique spectrum of *G. boninense* compared to healthy oil palm trunk tissues. Significant spectral differences between *G. boninense* and healthy trunk tissue can be seen in the range of 3500-3200 cm^{-1} (Region A), 1650-1390 cm^{-1}(Region B) and 1250-1000 cm^{-1}(Region C) respectively. These clear differences provide a preliminary indication of possible spectral parameters for detection of *G. boninense.* The result was also supported by the fact that fungi pathogens display typical infrared spectra that differ from spectra of substrate material such as plant fibers, which make it is possible to detect and identify such pathogens directly from the infected tissue [7].

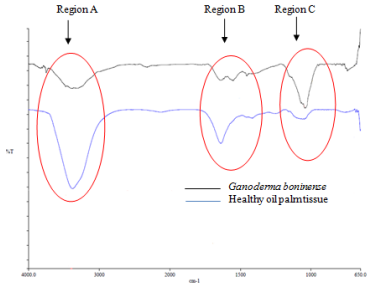

Fig. 1: FTIR spectra at the region 650-4000 cm^{-1} of *Ganoderma boninense* and healthy oil palm trunk tissues.

Table 1 summaries the FTIR spectrum functional group analysis of *Ganoderma* and healthy tissues. The result shows the presence of several functional groups in *Ganoderma* which are not detected in healthy tissue sample. The identified functional group are N-H, C=N, C=H and C-O-C. This finding was in agreement with previous study conducted by [1] which found CH_3, CN and C-O-C in *Ganoderma*'s fruiting bodies. Most of the functional groups in *Ganoderma* are detected in the area ranged between 1000 to 1800 cm^{-1}, which can be considered as an important area for easy and reliable discrimination with the healthy trunk tissue.

Table 1: Functional Group of *G. boninense* mycelia and healthy oil palm trunk tissues.

Wave-length (cm^{-1})	Region	Functional group		Possible Functional group for indicator of *Ganoderma* infection
		Ganoderma boninense	Healthy trunk tissue	
3500-3200	A		O-H (phenol)	
3400-3200	A	N-H (amine)		N-H (amine)
1650-1600	B	C=O (amide)	C=O (amide)	
1580-1500	B	C=N (imine)		C=N (imine)
1470-1450	B	C-H (alkane)		C-H (alkane)
1400-1390	B		C-O(carboxylic acid)	
1250-1000	C	C-O-C (ether)		C-O-C (ether)
1100-1000	C		Si-O (silicone)	

Empty box indicate absence of the respective compound in the sample(s).

Conclusion:

In conclusion, the present study shows that there is a great potential of FTIR spectroscopy for an easy and rapid detection of *G. boninense* infection in oil palm due to the specific functional groups that exist in the *Ganoderma boninense* which cannot be found in healthy oil palm tissue. Detection of BSR infection in oil palm at early stage is crucial for management of this disease.

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