Sensitivity analysis of the detection of *Ganoderma boninense* infection in oil palm using FTIR

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A b s t r a c t

One of the main issues in oil palm plantation is the infection of *Ganoderma boninense* causing basal stem rot disease. Huge monetary losses were reported in the industry by the main producer countries such as Malaysia and Indonesia. Many efforts have been carried out to detect the fungus at the early stage of infection with less practical achievement so far. Recently, detection of the pathogenic fungi using Fourier Transform Infrared Spectroscopy (FTIR) has been investigated by the authors. This paper examines the sensitivity of the detection method and correlates the results with the practicality in field scenario. It was found that percentage content of *G. boninense* cells in oil palm tissues of 5% is detectable using FTIR technique. The results presented in this study indicated that FTIR could be a solution to early detection of *G. boninense* infection in oil palm especially if the instrument can be made portable and robust for field application.

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is one of the world major crops which are grown for the production of vegetable oil used in foods, washing powders, cosmetics and biodiesel. The palm is of major economic importance in Southeast Asia where it is grown extensively in Malaysia and Indonesia (Paterson *et al.*, 2009). Currently, Indonesia is the largest producer and exporter of palm oil and its products, followed by Malaysia. However, the oil palm industries are being jeopardized with one major problem which is basal stem rot (BSR) disease caused by *Ganoderma boninense*. This disease not only causes reduction of yield of infected palms but it also resulted in direct loss of stand due to palm death. The economic losses are between $68 and $455 million a year in Malaysia alone (Chong, 2012). To date, there is no effective control or cure reported to combat this disease. Many researchers agree that the limiting factor in the control of BSR is due to the lack of early disease detection (Naher *et al.*, 2013; Dayou *et al.*, 2014a,b). Until recently, the detection of the disease was based on external symptoms. Observation of such symptoms in the field such as wilting of mature leaves and falling through malnutrition or the presence of basidiomata of the pathogen on the tree have been taken as an

indicator of *Ganoderma* infection (Lelong *et al.*, 2010). However, visible disease symptoms of Basal Stem Rot (BSR) only appear at the very late stage of infection, where more than 60 % of internal tissues are already rotten (Sundram *et al.,* 2006), leaving no chance for any treatment to take place. Therefore, early detection technique of this disease is essential to help us in managing and controlling this disease at early stage.

Some conventional diagnostic tools have been developed for early diagnosis of *G. boninense* such as semi-selective media for *Ganoderma* cultures from oil palms (Darus *et al*., 1993) and *Ganoderma* Selective Media (GSM) (Darus & Abu Seman, 1992), which were claimed to able to detect *Ganoderma* in infected oil palm but have not shown any external symptoms. However, these methods were less-accurate as other basidiomycete fungi also can grow on these media, therefore not recommended for large scale application. Concern on the inaccuracy of these methods, a more advance molecular techniques have been innovated with more accuracy of detection and *Ganoderma* identification. Molecular and immunological methods such as enzyme-linked immunosorbet assay (ELISA) and polymerase chain reaction (PCR) using specific deoxyribose nucleic acid sequences are two of the methods commonly used in *Ganoderma* detection (Kandan *et al.,* 2009; Utomo & Niepold, 2000; Chong *et al*., 2012). However, their application in the routine analysis is limited by their protocol complexities, reagent cost, sensitivity to contamination and the requirement of highly skilled personal. Currently, development of device system in agricultural technology such as remote sense system or e-nose system has been reported to be able to detect *G. boninense* in the field (Markom *et al*., 2009; Naher *et al.,* 2013). This method provides a fast result on field, however, this method is not specifically designed to detect infection by *G. boninense* alone, it also generated odour profiles when palms are infected by other pathogens. Most recently, an approach that is based on Fourier Transform Infrared (FTIR) spectroscopy have been proposed for identification of microorganisms. Several studies have showed that FTIR spectra can provide highly specific spectroscopic fingerprints of microorganisms allowing an accurate identification of species down to strain level (Mariey *et al.,* 2001; Maquelin *et al*., 2003; Taha *et al*., 2013). This technique offer a convenient, accurate and non time consuming analysis, therefore can be applied for *Ganoderma* detection in oil palm. A preliminary study by Dayou *et al.* (2014a,b) also showed that FTIR spectroscopy was able to detect and discriminate *G. boninense* from healthy oil palm tissue based on their unique fingerprint. The present study was conducted to evaluate FTIR spectroscopy as a sensitive and effective assay for the identification of *G. boninense*.

Methodology

Fungal culture and preparation

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 (Chong *et al*., 2012). The pure culture was then sub-cultured

and grown on Potato Dextrose Agar (PDA) for 7 days at 27ºC. After 7 days incubation, small plugs containing the fungus were transferred from the PDA culture into a liquid medium PDB (Potato Dextrose Broth). The cultures were grown for 21 days at 27ºC to get bulky mycelia. The pathogen was grown in 3 replicates.

After incubation, the fungal samples were first filtered using muslin cheese cloth to harvest the mycelia and then washed with distilled water twice to remove any media leftover on the mycelia surface. The samples were then air dried overnight under laminar flow until all water had evaporated. Samples were kept in drying chamber at 60ºC for overnight to remove moisture inside the mycelia. After that, samples were crushed in liquid nitrogen using mortar and pestle in order to obtain fine powder.

Tissue sample preparation

Oil palm tissues samples were collected from oil palm plantation in Sandakan, Sabah, Malaysia. Collection of trunk tissues was carried out following the method described by Chong (2012). Ethanol sterilization was taken to eliminate the possibility of contamination from unwanted saprophytes during trunk tissues collection. Healthy tissues were confirmed free from *Ganoderma* based on ergosterol analysis (Chong *et al*., 2012) and *Ganoderma* Selective Media (GSM) (Darus & Abu Seman, 1992). Samples were kept in drying chamber at 60ºC overnight to remove moisture content. After that, tissues samples were homogenized into fine powder using commercial blender.

FTIR analysis

For FTIR analysis, the oil palm tissues samples were mixed with *Ganoderma* powder in five different concentrations: 5%, 10%, 15%, 20% and 25% respectively. Mixture was vortex before subjected to analysis to ensure that the mixture was evenly mixed. The samples were analyzed using a Perkin Elmer 2000 Series Fourier Transform Infrared (FTIR) spectrometer. The spectrum resolution was set at 4 cm^{-1} and the scanning range was selected from 650 to 4000 cm^{-1}. Approximately 100 mg of sample was placed onto the sample holder and the spectra were collected. Three independent replicate of each mixture including healthy tissues for control were measured.

Results and Discussion

Study on the sensitivity of FTIR spectroscopy in detecting *G. boninense* at different levels of concentration could be highly important for future detection and identification of this pathogen at early stage. The results presented in Figure 1 shows that there was no obvious differences among all five concentrations (5%, 10%, 15%, 20% and 25%). High spectral similarity among different concentrations reinforce the previous finding by Dayou *et al*. (2014a,b) that FTIR spectroscopy technique provide unique spectral fingerprint specific to *G. boninense* which discriminate from

healthy oil palm tissues. All the concentration tested closely matching the *G. boninense* spectral reported by Dayou *et al*. (2014a,b).

Based on the results, FTIR spectroscopy can detect the presence of *G. boninense* as low as 5% concentration. A study by Naumann *et al*. (2005) shows that FTIR spectroscopy was able to localize and identify two wood-rooting fungi, *Trametes versicolour* and *Schizophyllum commune* which was prior experimentally infected wood blocks. This clearly showed that FTIR has the ability to be used for characterization of wood-rooting fungi and detection of relative distribution within wood even at very low concentration. Similarly, Sandt *et al*. (2003) demonstrated that FTIR spectroscopy is potent enough to identify *Candida albicans* with high sensitivity down to 10 µl cell suspension. Consistent spectral pattern at different concentrations of *Ganoderma* in Figure 1 shows that FTIR could provide an accurate identification regardless of the amount of the pathogen presence in the tissues. This is in parallel with the study by Mura *et al*. (2012) which detect identical and consistent FTIR spectral of *Escherichia coli* at different concentrations ranging from 10^{-8} - 10^{-2} (Colony Forming Unit) CFU/ml. FTIR approach represents an analytical, nondestructive, and dynamic method to investigate a cell population with little biomass (Naumann *et al*., 1991).

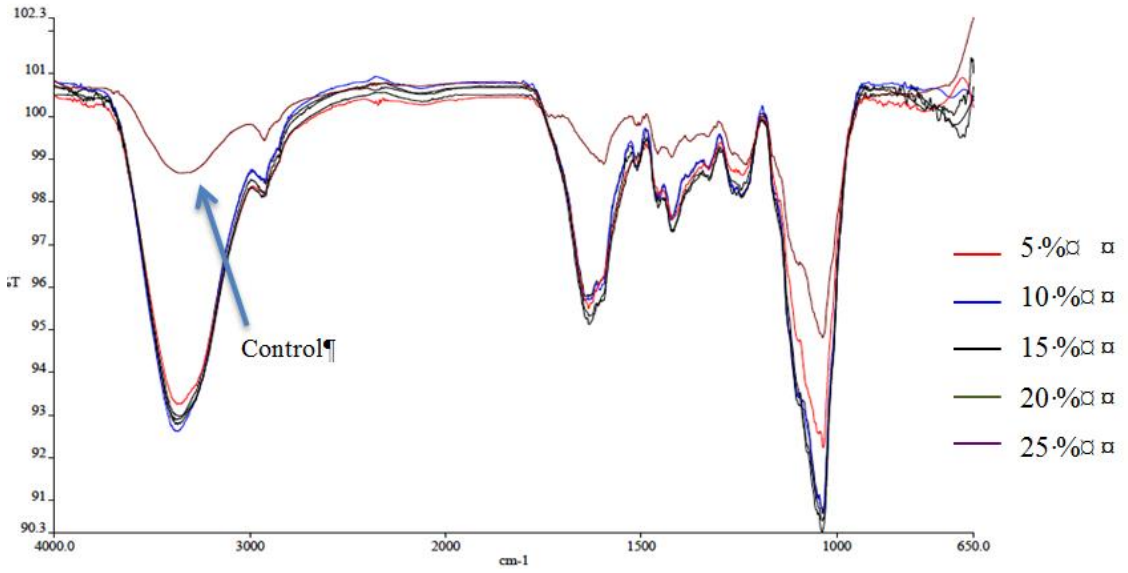

Figure 1: FTIR spectrum of oil palm trunk tissue (control) and tissues with different concentrations of *G. boninense* (5%, 10%, 15%, 20% and 25% respectively) at 4000-650 cm^{-1}.

Due to its high sensitivity characteristic, FTIR spectra can reflect small variations due to culture parameters such as type of media used, temperature, storage mode and age of culture. Therefore, a standardized preparation procedure should be taken into consideration to achieve a high level of spectrum reproducibility that is crucial to avoid misidentification (Santos *et al*., 2010).

Conclusion

The sensitivity of FTIR could possibly fulfill the demand of fast, sensitive and accurate for the early detection of *G. boninense.* As this technique is not time consuming and laborious, it provides considerable saving in terms of economic benefits and productivity. FTIR could be a solution to early detection of *G. boninense* infection in oil palm tree especially if the instrument can be made portable and robust for field application.

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