

1 Volatile Organic Compounds (VOC) Produced by *Paraconiothyrium* 2 *archidendri* F10 as Biofungicidal Materials for *Ganoderma boninense*

3 Anisa Lutfia^{1*}, and Bedah Rupaedah¹

4 Abstract

5 *Ganoderma boninense* Pat. is a persistent soil-borne pathogen that causes significant losses in
6 oil palm (*Elaeis guineensis* Pat.) productivity. An effective control strategy that can be
7 employed involves the use of biological control agents (BCAs), particularly fungi which
8 produced antifungal metabolites. In this study, a soil fungus isolated from a healthy, disease-
9 free oil palm plantation was evaluated for its inhibitory activity *in vitro*, with the aim of
10 assessing its effectiveness as a bioinoculant for future field control applications. The soil fungus
11 was sequenced for the ITS-rDNA region, and its similarity was analyzed through bioinformatics
12 using BLASTn searches and phylogenetic tree construction. Volatile organic compounds
13 (VOCs) were produced through batch fermentation on Potato Dextrose Agar (PDA), extracted,
14 and concentrated. The inhibitory activity against the radial growth of *G. boninense* was
15 evaluated using the vapour assay method. The VOC profile and other metabolites were analyzed
16 using GC-MS. The inhibitory mechanism between VOCs and target proteins was studied
17 through *in silico* analysis. VOCs produced by *P. archidendri* F10 were found to inhibit *G.*
18 *boninense* mycelium growth by up to 55.8% in four days, with the mycelium exhibiting wavy,
19 non-smooth, and wrinkled morphology, abnormal branching, fused, defective hyphae, and lysis
20 through microscopy imaging. The major VOC components were esters, with 7,9-ditert-butyl-
21 1-oxaspiro[4.5]deca-6,9-diene-2,8-dione being the most abundant (16.72%). The other top-
22 ranking components were 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate (8.71%), methyl
23 heptadecanoate (8.66%), and butyl acetate (5.66%), with minor components comprising less
24 than 5% of the total VOCs. The molecular docking analysis revealed that among the tested
25 ligands, 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione had the strongest binding
26 affinity at -8.5 kcal/mol, forming one hydrogen bond with Tyr646 at a distance of 2.98 Å.
27 Another notable ligand was 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, with a binding affinity
28 of -5.6 kcal/mol and one hydrogen bond with His698 at 3.05 Å. The remaining ligands showed
29 lower binding affinities and did not form hydrogen bonds. Our findings suggest that *P.*
30 *archidendri* F10 has potential as a biofungicide for controlling *G. boninense* in the future.

¹ Research Center for Applied Microbiology, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor
Km. 46, Cibinong, Bogor, West Java 16911, Indonesia.

*Corresponding author; e-mail: anis031@brin.go.id

1 **Key words:** 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, GC-MS, SEM, vapour
2 assay, VOC.

3
4 **INTRODUCTION**

5 The pathogenic fungus, *Ganoderma boninense* Pat., is a significant issue in industrial plants,
6 leading to basal stem rot disease and a decrease in oil palm production. *Ganoderma boninense*
7 Pat. is a soil-borne fungal pathogen that causes basal stem rot (BSR) disease in oil palm
8 (Paterson, 2019). The pathogen is difficult to control, and the use of synthetic fungicides is not
9 a sustainable solution, given their adverse impact on the environment and public health.
10 Synthetic fungicides, such as dazomet and hexacanazole, offer a temporary solution for
11 controlling *G. boninense* (Maluin *et al.*, 2020). However, the prolonged use of synthetic
12 antifungal agents can lead to the antifungal resistance, death of non-target microorganisms, and
13 degradation of ecosystem function (Fang *et al.*, 2018). One approach that has gained attention
14 in recent years is biological control, which involves the use of microbial biocontrol agents
15 (BCAs) to control plant diseases. Fungi have been shown to be effective biocontrol agents
16 against a wide range of phytopathogens due to their diverse mechanisms, including antibiosis,
17 host resistance induction, mycoparasitism, and niche competition for nutrients and space (Latz
18 *et al.*, 2018). One promising area of research in fungal BCAs is volatile organic compounds
19 (VOCs) that exhibit strong inhibitory activity against phytopathogenic microbes. These
20 compounds are defined as small, carbon-based molecules that have a low water solubility and
21 a high vapour pressure, which allows them to be present in a gaseous state under normal
22 ambient conditions, such as at a pressure of 1 atm and a temperature of 25 °C. VOCs are a blend
23 of volatile metabolites produced by both microbial and plant sources, which is referred to as
24 "volatilome" (Farbo *et al.*, 2018). These compounds are distinguished by functional effects in
25 the soil, greater ability to disperse, and stronger antifungal properties. *Muscodor albus*
26 (Xylariaceae) was the first commercially and successful fungus being a BCA, known for its
27 bioactive volatilome. This endophytic fungus, found in *Cinnamomum zeylanicum*, produces a
28 range of volatiles, including acids, alcohols, esters, and terpenoids that exhibit antimicrobial
29 activity against post-harvest pathogens responsible for the decay of perennial fruit trees (Saxena
30 and Strobel, 2021). In more recent studies, some fungal species have been investigated and
31 reported to produce antifungal VOCs such as *Aureobasidium pullulans* (Sarcotheciaceae) (Don
32 *et al.*, 2021), *Lasiodiplodia avicenniae* (Botryosphaeriaceae) (Hartanto *et al.*, 2023),
33 *Sarocladium brachiariae* (Sarocladiaceae) (Yang *et al.*, 2021), *Trichoderma atroviride*
34 (Hypocreaceae) (Rao *et al.*, 2022), and *Trichoderma koningiopsis* (Kong *et al.*, 2022). In this

1 study, the development of effective BCAs for controlling *G. boninense* was investigated in soil
2 inhabitants of healthy and non-infected oil palm plantations. The aim of this study was to
3 evaluate the *in vitro* bioactivity of a soil fungus obtained from local oil palm plantations against
4 *G. boninense*. The results will contribute to the development of sustainable and eco-friendly
5 approaches to controlling BSR disease, ensuring the continued productivity of oil palm
6 cultivation while reducing the impact of synthetic biocides on the environment and public
7 health.

8

9 MATERIALS AND METHODS

10 Fungal Isolate and Molecular Identification

11 The soil fungus, *Paraconiothyrium* isolate F10, was isolated from a healthy and uninfected oil
12 palm plantation soils in Bogor, Indonesia. The pathogenic fungus, *G. boninense* strain SSU008
13 used in this study, is a collection of the Indonesian Oil Palm Research Institute (PPKS
14 Marihat), Simalugun Regency, North Sumatra, Indonesia. This study was conducted in April
15 2023 at the Laboratory of Microbiology, Research Centre for Applied Microbiology, National
16 Research and Innovation Agency (BRIN), Serpong, Indonesia. The isolates were maintained
17 in Potato Dextrose Agar (PDA) medium. Molecular identification was performed
18 commercially by sending the fungal specimen, isolate F10 to Macrogen, Inc. (Singapore). Raw
19 sequences was retrieved and checked for its similarity to online database using BLASTn for
20 ITS-rDNA region. A phylogenetic tree was constructed to assign the fungal species based on
21 clustering analysis among accessions using MEGAXI. The confirmed species was submitted
22 to GenBank and given with an accession code for *P. archidendri* F10 (OQ835627).

23

24 VOC Production in Submerged Fermentation

25 Potato dextrose broth (PDB) was used as a fermentation medium for VOCs production. The
26 fungus, *P. archidendri* F10 was grown in 50 mL of PDB in a 250-mL flask at 28 °C for 14 days.
27 The cell-free supernatant (CFS) was filtered using a Whatman filter paper No. 1 and centrifuged
28 at 10,000×g for 15 min. The resulting CFS was extracted thrice using a laboratory grade ethyl
29 acetate (EtOAc) in a ratio of 1 : 1 and shaken vigorously for 3 days. The EtOAc layer was
30 separated using a separator funnel and concentrated *in vacuo* using a Buchi Rotavapor® R-300.

31

32 Antifungal Vapour Assay

33 The antifungal activity of volatile organic compounds (VOCs) produced by *P. archidendri* F10
34 against *G. boninense* was assessed using a modified disc diffusion or vapour assay (Bismarck

1 *et al.*, 2019). An active-growing colony of *G. boninense* was placed in the center of PDA
2 medium, while a disc (Ø 6-mm) containing ethyl acetate (EtOAc) extract or saturated VOCs
3 was placed in the center of the lid of the agar plate. The plates were then incubated for five days
4 at 28°C while standing on their lids. A control plate was also prepared with only the colony of
5 *G. boninense* in the center. The assay was performed in triplicate. The percentage of radial
6 growth inhibition (%) was calculated as: (%) = [(D1 – D2)/ D1]×100%, where D1 is the radial
7 growth (mm) of the control plate and D2 is the radial growth of the treated plates. The surface
8 morphology of the treated colony or mycelium was examined using a scanning electron
9 microscope (JSM-6510LA JEOL SEM). The slide was fixed and coated with platinum (35 s:
10 30 mA) using 10 kV.

11 12 **GC-MS Profiling of VOC**

13 A qualitative analysis of the sample containing VOCs was conducted using an Agilent column
14 (Type 19091S-433: 93.92873 DB-5MS UI, 5% Phenyl Methyl Silox) with dimensions of 30 m
15 × 250 µm × 0.25 µm and a temperature range from 0 °C to 325 °C (with a final hold time of 1
16 min) for injection. The analytical instrument was using an Agilent-type 7890 (GC) and 5977A
17 (MSD).

18 19 **Molecular Docking of Antifungi as Anti-*Ganoderma boninense***

20 Major compounds as determined from the highest relative peak area were subjected to
21 molecular docking studies. Chemical structure of each compound was retrieved from an online
22 database. Protein target used in this study was chitin synthase as commonly involved in the cell
23 wall synthesis which serves as the factory of protective layer of *G. boninense*. The sequence of
24 protein target with an entry code: A0A5K1JXQ5 was retrieved from UniProt database
25 (<https://www.uniprot.org/>) and modelled utilizing the SWISS-MODEL web server
26 (<https://swissmodel.expasy.org/>).

27 28 **RESULTS AND DISCUSSION**

29 **Species Assignment of Isolate F10**

30 Molecular identification based on the ITS-rDNA sequence analysis and BLASTn results
31 showed that isolate F10 had the closest similarity (>99%) with *Paraconiothyrium archidendri*.
32 Further analysis through phylogenetic construction using a neighbor-joining tree showed that
33 isolate F10 was clustered together and had a similar resemblance with *P. archidendri* CBS
34 168.77 (Figure 1), a type fungus material isolated from its host plant, *Archidendron bigeminum*

1 (Fabaceae) in Myanmar (Verkley *et al.*, 2014). To our understanding, the discovery of *P.*
2 *archidendri* F10 may be regarded as a new report as a soil inhabitant, especially from healthy
3 oil palm plantation soils. Furthermore, it has been reported that certain related taxa belonging
4 to the genera *Paracamarosporium* and *Pseudopithomyces* have been found to act as leaf
5 endophytes, specifically in the petioles of *E. guineensis*. Notably, these taxa exhibit the ability
6 to produce exopolysaccharides under laboratory conditions (Yurnaliza *et al.*, 2021). It can be
7 implied that the presence of *Paraconiothyrium* members and other related taxa within the soils
8 may establish functional plant-microbe associations through a pathway that transitions from
9 saprotrophic to hemi-/biotrophic modes in the living tissue of oil palm. However, the specific
10 functional traits are yet to be fully uncovered. A more recent study had reported the occurrence
11 of *P. archidendri* as a saprophytic fungus in the plant litters of *Magnolia* sp. in China
12 (Tennakoon *et al.*, 2022). The presence of *P. archidendri* and other taxa within the
13 Didymosphaeriaceae family is expected to have a crucial impact on nutrient cycling in forest
14 ecosystems. This is because the majority of members within this family are cosmopolitan, and
15 most of them are known to function as saprobes (Zhang *et al.*, 2012). The ability of these fungi
16 to decompose organic matter, including plant litter, results in the release of crucial nutrients
17 back into the soil. This process, in turn, promotes the growth and development of new
18 vegetation, highlighting the critical role played by these fungi in the maintenance of healthy
19 and sustainable ecosystems (Wanasinghe and Mortimer, 2022). There is still limited research
20 on the role of *P. archidendri* as a biocontrol agent. However, other related species, like
21 *Paraconiothyrium brasiliense* LT161, have shown potential as biocontrol agents due to their
22 production of antifungal metabolites effective against various phytopathogens (Han *et al.*,
23 2012). Additionally, *Coniothyrium minitans*, reclassified as *Paraphaeosphaeria minitans*, has
24 demonstrated mycoparasitic activity against *Sclerotinia sclerotiorum* (Verkley *et al.*, 2014;
25 Patel *et al.*, 2021).

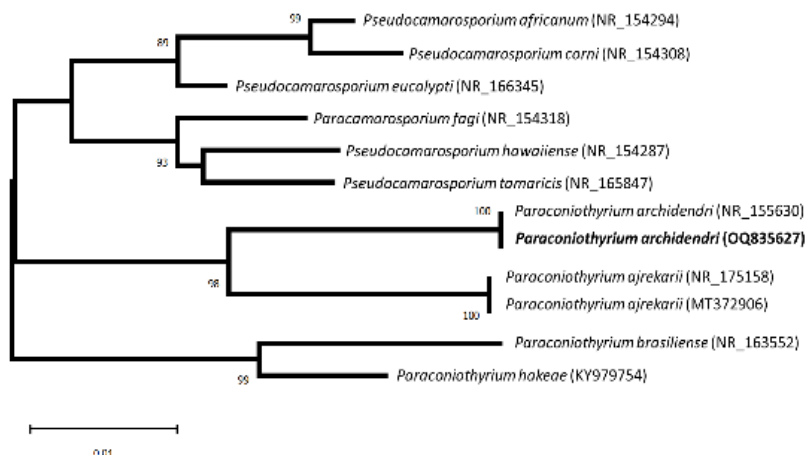
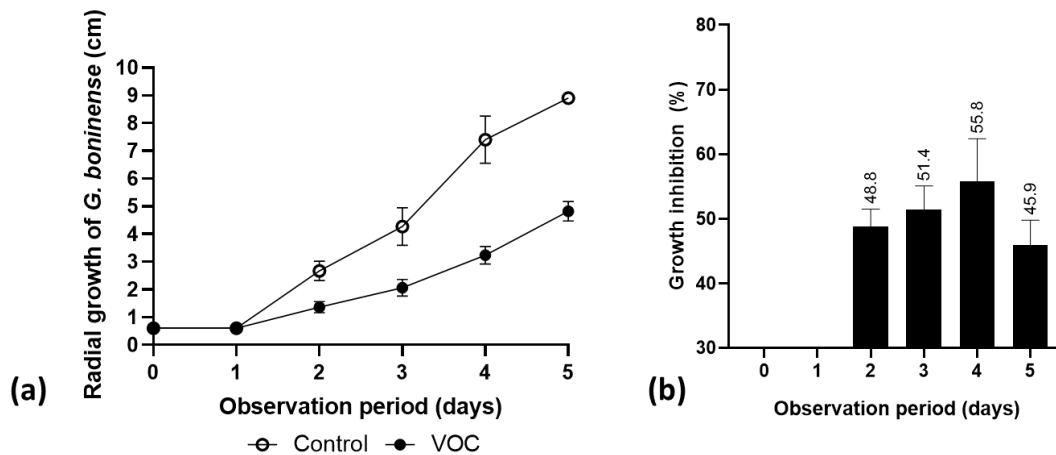


Figure 1. Phylogenetic tree of *P. archidendri* isolate F10 using ITS-rDNA for sequence homology studies. Sequence of reference strains was retrieved from GenBank accessions. Bootstrap value (%) of 1000 replications.

Inhibition of *Ganoderma boninense*

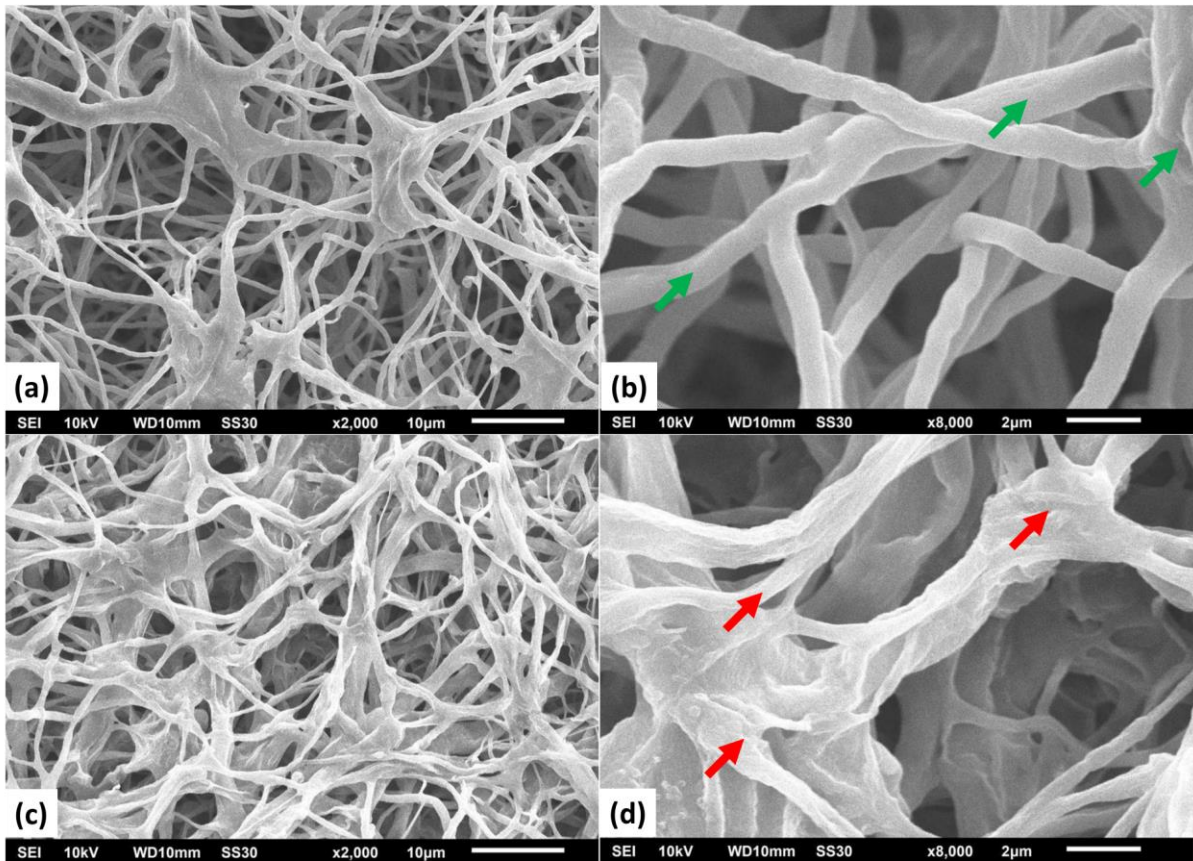
The production of VOCs by *P. archidendri* F10 was found to inhibit the growth of *G. boninense* mycelium (Figure 2). On PDA medium, the maximum growth of *G. boninense* was observed on the fifth day of incubation, reaching 9 cm. Conversely, on disc volatilization plate, the radial growth of *G. boninense* was halted at 4.81 cm. The inhibition commenced on the second day and peaked on the fourth day, with a recorded inhibition rate of 55.8%, which subsequently declined on the fifth day (45.9%). This decline may be attributed to the maximum growth of *G. boninense* colony (9 cm), which likely outcompeted the rate of inhibition induced by the VOCs. The observation of maximum inhibition of radial growth on the fourth day, followed by stagnation in inhibition thereafter, can be attributed to the assumption that the gaseous form of VOCs had diffused completely into the fungal colony and reached saturation. This may explain why there was no further inhibition of growth on the subsequent day. VOCs emission is a crucial antifungal mechanism exhibited by antagonistic microorganisms. The activity of these VOCs is observed to range from proximal interactions through water diffusion to distant interactions via air diffusion (Spadaro and Droby, 2016). Due to their volatile nature, microbial VOCs have shown great potential as biofumigants in air-tight environments (Tilocca *et al.*, 2020). These compounds possess physical properties that allow for the rapid saturation of the atmosphere with bioactive concentrations. When applied using an antagonistic isolate, the continuous exposure of VOCs may occur, leading to a potential permanent inhibition within a closed system, for example the porous soil against the soil-borne pathogen, *G. boninense*. The disc volatilization assay was initially employed to demonstrate the existence of VOCs produced

1 by *P. archidendri* F10, which could then be subjected to profiling using gas chromatography-
 2 mass spectrometry (GC-MS). The ultrastructure of *G. boninense* mycelium following exposure
 3 to *P. archidendri* F10 VOCs after five days is presented in Figure 3. Under 2,000×
 4 magnification, the branching pattern and hyphal diameter of mycelium in the control and VOCs
 5 treatment were found to be nearly indistinguishable. However, at 8,000× magnification,
 6 differences between the two treatments were observed. Specifically, in the control plate, the
 7 mycelium displayed normal diameter, smooth surface, and proper branching. On the other hand,
 8 in the VOCs treatment, the mycelium appeared to be wavy, non-smooth, and wrinkled, with
 9 abnormal branching and fused, defective hyphae that underwent lysis from within. This
 10 structural difference is likely responsible for the observed inhibition of maximum growth in *G.*
 11 *boninense* under the VOCs treatment. Similar observations of abnormal hyphal morphology
 12 and wrinkling have been reported in *G. boninense* exposed to VOCs produced by *Streptomyces*
 13 sp. GMR22 and *Nocardiopsis alba* GME01 and GME22 (Islamiati *et al.*, 2022; Widada *et al.*,
 14 2021).



15

16 **Figure 2.** (a) Radial growth of *G. boninense* exposure to VOCs produced by *P. archidendri*
 17 F10 and (b) Growth inhibition (%). The error bars indicate the standard deviation that was
 18 calculated from three replicates.



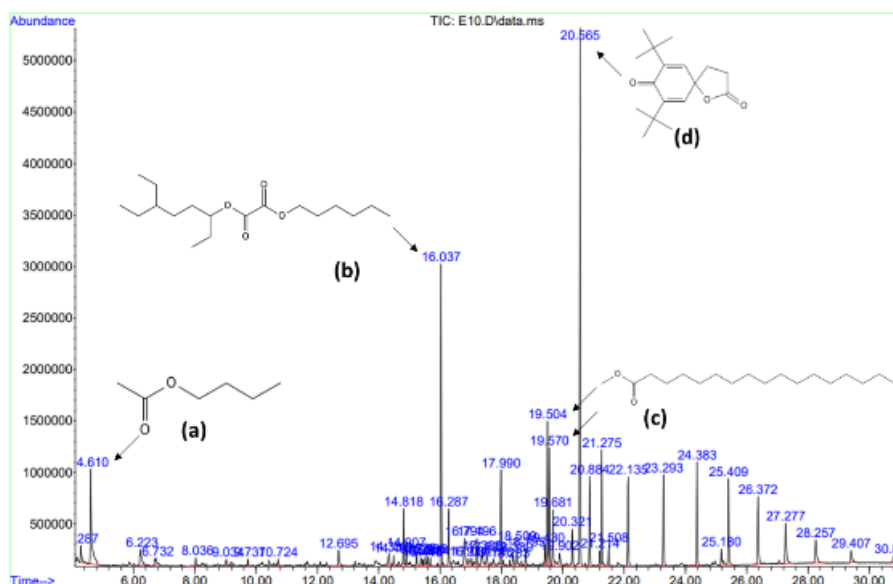
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2 **Figure 3.** (a) The ultrastructure of *G. boninense* in control plate at 2000× and (b) 8000×
3 magnification. The images as pointed with green arrows showed smooth, and well-branched
4 hyphal networks, indicative of healthy fungal growth without damage. (c) The ultrastructure of
5 *G. boninense* after 5-d exposure to VOCs at 2000× and (d) 8000× magnification. The images
6 as pointed with red arrows showed thinning, shrinkage, and reduced network density, with some
7 sections collapsed or broken.

8
9

1 Volatile Organic Compounds (VOC) as Antifungal Metabolites

2 GC-MS analysis was performed to determine the profile of VOCs produced by *P. archidendri*
3 F10 which produced a total of 54 detections (Figure 4). A total of 27 VOCs was identified in
4 the aromatic groups such as alcohols, alkanes, esters, ketones, and lipids. The major chemical
5 components were esters while the relative abundance based on the percentage of peak area were
6 ranked as follow: 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione (16.72%), followed
7 by 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate (8.71%), methyl heptadecanoate (8.66%), butyl
8 acetate (5.66%), and other minor components (<5%). The existence of 7,9-ditert-butyl-1-
9 oxaspiro[4.5]deca-6,9-diene-2,8-dione has been documented from various plant sources such
10 as *Allium chinense* (Amaryllidaceae) (Rhetso *et al.*, 2021), *Garcinia cambogia* and *Garcinia*
11 *indica* (Clusiaceae) (Jayakar *et al.*, 2020), *Nigella sativa* (Ranunculaceae) (Pachaiappan *et al.*,
12 2022), *Portulacaria afra* (Didiereaceae) (Tabassum *et al.*, 2023), and a seaweed, *Chara baltica*
13 (Characeae) (Tatipamula *et al.*, 2019). Despite the lack of information towards its antifungal
14 properties, this compound has been reported to exhibit several pharmacological activities,
15 including antibacterial, antioxidant, anti-inflammatory, anti-analgesic, and cytotoxic effects
16 (Pachaiappan *et al.*, 2022; Tabassum *et al.*, 2023; Tatipamula *et al.*, 2019). The second most
17 abundant compound, oxalic acid, 6-ethyloct-3-yl hexyl ester has been reported to display a
18 broad spectrum of antifungal activity against *Aspergillus niger*, *Fusarium oxysporum*,
19 *Penicillium funiculosum*, and *Trichoderma reesei* in the form of phytosterols from *Anogeissus*
20 *pendula*: Combretaceae (Sharma *et al.*, 2019). Methyl heptadecanoate, a member of the fatty
21 acid methyl ester (FAME) family, shows promise as a biofungicide against *G. boninense*. These
22 esters have also been designated as biomarkers to screen for resistant oil palm progenies, as
23 they exhibit elevated expression levels during *G. boninense* interactions (Rozlianah *et al.*,
24 2015). Fatty acid derivatives have been identified as regulators of various responses to *G.*
25 *boninense* in both non-infected and infected roots. The increased abundance of these
26 metabolites in infected roots is attributed to their crucial role in pathogen defense mechanisms
27 (Isha *et al.*, 2020). Therefore, the external application of *P. archidendri* F10 into oil palm
28 seedlings may be prospective in the future. Butyl acetate is an ester form of acetic acid which
29 is found to exhibit diverse antifungal activities. For instance, the compound can initiate
30 apoptotic cell death mechanisms in baker's yeast, *Saccharomyces cerevisiae* (Rego *et al.*, 2014).
31 Additionally, a mixture of volatile organic compounds (VOCs) produced by *Nocardiosis alba*,
32 including acetic acid and its derivatives, has been shown to be effective against *G. boninense*
33 (Widada *et al.*, 2021). In another study, the VOCs produced by *Hanseniaspora uvarum*

1 (Saccharomycodaceae) were found to effectively control the incidence of *Botrytis cinerea* in
 2 cherries and strawberries (Ruiz-Moyano *et al.*, 2020). The VOCs produced by *Phaeosphaeria*
 3 *nodorum* (Phaeosphaeriaceae) contained a significant proportion of acetic acid and its
 4 derivatives, which inhibit the growth of post-harvest phytopathogenic fungi, such as *Monilinia*
 5 *fruticola* (Pimenta *et al.*, 2012). Based on the SEM analysis results, it can be inferred that the
 6 inhibitory mechanism of the VOCs may involve the activation of multiple mechanisms and
 7 targets, working together synergistically to control the mycelium mass. The observed reduction
 8 in hyphal size and wrinkling of the mycelial network may be a result of signal molecules from
 9 external sources (i.e., VOCs) triggering intrinsic mechanisms that lead to delayed growth. It is
 10 also possible that these mechanisms target less common components beyond the general aspects
 11 of fungal cell walls, such as chitin, mannoproteins, and glucans, which are usually the first
 12 structures to be inhibited (Ibe and Munro, 2021).



13
 14 **Figure 4.** GC-MS spectra of EtOAc extract of *P. archidendri* F10 showing three major
 15 compounds based on the highest relative peak area (%). (a) butyl acetate, (b) 2-*O*-(6-ethyloctan-
 16 3-yl) 1-*O*-hexyl oxalate, (c) methyl heptadecanoate, and (d) 7,9-ditert-butyl-1-
 17 oxaspiro[4.5]deca-6,9-diene-2,8-dione.

18 19 Docking Study of Antifungal Compounds

20 Chemical information of selected compounds or VOCs produced by *P. archidendri* F10 and a
 21 standard antifungal compound in the field, dazomet, is presented in Table 1. In Table 2, the data
 22 of protein modeling was provided based on the representative criteria or descriptions given in
 23 the web server. The model was solely chosen for its high GMOE and sequence identity based
 24 on the available model from *Ganoderma sinense* ZZ0214-1 (Figure 5). The protein model was

1 validated using the Ramachandran plot, constituting a high score of core value, which was 80%,
 2 to represent an excellent quality of target protein (Figure 5). The binding affinity of each VOC
 3 produced by *P. archidendri* F10 produced a higher score than dazomet as a control (Table 3).
 4 The interactions between ligands or VOCs with the target protein are presented in Figures 6
 5 and 7. Visualization of the docking results revealed distinct patterns of chemical bonding
 6 interactions for each compound, where all compounds tended to bind to the hydrophilic region
 7 of the target protein. Only methyl heptadecanoate demonstrated a tendency to interact with the
 8 hydrophobic region. These differences were due to the different types of amino acids involved
 9 in the interactions and their residues.

10 **Table 1.** PubChem CID, molecular weight and formula of tested compounds.

No.	Compound(s)	PubChem CID	Molecular weight (g/mol)	Molecular formula
1.	Dazomet	10788	162.3	C ₅ H ₁₀ N ₂ S ₂
2.	Butyl acetate	31272	116.16	C ₆ H ₁₂ O ₂
3.	2- <i>O</i> -(6-ethyloctan-3-yl) 1- <i>O</i> -hexyl oxalate	6420420	314.5	C ₁₈ H ₃₄ O ₄
4.	Methyl heptadecanoate	15609	284.5	C ₁₈ H ₃₆ O ₂
5.	7,9- <i>ditert</i> -butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	545303	276.4	C ₁₇ H ₂₄ O ₃

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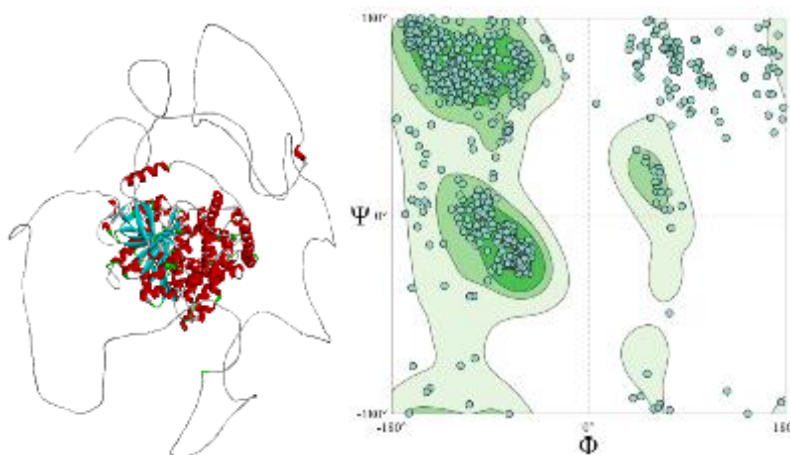
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Table 2. The information of 3D structure model of target protein.

Protein name (Sequence ID)	GMQE	Amino acid(s)	Sequence similarity	Sequence identity (%)	Description (Sequence ID)
Chitin synthase (A0A5K1JXQ5)	0.66	1102	0.60	95.17	Chitin synthase (A0A2G8SQ05)

13

14



15

16 **Figure 5.** Three dimensional structure of the protein model, chitin synthase (Left).
 17 Ramachandran plot of a model chitin synthase of *Ganoderma boninense* (Right).

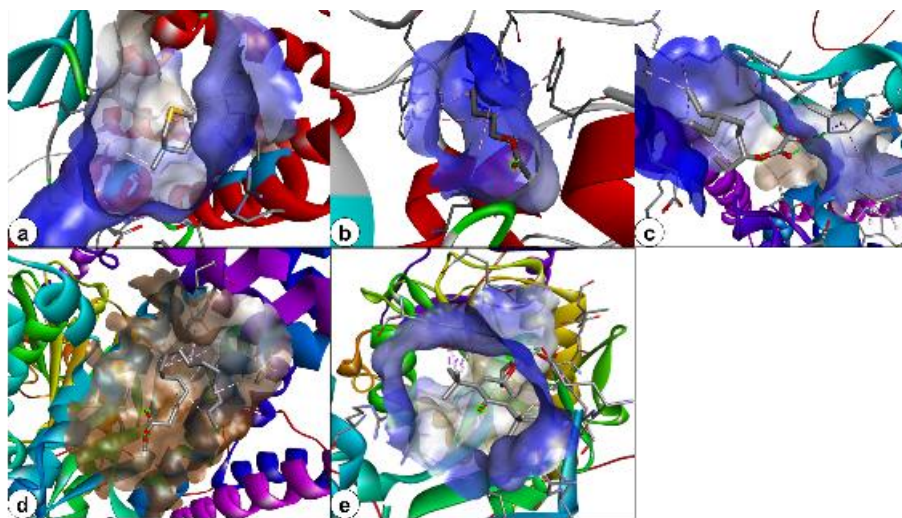
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1 **Table 3.** Docking profile of tested compounds against chitin synthase of *G. boninense*.

Ligand(s)	Binding affinity/ ΔG (kcal/mol)	Number of H-bond (Residue)	Interaction of hydrogen with amino acid residues (Distance)
Dazomet	-4.1	-	-
Butyl acetate	-4.4	-	-
Methyl heptadecanoate	-4.6	-	-
2- <i>O</i> -(6-ethyloctan-3-yl) 1- <i>O</i> -hexyl oxalate	-5.6	1	His698 (3.05 Å)
7,9- <i>di</i> tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	-8.5	1	Tyr646 (2.98 Å)

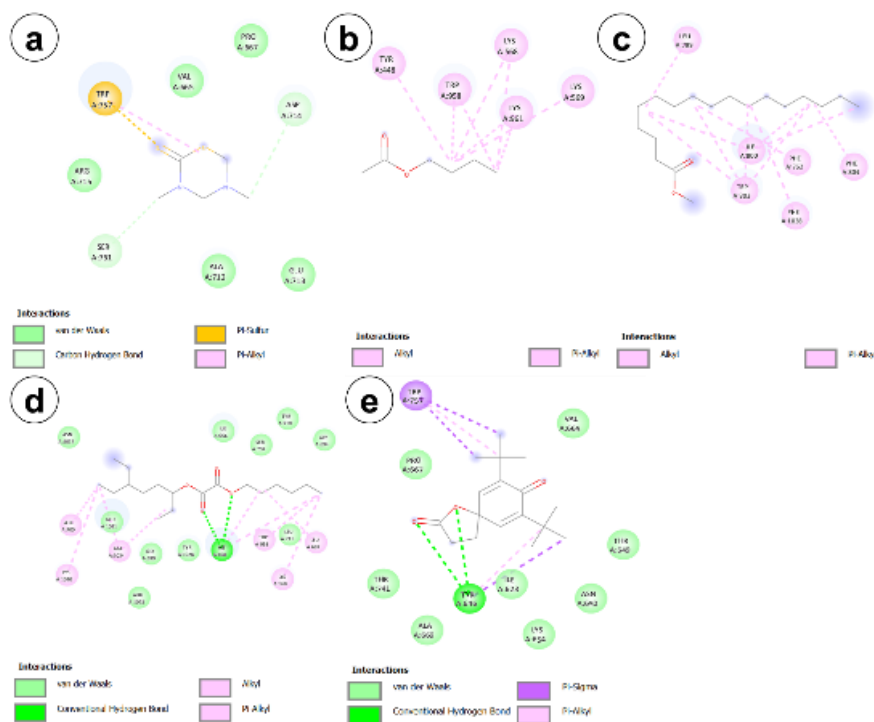
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4 **Figure 6.** Three dimensional interaction between VOC and chitin synthase from *G. boninense*.
 5 Interacting pockets represent the degree of hydrophobic region of target protein ranging from
 6 high (brown) to low (blue): (a) Dazomet, (b) Butyl acetate, (c) 2-*O*-(6-ethyloctan-3-yl) 1-*O*-
 7 hexyl oxalate, (d) Methyl heptadecanoate, and (e) 7,9-*di*tert-butyl-1-oxaspiro[4.5]deca-6,9-
 8 diene-2,8-dione.

9



1
2 **Figure 7.** Two dimensional interaction between VOC and chitin synthase from *G. boninense*.
3 Possible bonds were visualized between receptor or amino acid residues with the ligands: (a)
4 Dazomet, (b) Butyl acetate, (c) 2-*O*-(6-ethyloctan-3-yl) 1-*O*-hexyl oxalate, (d) Methyl
5 heptadecanoate, and (e) 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione.

6
7 The obtained binding affinity values (ΔG) for the ligands in this study provide insights into
8 their potential interactions with the target protein. The negative ΔG values indicated
9 thermodynamically favorable binding, with more negative values representing stronger
10 interactions. The ligand with the most negative ΔG value, 7,9-ditert-butyl-1-oxaspiro[4.5]deca-
11 6,9-diene-2,8-dione, suggested the strongest binding affinity to the target protein. The absence
12 of reported hydrogen bonds and interaction distances for Dazomet and Butyl acetate might
13 suggest that their binding may be primarily driven by hydrophobic interactions or other non-
14 covalent forces. On the other hand, Methyl heptadecanoate showed a higher binding affinity
15 and a possible hydrophobic interaction, possibly implying that the hydrophobic region of the
16 target protein plays a role in its binding. In contrast, 2-*O*-(6-ethyloctan-3-yl) 1-*O*-hexyl oxalate
17 and 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione exhibited hydrogen bonding
18 interactions with specific amino acid residues. This suggests the presence of potential hydrogen
19 bond donor and acceptor sites in the ligands and the target protein, indicating specific binding
20 pockets. The distances observed between the ligands and the interacting amino acid residues
21 are consistent with typical hydrogen bond lengths, reinforcing the possibility of these
22 interactions. Overall, the diversity in binding affinities and interactions observed among the

1 ligands reflects the complexity of ligand-protein interactions and highlights the role of different
2 forces contributing to binding. Fungal cell wall (FCW) is a fundamental element of hyphae,
3 essential for providing structural support, maintaining cellular morphology, and defending
4 against environmental stresses. FCW is primarily composed of polysaccharides, including
5 chitin and β -glucans, as well as proteins and lipids (Gow *et al.*, 2017). Disruption or inhibition
6 of these cell wall components can result in significant morphological abnormalities,
7 undermining the integrity of the hyphae and ultimately leading to cellular death (Zhang *et al.*,
8 2019). Molecular docking analysis indicates that the identified volatile organic compounds
9 (VOCs) may interact with specific targets within the fungal cell wall or associated proteins,
10 potentially leading to the observed structural damage. For example, VOCs such as Dazomet
11 and Butyl acetate exhibit significant hydrophobic interactions with the target proteins, despite
12 the absence of specific hydrogen bond interactions. These hydrophobic interactions are likely
13 to disrupt the hydrophobic domains within cell wall proteins or enzymes involved in cell wall
14 synthesis (Dover *et al.*, 2007). The disruption may compromise the integrity of the cell wall,
15 resulting in thinning and weakening of its structure. This weakening renders the cell wall more
16 susceptible to environmental stress and mechanical damage, as evidenced by the alterations
17 observed in the treated hyphae. Butyl acetate and its derivatives exhibit both antifungal and
18 fungal-stimulating properties, which vary depending on the target species and source. In co-
19 cultures of *Trichoderma sp.* and *Bacillus subtilis*, butyl acetate was identified as the
20 predominant compound exerting antifungal activity against *Colletotrichum gloeosporioides*,
21 effectively inhibiting its growth and spore formation (Emanuel *et al.*, 2020). Conversely, butyl
22 acetate and other acetate esters derived from apple fruit were found to stimulate the adhesion
23 and germination of *Botrytis cinerea* conidia, indicating a potential role in the fungal life cycle
24 (Filonow, 2002). In contrast, VOCs like 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate and 7,9-
25 ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione demonstrate strong binding affinities and
26 form specific hydrogen bonds with amino acid residues such as His698 and Tyr646. These
27 interactions likely occur at critical sites on fungal enzymes or structural proteins. The binding
28 of these VOCs to key residues may inhibit the function of enzymes involved in chitin or glucan
29 synthesis, which are crucial for cell wall construction. This inhibition can lead to defective
30 synthesis and, consequently, a weakened cell wall structure.

31

32

1 CONCLUSIONS

2 A soil-borne fungus, *P. archidendri* F10 isolated from healthy oil palm plantation soils showed
3 antifungal activity against the basal stem rot agent, *G. boninense* as evidenced through *in vitro*
4 assay, surface morphology of abnormal hypha formation, and potent bioactive VOCs revealing
5 four major components. i.e., 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, 2-*O*-(6-
6 ethyloctan-3-yl) 1-*O*-hexyl oxalate, methyl heptadecanoate, butyl acetate, and other minor
7 components. Based on the *in silico* evaluation, four VOCs may have targeted the cell wall
8 integrity by binding with the chitin synthase as a mode of antifungal action.

9

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