

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

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## 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.

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## INTRODUCTION

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

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

## MATERIALS AND METHODS

### Fungal Isolate and Molecular Identification

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

### VOC Production in Submerged Fermentation

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

### Antifungal Vapour Assay

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

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

### GC-MS Profiling of VOC

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

### Molecular Docking of Antifungi as Anti-*Ganoderma boninense*

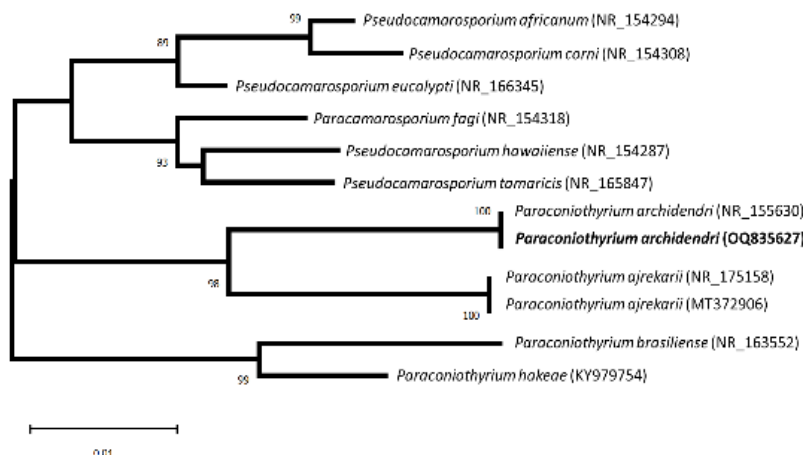
Major compounds as determined from the highest relative peak area were subjected to molecular docking studies. Chemical structure of each compound was retrieved from an online database. Protein target used in this study was chitin synthase as commonly involved in the cell wall synthesis which serves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

## RESULTS AND DISCUSSION

### Species Assignment of Isolate F10

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

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



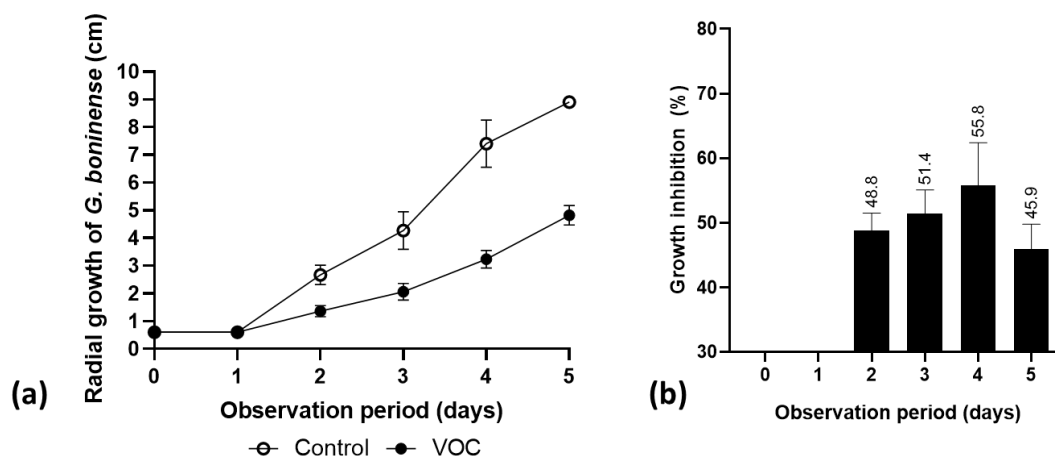
**Figure 1.** Phylogenetic tree of *P. archidendri* isolate F10 using ITS-rDNA for sequence homology studies. Sequence of reference strains was retrieved from GenBank accessions. Bootstrap value (%) of 1000 replications.

### Inhibition of *Ganoderma boninense*

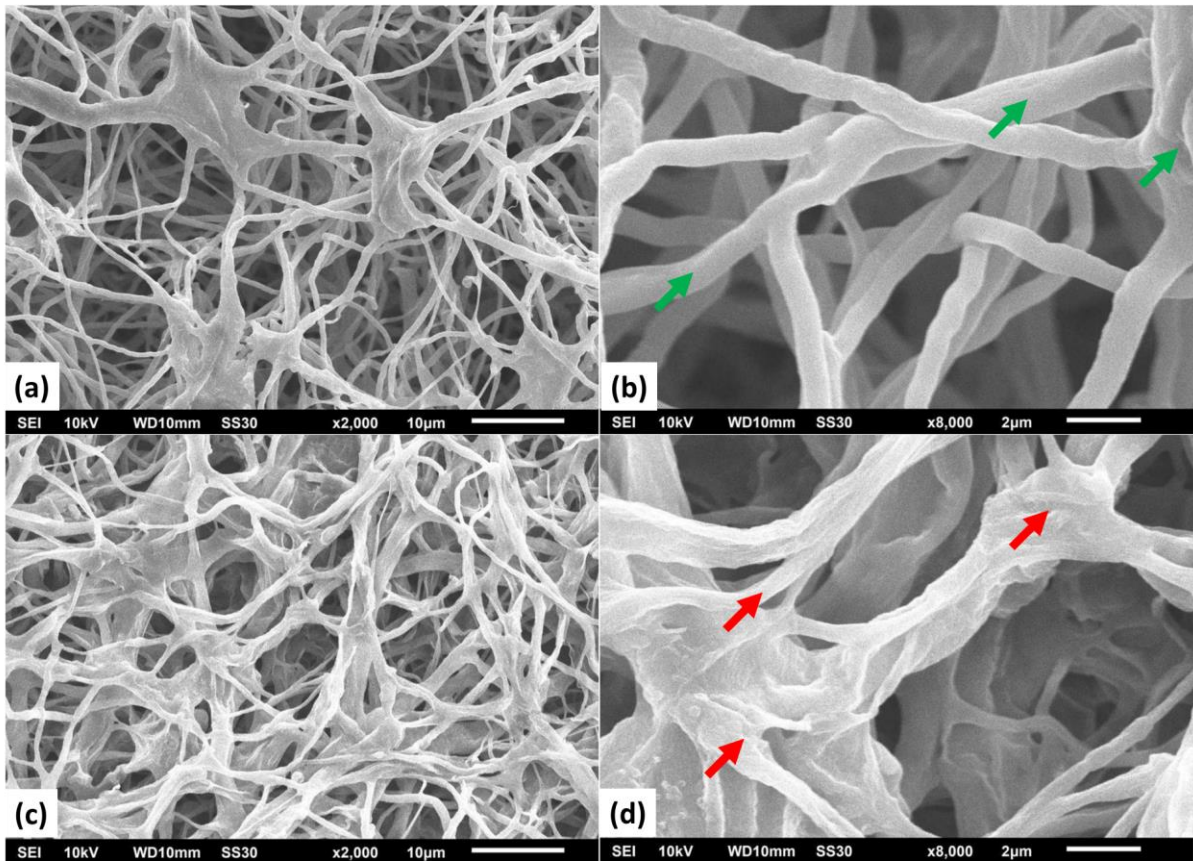
The production of VOCs by *P. archidendri* F10 was found to inhibit the growth of *G. boninense* mycelium (Figure 2). On PDA medium, the maximum growth of *G. boninense* was observed on the fifth day of incubation, reaching 9 cm. Conversely, on disc volatilization plate, the radial growth of *G. boninense* 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



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



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



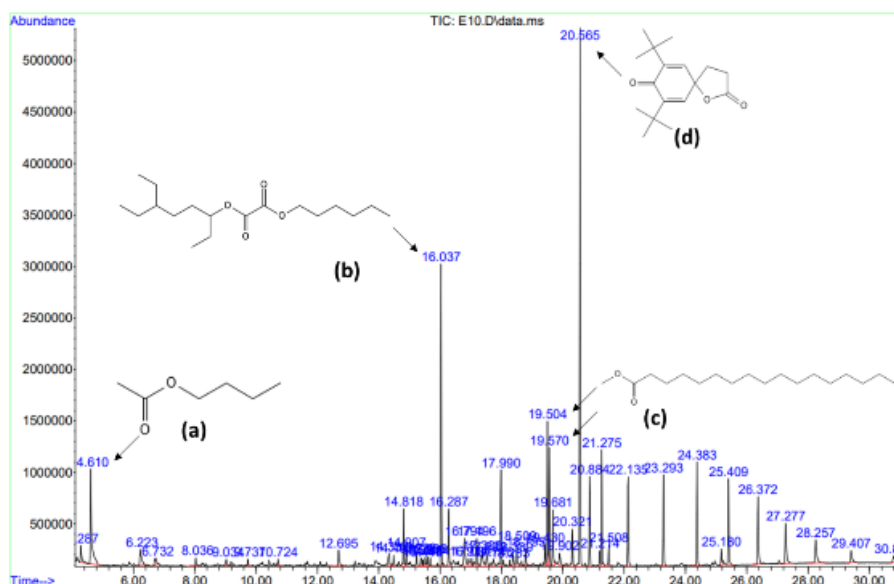
**Figure 3.** (a) The ultrastructure of *G. boninense* in control plate at 2000× and (b) 8000× magnification. The images as pointed with green arrows showed smooth, and well-branched hyphal networks, indicative of healthy fungal growth without damage. (c) The ultrastructure of *G. boninense* after 5-d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



**Volatile Organic Compounds (VOC) as Antifungal Metabolites**

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

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



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

### Docking Study of Antifungal Compounds

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

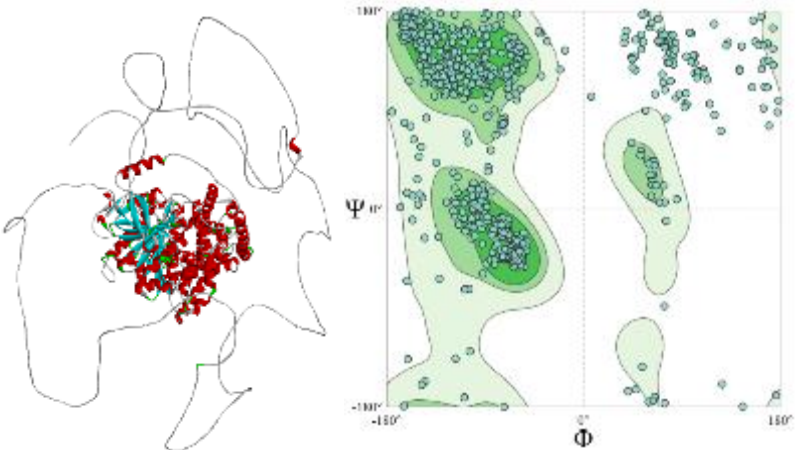
validated using the Ramachandran plot, constituting a high score of core value, which was 80%, to represent an excellent quality of target protein (Figure 5). The binding affinity of each VOC produced by *P. archidendri* F10 produced a higher score than dazomet as a control (Table 3). The interactions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

**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 <sub>5</sub> H <sub>10</sub> N <sub>2</sub> S <sub>2</sub>
2.	Butyl acetate	31272	116.16	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>
3.	2- <i>O</i> -(6-ethyloctan-3-yl) 1- <i>O</i> -hexyl oxalate	6420420	314.5	C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>
4.	Methyl heptadecanoate	15609	284.5	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
5.	7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	545303	276.4	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>

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

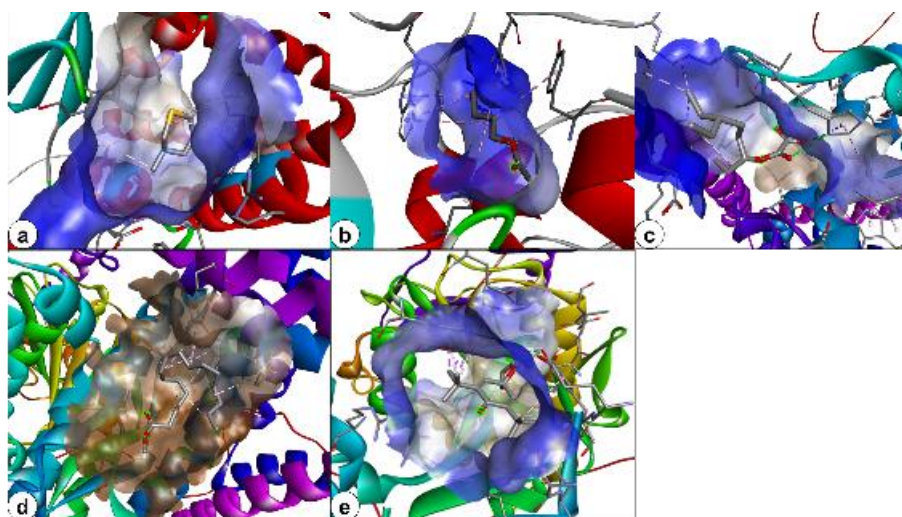
Protein name (Sequence ID)	GMQE	Amino acid(s)	Sequence similarity	Sequence identity (%)	Description (Sequence ID)
Chitin synthase (A0A5K1JXQ5)	0.66	1102	0.60	95.17	Chitin synthase (A0A2G8SQ05)



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

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

Ligand(s)	Binding affinity/ $\Delta G$ (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> <i>tert</i> -butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	-8.5	1	Tyr646 (2.98 Å)



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



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



## CONCLUSIONS

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

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