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A FEASIBILITY STUDY ON VOLATILE ORGANIC COMPOUNDS PROFILING OF OIL PALM-*Ganoderma* INFECTED WOOD FOR BASAL STEM ROT DETECTION

(Kajian Kebolehlaksanaan Pemprofilan Sebatian Organik Meruap daripada Kayu Sawit yang Dijangkiti *Ganoderma* bagi Mengesan Penyakit Reput Pangkal Batang)

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MJAS Vol 24 No 4 (2020)

Abstract

Volatile organic compounds (VOCs) are commonly released during plant-pathogen interactions due to pathogenicity or plant defence responses. Basal stem rot (BSR) disease caused by *Ganoderma boninense* fungus remains a serious threat to oil palm cultivation. This study aimed to investigate the feasibility of detecting VOCs released from oil palm wood tissue infected by *G. boninense* using headspace solid-phase microextraction (HS-SPME) method combined with gas-chromatography mass-spectrometry (GC-MS). Several factors affecting the HS-SPME extraction efficacy were investigated; 0.25 g of sample homogenised in liquid nitrogen was shown to be the most useful preparation procedure, while fibre divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) was shown as the optimum extraction phase. The optimised method was capable of sampling VOCs with high reproducibility with a balanced VOCs profile of various chemical classes. The most abundant VOCs detected were the aliphatic compounds with eight-carbon atoms, 1-octen-3-ol (69.43%) and 3-octanone (10.34%). This preliminarily study reveals that the detected VOCs could also be used as biomarker candidates for basal stem rot disease detection. However, further study is required.

Keywords: basal stem rot, *Ganoderma*, oil palm, 1-octen-3-ol, 3-octanone

Abstrak

Sebatian-sebatian organik meruap (VOCs) biasanya dibebaskan semasa interaksi tumbuhan-patogen kesan kepatogenan atau tindak balas pertahanan tumbuhan. Penyakit reput pangkal batang (BSR) yang disebabkan oleh kulat *Ganoderma boninense* masih lagi menjadi ancaman serius terhadap penanaman sawit. Kajian ini dijalankan bertujuan menyelidik kebolehlaksanaan pengesanan VOC yang terbebas dari tisu kayu kelapa sawit yang dijangkiti oleh *G. boninense* menggunakan kaedah mikroekstraksi fasa pepejal-ruang tutupan (HS-SPME) yang digabung bersama kromatografi gas-spektrometri jisim (GC-MS). Beberapa faktor yang mempengaruhi keberkesanan pengambilan HS-SPME telah dikaji; 0.25 g sampel yang dihomogenkan dalam nitrogen cair terbukti sebagai prosedur penyediaan yang paling sesuai, sementara gentian divinilbenzena/karboksena/polidimetilsikloheksana (DVB/Car/PDMS) dibuktikan sebagai fasa pengekstrakan yang optimum. Kaedah yang optimum ini mampu mengekstrak VOCs dengan kebolehulangan yang tinggi dengan profil VOCs yang seimbang dari pelbagai kelas kimia. VOCs yang paling banyak dikesan adalah sebatian-sebatian alifatik dengan atom-atom lapan-karbon, 1-okten-3-ol (69.43%) dan 3-oktanon (10.34%). Kajian awal ini mendedahkan bahawa VOCs yang dikesan juga berpotensi digunakan sebagai penanda biologi bagi pengesanan penyakit reput pangkal batang. Walau bagaimanapun, kajian lanjutan adalah diperlukan.

Kata kunci: reput pangkal batang, *Ganoderma*, sawit, 1-okten-3-ol, 3-oktanon

MJAS Vol 24 No 4 (2020)

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is one of the major economic crops feeding the world. In 2018, the main palm oil producing countries, Indonesia and Malaysia, contributed 85% of the palm oil supply worldwide, accounted for 34% of the global vegetable oils consumption [1]. Oil palm as a monoculture plantation crop is exposed to serious threats by pests and diseases. In Southeast Asia, the basal stem rot (BSR) disease due to *Ganoderma boninense* is a major disease of oil palm plantation. BSR infection starts with the penetration of mycelia into oil palm roots and slowly spread to the stem bole [2]. The BSR infection can only be recognised at a very late stage with severe foliar chlorosis and breakage at older fronds, presence of decayed tissues at palm base and production of fruiting bodies [3]. BSR disease has caused severe economic losses due to reduction in oil yield, number of stands, and replanting cycle of new palms [4]. Therefore, rapid and accurate detection of *Ganoderma* fungus at the early stage of infections is desired to assist more efficient management practices for the reduction of disease spread.

Volatile organic compounds (VOCs) have gained attention for their application in both plant disease detection and discrimination. VOCs analysis for recognition of infected plant materials has been achieved on several economically important crops species such as apple and pear plants [5], grapevine plants [6], strawberries [7] and also potato tubers [8-10]. VOCs are carbon compounds with low molecular weight and high vapour pressure, making them easily evaporate at room temperature [11].

Detection of BSR disease in oil palm has been previously reported through the use of an electronic nose; but the VOCs identity has not yet been examined [12]. The determination of VOCs is claimed to be crucial in the development of electronic sensor systems, as shown in the studies of soft rot diseases [13, 14]. Therefore, the profiling of VOCs from oil palm infected with *G. boninense* is necessary to produce more effective electronic noses for BSR disease detection so that the disease spread can be reduced and suitable control management can be carried out. Sampling of VOCs is challenging as the VOCs profiles are highly dependent on the extraction method chosen, particularly when high temperature application is involved, pyrolysis and thermal rearrangement of VOCs may take place [15]. Because of that, solid phase microextraction (SPME) has been the most favourable method in the analysis of VOCs due to its simple preparation technique, particularly the ability to pre-concentrate analytes in the absence of solvent, and rapid detection. SPME is generally employed together with headspace analysis of VOCs because it is the most useful method to obtain a true volatile species at a given time. Thus, headspace-SPME (HS-SPME) are often demonstrated as the method of choice for VOCs detection from fungi and plants [6-8, 15-18]. However, optimisation of HS-SPME is required to ensure its extraction efficiency, which includes the sample preparation method, the sample weight and the type of SPME fibre.

The present work demonstrates VOCs released from *in vitro* infection of *Ganoderma* on oil palm wood tissues that were extracted through HS-SPME and profiled by gas chromatography-mass spectrometry (GC-MS). The main objective was to conduct preliminary assessment on the sampling method to obtain VOCs profile from the oil palm-*Ganoderma* infected wood tissues.

Materials and Methods

Preparation of oil palm-Ganoderma infected samples

Ganoderma boninense strain PER71 was obtained from the internal culture collection of Malaysian Palm Oil Board (MPOB). *G. boninense* (PER71) was grown on a sterilised cellophane membrane on top of potato dextrose agar (PDA) of pH 5.5 in 145 mm petri plate and incubated at 28±2 ºC for 7 days. Oil palm wood shavings were collected from areas in MPOB Kluang Research Station, Johor plantation with no *Ganoderma* incidence and autoclaved at 121 ºC, 103.4 kPa for 15 min. *In vitro* infection of oil palm wood was attained by placing the sterilised oil palm wood shavings on PDA with 7-day-old *G. boninense* and further incubated at 28±2 ºC for one week (Figure 1). For headspace analysis of VOCs, after 14 days of incubation, samples of oil palm-*Ganoderma* infected were collected and subjected to two types of sample preparation methods. The first sample preparation method was without any mechanical force, while the other preparation method was with mechanical force application where oil palm-*Ganoderma* infected sample was crushed into fine powder in the presence of liquid nitrogen. Each sample type was distributed into 0.25 g, 0.50 g and 1.00 g in 20 ml headspace vials. Headspace vials were sealed with PTFE/silicone-coated septa and aluminium crimp cap. Blank headspace vials were set up as controls. All samples were stored at -80 ºC until required.

Extraction of fungal VOCs

An SPME manual holder and four types of fibres *i.e.* 100 µm polydimethylsiloxane (PDMS), 85 µm carboxen/polydimethylsiloxane (CAR/PDMS), 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 50/30 µm divinylbenzene/carboxen/polydimethyl siloxane (DVB/Car/PDMS) were purchased from Supelco (Bellefonte, PA, USA). All the SPME fibres were conditioned according to manufacturer's specification before VOCs sampling. SPME fibres were directly thermally desorbed in a GC inlet heated to 250 ºC for 30 min to eliminate contaminants. For analysis of headspace VOCs, *in vitro* oil palm-*Ganoderma* infected sample was preincubated for 30 min at 40 ºC before being exposed to SPME fibres (Figure 2). After 15 min of exposure, the SPME fibre was immediately desorbed into GC heated injection port, heated to 250 ºC in splitless mode. HS-SPME analyses were repeated four times.

GC-MS analysis

GC analysis was performed on an Agilent Technologies (CA, USA) GC 7890A, equipped with an inert MS Triple-Axis detector model 5975C. VOCs were separated on a fused-silica bonded-phase capillary column Zebron^{TM} ZB-5HT Inferno, 30 m in length by 0.25 mm in internal diameter, with 0.25 µm of film thickness (5% phenyl 95% dimethylpolysiloxane). Helium was used as the carrier gas and the flow rate was 1 mL/min. The following three-step heating oven programme was applied: (1) 45 ºC for 1 minute; (2) 10 ºC min^{-1} temperature increased to 260 ºC; (3) 260 ºC for 30 minutes. The transfer line temperature was 280 ºC. A mass range from 20–400 m/z was scanned in full scan mode at 70 eV ionisation energy. The chromatograms were analysed using Agilent Enhanced MSD ChemStation (Version E.02.00.493) and tentative identification of VOCs was performed by comparing their mass spectra and retention indices with the NIST data library.

Statistical analysis

Raw data generated from GC-MS analyses were extracted and imported into Microsoft Excel to create a data matrix. The differences between VOCs concentrations were examined using analysis of variance (ANOVA) at significant level of $p < 0.05$ (IBM SPSS Statistics version 20.0).

MJAS Vol 24 No 4 (2020)

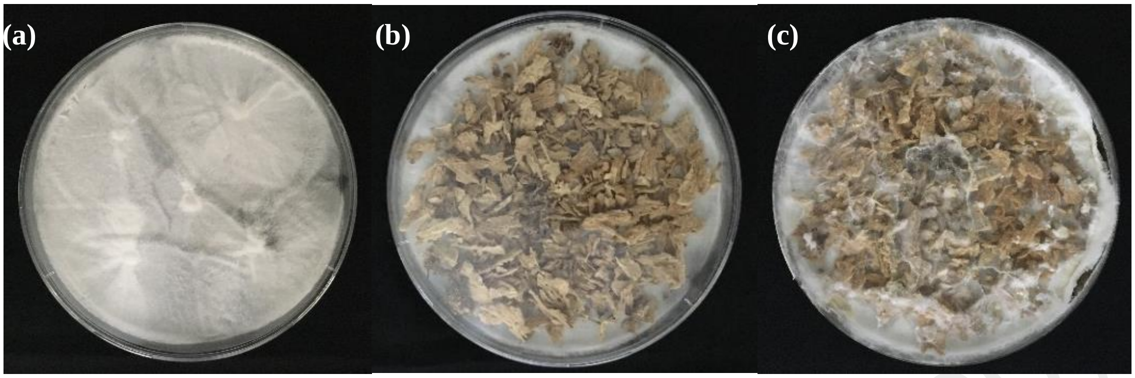


Figure 1. Preparation of artificial infection of oil palm wood tissues by *Ganoderma boninense*. Pure culture of *G. boninense* after 7 days of incubation at 28±2 ºC (a), sterilised oil palm wood on top of fully grown *G. boninense* (b), and oil palm wood colonised by *G. boninense* after 14 days (c)

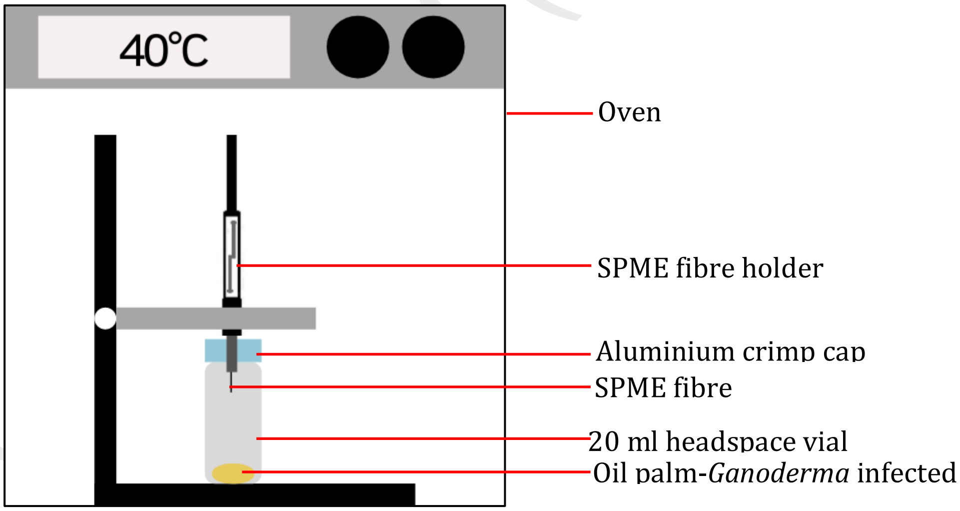


Figure 2. Schematic diagram of experimental setup for headspace VOCs extraction from oil palm-*Ganoderma* infected tissues using SPME in an oven at 40 ºC.

MJAS (2023)

Results and Discussion

Optimisation of HS-SPME sampling protocol

The performance of a given SPME application was highly dependent on the selected extraction phase which determined the selectivity and the reliability of the method. However, analysis of VOCs using SPME in biological studies should also examine sample stability and integrity to obtain a consistent profile because of their dynamic nature due to enzymatic and non-enzymatic reactions. Therefore, preliminary studies were conducted to analyse the experimental conditions that may influence the VOCs profiles generated.

Sample preparation method

The first factor investigated was the method of sample preparation, either with or without the application of mechanical force with the use of liquid nitrogen. Results showed that higher and well-balanced GC profile with wider range of chemical classes were obtained when biological tissues were disrupted at ultra-low temperature. In addition, the chromatograms of all replicates demonstrated consistent sequence of VOCs, which were hexanal, benzaldehyde, 3-octanone, 2-pentylfuran, octanal, (E)-2-octenal, 1-octanol, nonanal, naphthalene, dodecane, tetradecane and α-cardinene, eluted from the column (Figure 3). Constant chromatogram profiles indicated the sample was stable, and therefore, was used for further analyses.

This study reveals oil palm-*Ganoderma* infected samples crushed in the presence of liquid nitrogen were more stable and produced wider range of GC profile with various chemical classes compared to samples that were not given any pre-treatment. Similar observations were reported in the development of HS-SPME-GCMS protocol for herbivore-induced volatiles in *Myrcia splendens* [19]. This is due to the use of liquid nitrogen that has allowed tissue disruption to take place at very low temperature, minimising the loss of highly volatile compounds, and thus, providing higher exposure of biological cells that gives higher volatile emission. More importantly, processing samples at ultra-low temperature also limited the activities of the enzymes by quenching, subsequently reducing changes in compound composition due to internal and external environmental effects.

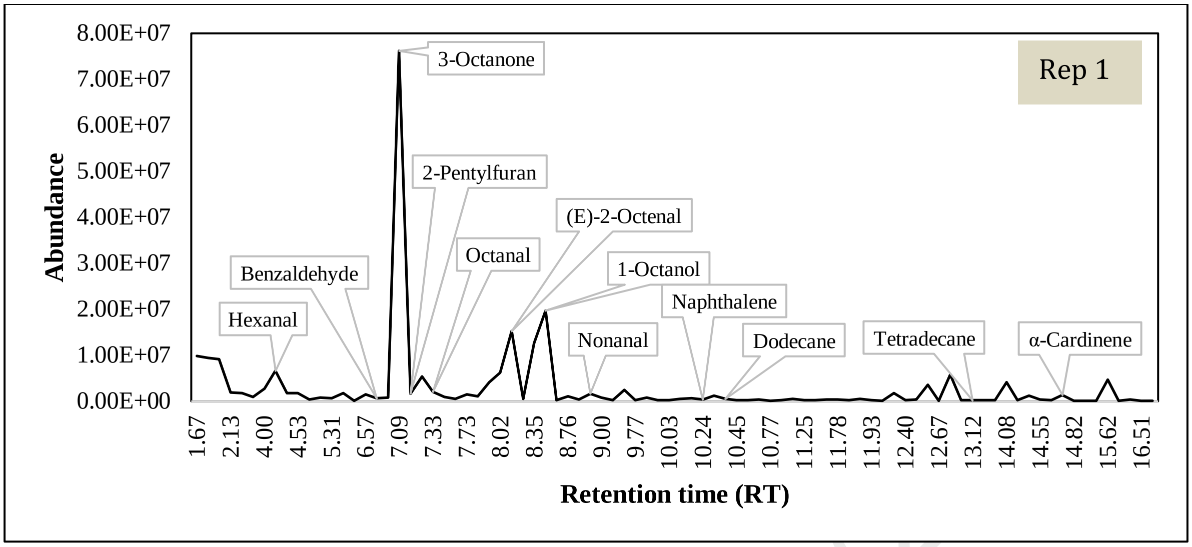
Sample weight

Sixteen commonly reported VOCs from fungi, i.e. hexanal, benzaldehyde, 1-octen-3-ol, 2-pentylfuran, (E)-2-octenal, 1-octanol, undecane, nonanal, naphthalene, dodecane, tridecane, butyl butanoate, tetradecane, 2,4-di-tert-butylphenol, α-cardinene and β-cardinene were chosen to perform HS-SPME optimisation using different sample weight [15, 17, 19-27]. VOCs were selected based on the highest total average peak area of the gas chromatogram with minimal variation between samples. The effect of sample weight is presented in Figure 4, where results were the average response of quadruplicate of extractions and normalised to the response obtained for 0.25 g.

The concentration or amount of sample injected into GC was generally increased to obtain better signal, thereby increasing the signal strength. However, in headspace analysis, increasing sample weight did not necessarily resulted in increased peak areas as greater sample weight caused reduction in the actual headspace volume [28, 29].

The oil palm-*Ganoderma* infected tissues released various compounds with different chemical and physical properties which explained the different responses observed across the headspace volumes tested (Figure 4). This work demonstrates the small sample weight that implied higher headspace volume in 20 mL headspace vial, making 0.25 g the most appropriate weight since the signal intensities observed was sufficient to achieve the satisfactory peak areas for the diverse chemical natures of the selected compounds. Thus, 0.25 g was chosen as the sample weight for subsequent experiments.

MJAS Vol 24 No 4 (2020)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3.00E+07
2.00E+07
1.00E+07
0.00E+00
3-Octanone
2-Pentylfuran
(E)-2-Octenal
Octanal
1-Octanol
Benzaldehyde
Naphthalene
Hexanal
Nonanal
Dodecane
Tetradecane
α-Cardinene
1.67
2.13
4.00
4.53
5.31
6.57
7.09
7.33
7.73
8.02
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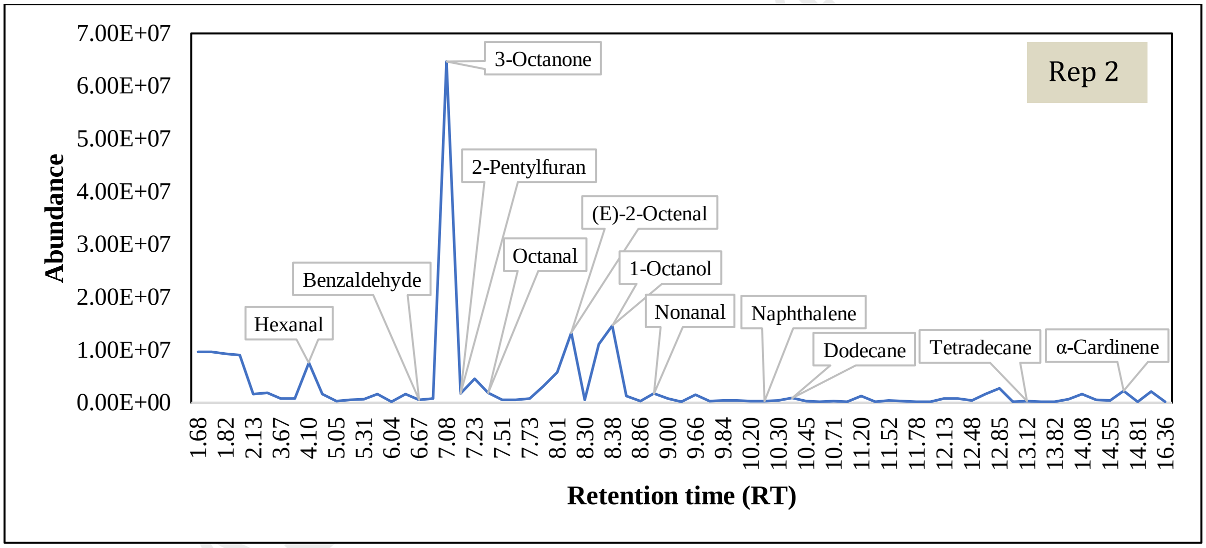
Retention time (RT)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2-Pentylfuran
(E)-2-Octenal
Octanal
1-Octanol
Benzaldehyde
Hexanal
Nonanal
Naphthalene
Dodecane
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Retention time (RT)

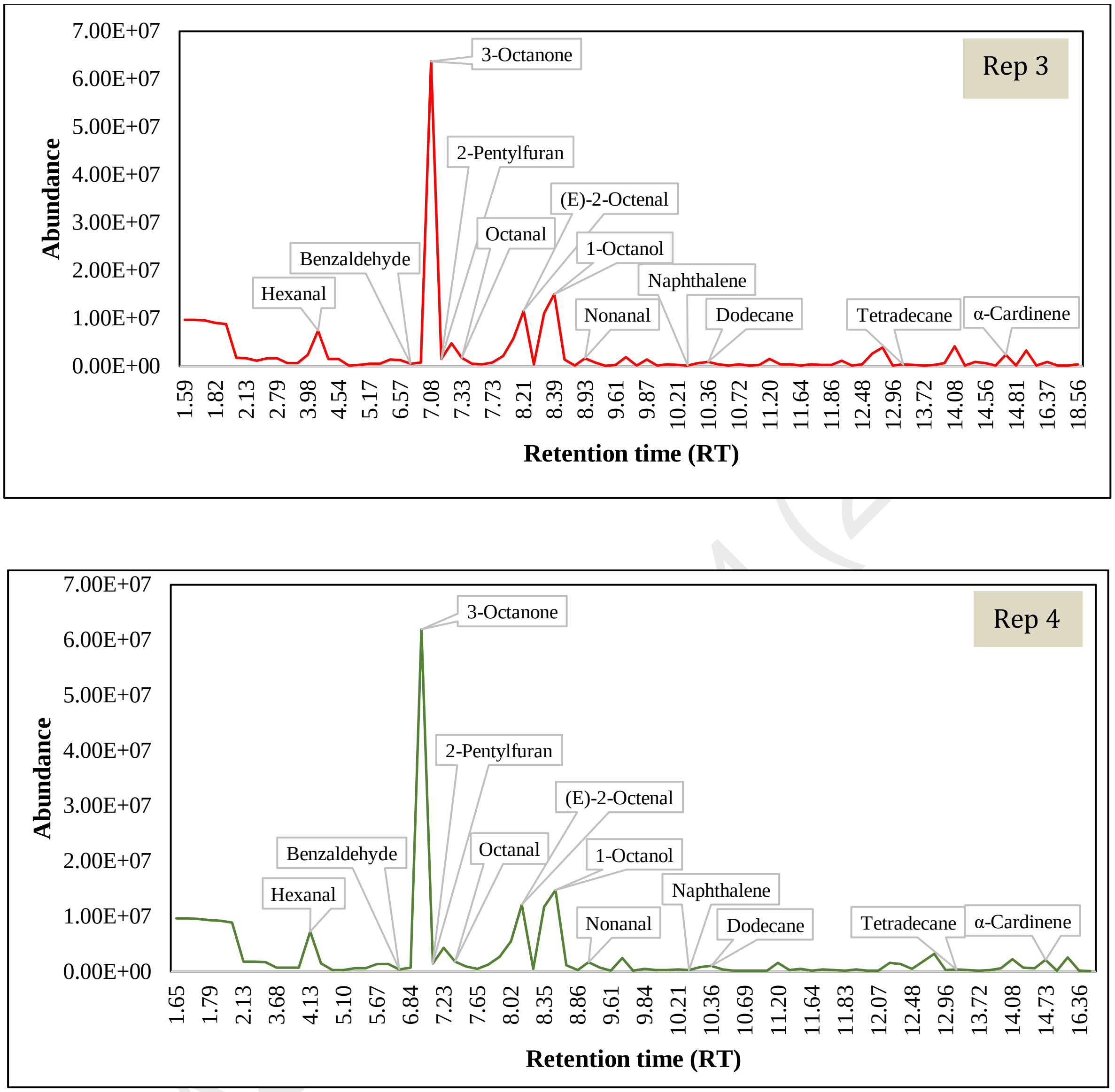


Figure 3. Consistent total ion current chromatograms across HS-SPME extraction replicates using 100 µm PDMS extraction phase from 0.25 g of oil palm-*Ganoderma* infected tissues homogenised to fine powder using liquid nitrogen

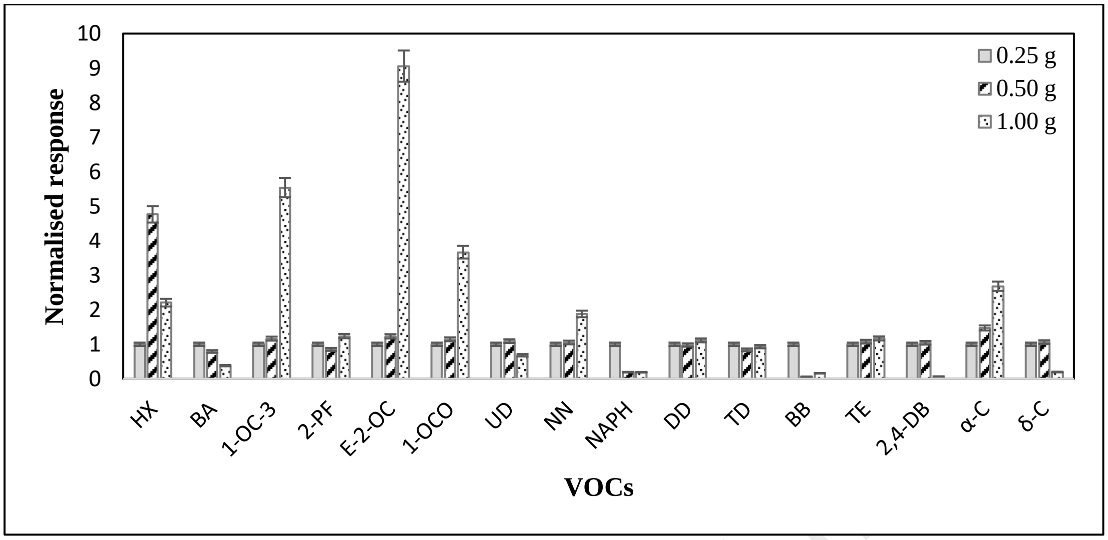


Figure 4. Effect of sample weight for selected compounds (HX = hexanal; BA = benzaldehyde; 1-OC-3 = 1-octen-3-ol; 2-FP = 2-pentylfuran; E-2-OC = (E)-2-octenal; 1-OCO = 1-octanol; UD = undecane; NN = nonanal; NAPH = naphthalene; DD = dodecane; TD = tridecane; BB = butyl butanoate; TE = tetradecane; 2,4-DB = 2,4-di-tert-butylphenol; α-C = α-cardinene and β-C = β-cardinene) obtained by HS-SPME-GCMS analysis of oil palm-*Ganoderma* infected tissues using 100 µm PDMS extraction phase. Response shown as normalised to response obtained for 0.25 g. Error bars represent standard deviation for four replicate ran of each weight.

MJAS Vol 24 No 4

Selection of SPME fibre extraction phase

Figure 5 summarises the average normalised areas of the total selected compounds obtained from different SPME fibre extraction phases. Higher absorption was generally observed in combined extraction phases in comparison to PDMS. ANOVA test showed significant differences between the SPME fibre extraction phases tested. No significant difference was observed between the responses obtained between CAR/PDMS and PDMS/DVB ($p > 0.05$). The average normalised areas of total selected compounds of CAR/PDMS and PDMS/DVB were almost six times higher than PDMS, while extraction using DVB/Car/PDMS had shown nearly seven times higher than PDMS. Therefore, the composite DVB/Car/PDMS extraction phase exhibited superior extraction efficiency compared to other fibre extraction phases tested (Figure 5).

Fibre coating selection was the most important factor in any development of SPME method. Assorted kinds of SPME fibres were introduced commercially with different extraction phase polarity and thickness to favour the adsorption of analytes for various classes and molecular weights during extraction. Unlike absorbents, in the solid coating, analytes were extracted via adsorption where analytes moved into the pores of the absorbent and got physically trapped or chemically bonded with the surface of the absorbent. Being a porous material, it is important to note on the degree of pore distribution and pore size since their ability to retain an analyte was dependent on the pore size diameter [30]. For instance, carboxen was generally developed for the adsorption of analytes with a molecular weight of less than 90 m/z due to its micropores with an opening diameter of 2 to 20 Å, making it less efficient for adsorption and desorption of large molecular weight analytes. In comparison, DVB was designed for the entrapment of larger analytes with high degree of mesopores (20 to 500 Å) and therefore, did not suitable for low molecular weight analytes extraction. Thus, the

MJAS Vol 24 No 4 (2020)

combination of both adsorbents' properties coating in DVB/Car/PDMS has enabled excellent fibre coating for wide range molecular weight analytes during a single extraction [31].

Figure 5 presents the average normalised areas of total selected compounds of CAR/PDMS and PDMS/DVB that is almost six times higher than PDMS. In contrast, composite coating DVB/Car/PDMS exhibited superior extraction efficiency compared to other fibre coating extraction phase. This difference might be due to monoterpenes being compounds with molecular weights closed to the upper limit of the carboxen adsorbent, and thus were extracted by the fibre containing carboxen and/or DVB coating. Compounds such as 2-pentylfuran, naphthalene, α-terpinol, N,N-dibutylformamide, chloroxylenol, and dibutylhydroxytoluene were only detected if DVB-containing fibre was used. In DVB/Car/PDMS fibre, the layers were assembled with carboxen layer located on the inside and DVB layer on the outside of the coating, which means that molecules of both high and low molecular weight might be successfully adsorbed as DVB layer was efficient for large molecules but less efficient in the entrapment of smaller molecules [31]. The small molecules could then migrate to the inner part of the coating and be fully adsorbed by the carboxen layer [30]. Similar studies on fibre extraction phase selection in the development of SPME methods have demonstrated DVB/Car/PDMS as the most efficient extraction fibre [19, 32]. Based on these results, DVB/Car/PDMS was selected as the most efficient fibre extraction phase to extract VOCs from the oil palm-*Ganoderma* infected tissues.

Tentative VOCs profile

The optimised HS-SPME-GCMS condition was employed for the analysis of VOCs released from *in vitro* oil palm-*Ganoderma* infected tissues. The composition of VOCs released was expressed in percentage of the total ion count (TIC) and presented in Table 1. A total of 39 compounds were found in the headspace of oil palm-*Ganoderma* infected tissues. They belong to alcohols, alkanes, volatile acids, ketones, aldehydes, esters, sesquiterpenes and polycyclic aromatic hydrocarbon groups. Figure 6 shows the molecular structures of the compounds.

Qualitatively, the largest absorption of VOCs was with DVB/Car/PDMS fibre: chromatograms demonstrated that VOCs with low molecular mass were eluted first with short retention times, followed by long chain alcohols, aldehydes and ketones, then long chain hydrocarbons and acids, then terpenes and sesquiterpenes, and finally organic esters at long retention times (Table 1).

Some of the VOCs found in the oil palm-*Ganoderma* infected tissues were the aliphatic C_8-complex that were found ubiquitous in truffle species and fungi, i.e. 1-octen-3-ol, 3-octanone and 1-octanol. They had been described as the key contributors to mushroom flavour and fungal aroma giving the scent or the odour ranging from sweet, fruity, musty, lavender, detergent and soap to orange like [23]. The most abundant VOC detected, 1-octen-3-ol (sometimes referred to as "mushroom alcohol"), was reported to emit by decomposers and wood-rotting fungi, and the alcohol biosynthesis from 10-hydroperoxide isomer of linoleic acid was well discussed in several literature [15, 23, 33-35]. The detection of volatile compound 1-octen-3-ol in this study is expected considering *G. boninense* is a basidiomycete and a white rot fungi.

Similar findings on the detection of hexanal is shown in the study of VOCs emitted from wood-rotting fungi *Serpula lacrymans* on the shavings of *Pinus sylvestris* (pine) timber [15]. The aromatic compound benzaldehyde was released in the study of volatile constituents of wood-rotting basidiomycetes *Piptoporus betulinus*, while in *Fomitopsis pinicola* and *Trametes suaveolens*, another aromatic compound, 2-pentylfuran, was also detected [20]. A preliminary head-space gas chromatographic analysis of volatile flavour compounds released from *Ganoderma sinense* (a medicinal mushroom) emitted octanoic acid and 2,4-di-tert-butylphenol in the mushroom broth while in the mycelium, only the latter compound was found [25, 26]. Among the VOCs detected in this study was the two-ring polycyclic aromatic hydrocarbon naphthalene which is known as effective insect repellent. Endophytic fungus, *Muscodor vitigenus* is also claimed to produce naphthalene almost exclusively [36]. Naphthalene derivatives, α-cadinene and δ-cadinene, are released in the *in vitro* oil palm-*Ganoderma* infected tissues. The same volatile compounds are released when *Myrcia splendens* is attacked by gall-inducing insects, as a signal of plant-insect interaction [19]. Therefore, the same VOCs released in this study could be due to the metabolic changes that take place in oil palm (host plant) when it is under attack by *G. boninense* (plant pathogen).

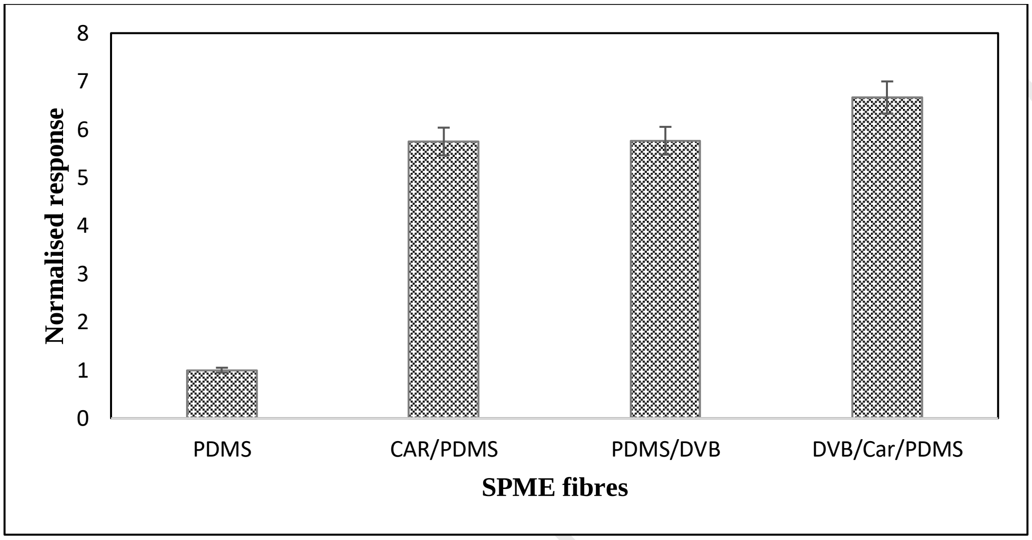


Figure 5. Effect of SPME fibre extraction phases (PDMS = polydimethylsiloxane; CAR/PDMS carboxen/polydimethylsiloxane; PDMS/DVB = polydimethylsiloxane/divinylbenzene and DVB/Car/PDMS = divinylbenzene/carboxen/polydimethylsiloxane) on extraction efficiency of headspace VOCs released from oil palm-*Ganoderma* infected tissues. Average areas of compounds obtained were normalised to response obtained for PDMS. Error bars represent standard deviation of four replicate ran for each SPME fibre.

Table 1. Overview of tentative VOCS emitted by oil palm-*Ganoderma* infected tissues using the optimised HS-SPME-GCMS protocol

#	RT*	Compound Name	Molecular Formula	CAS	m/z	Percentage of Total Ion Current‡
1	3.72	Toluene	C_7H_8	108-88-3	92	0.580
2	4.12	Hexanal	$C_6H_{12}O$	66-25-1	100	7.162
3	5.17	m-Xylene	C_8H_{10}	108-38-3	106	0.321
4	5.30	p-Xylene	C_8H_{10}	106-42-3	106	0.362
5	5.84	Methyl (Z)-N-hydroxybenzene-carboximidate	$C_8H_9NO_2$	1000222-86-6	151	1.006
6	6.68	Benzaldehyde	C_7H_6O	100-52-7	106	0.181
7	6.97	1-Octen-3-ol	$C_8H_{16}O$	3391-86-4	128	69.43
8	7.08	3-Octanone	$C_8H_{16}O$	106-68-3	128	10.34
9	7.16	2-Pentylfuran	$C_9H_{14}O$	3777-69-3	138	0.270

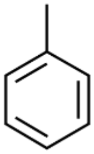
MJAS Vol 24

Table 1 (cont'd). Overview of tentative VOCS emitted by oil palm-*Ganoderma* infected tissues using the optimised HS-SPME-GCMS protocol

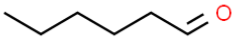
#	**RT**[*]	**Compound Name**	**Molecular Formula**	**CAS**	**m/z**	**Percentage of Total Ion Current**[‡]
10	7.33	Octanal	$C_8H_{16}O$	124-13-0	128	0.554
11	7.65	4-Methyldecane	$C_{11}H_{24}$	2847-72-5	156	0.148
12	7.83	1,3-Hexadiene, 3-ethyl-2-methyl	C_9H_{16}	61142-36-7	124	0.770
13	8.21	(E)-2-Octenal	$C_8H_{14}O$	2548-87-0	126	2.100
14	8.30	Eicosane	$C_{20}H_{42}$	112-95-8	282	0.047
15	8.35	2-Octen-1-ol	$C_8H_{16}O$	22104-78-5	128	1.457
16	8.39	1-Octanol	$C_8H_{18}O$	111-87-5	130	2.520
17	8.76	4-Octanyl butyrate	$C_{12}H_{24}O_2$	20286-46-8	200	0.231
18	8.86	Undecane	$C_{11}H_{24}$	1120-21-4	156	0.042
19	8.93	Nonanal	$C_9H_{18}O$	124-19-6	142	0.293
20	9.77	(E)-2-Nonenal	$C_9H_{16}O$	18829-56-6	140	0.033
21	9.85	Octanoic acid	$C_8H_{16}O$	124-07-2	144	0.136
22	10.20	1-(2-Butoxyethoxy)ethanol	$C_8H_{18}O$	54446-78-5	162	0.063
23	10.24	Naphthalene	$C_{10}H_8$	91-20-3	128	0.038
24	10.30	α-Terpineol	$C_{10}H_{18}$	98-55-5	154	0.100
25	10.36	Dodecane	$C_{12}H_{26}$	112-40-3	170	0.113
26	11.20	1,3-Ditert-butylbenzene	$C_{14}H_{22}$	1014-60-4	190	0.151
27	11.25	UNK[§] *m/z* 314	-	-	314	0.064
28	11.52	7-Hexylicosane	$C_{26}H_{54}$	55333-99-8	366	0.047
29	11.71	2-Undecanone	$C_{11}H_{22}$	112-12-9	170	0.070
30	11.86	N,N-dibutylformamide	$C_9H_{19}NO$	761-65-9	157	0.050
31	12.85	Butyl butanoate	$C_8H_{16}O_2$	109-21-7	144	0.460
32	12.95	Chloroxylenol	C_8H_9ClO	88-04-0	156	0.046
33	13.12	Tetradecane	$C_{14}H_{30}$	629-59-4	198	0.039
34	14.55	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	96-76-4	206	0.079
35	14.63	Dibutylhydroxytoluene	$C_{15}H_{24}O$	128-37-0	220	0.013
36	14.73	α-Cardinene	$C_{15}H_{24}$	483-75-0	204	0.239
37	14.81	δ-Cardinene	$C_{15}H_{24}$	483-76-1	204	0.032
38	15.62	UNK[§] *m/z* 286	-	-	286	0.395
39	15.63	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	$C_{16}H_{30}O_4$	74381-40-1	286	0.020

[*]RT, retention time; [‡]The percentages were calculated using the peak area for each VOC relative to the total average normalised areas of the total ion current recorded; [§]UNK, unknown.

MJAS Vol 24 No 4 (2020)



Toluene



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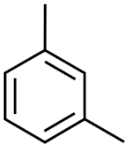
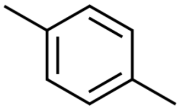


m-Xylene



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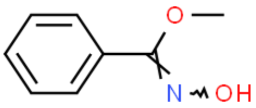
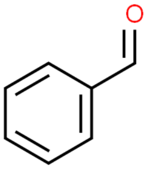
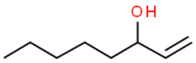


Methyl (Z)-N-hydroxybenzene-carboximidate

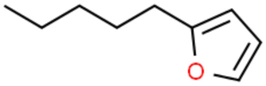


Benzaldehyde



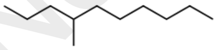
1-Octen-3-ol

3-Octanone

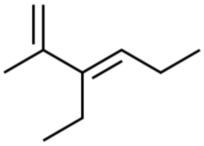


2-Pentylfuran

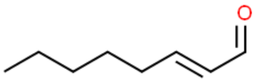
Octanal



4-Methyldecane

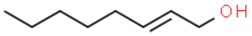


1,3-Hexadiene, 3-ethyl-2-methyl



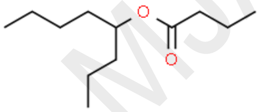
(E)-2-Octenal

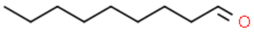
Eicosane

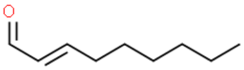


2-Octen-1-ol

1-Octanol







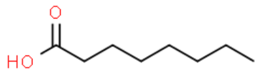
MJAS Vol 24 No 4 (2020)

4-Octanyl butyrate

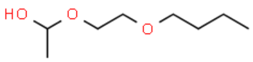
Undecane

Nonanal

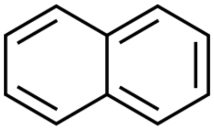
(E)-2-Nonenal



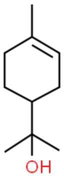
Octanoic acid



1-(2-Butoxyethoxy)ethanol

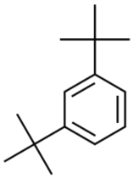


Naphthalene

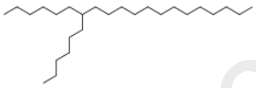


α-Terpineol

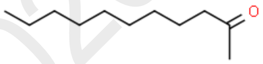
Dodecane



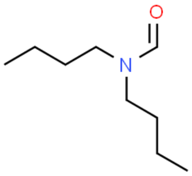
1,3-Ditert-butylbenzene



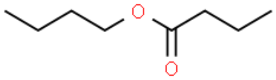
7-Hexylicosane



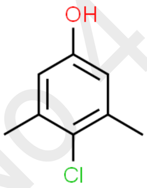
2-Undecanone



N,N-dibutylformamide

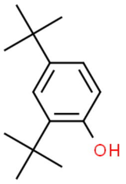


Butyl butanoate

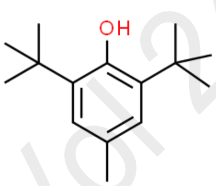


Chloroxylenol

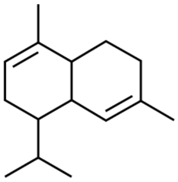
Tetradecane



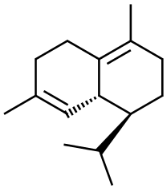
2,4-Di-tert-butylphenol



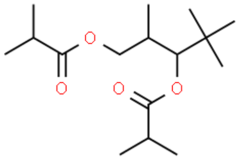
Dibutylhydroxytoluene



α-Cardinene



δ-Cardinene



Propanoic acid, 2-methyl-,
1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester

Figure 6. Molecular structures of tentative VOCs emitted

MJAS Vol 24 No 4 (2020)

MJAS Vol 24 No 4 (2020)

Conclusion

The results showed the initial feasibility application of HS-SPME extraction method for VOCs profiling of *in vitro* oil palm-*Ganoderma* infected tissues. The HS-SPME-GCMS protocol developed here is capable of detecting a diverse volatile chemical profile with high reproducibility. Future research should focus on the VOC profiles of *G. boninense* culture and oil palm wood to complement the current study for the VOCs to be developed as biomarker candidates for BSR disease detection. Coupled with the available electronic tools such as handheld electronic noses or any portable air monitoring systems, it could raise the possibility for the on-site detection of BSR disease.

Acknowledgement

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MJAS Vol 24 No 4 (2020)

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MJAS Vol 24 No 4 (2020)