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ANTIFUNGAL ACTIVITY OF VOLATILE ORGANIC COMPOUNDS (VOC) BY AN ENDOPHYTIC FUNGUS, *Lasiodiplodia avicenniae* P2P4 FROM *Avicennia alba* AGAINST *Fusarium oxysporum*

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ABSTRACT

Fusarium oxysporum is a significant phytopathogenic fungus in the agricultural sector which causes a substantial loss to the yield of commercial crops and economical benefits. The fungal species is a primary agent of vascular wilt diseases on various crops that are currently mitigated through the application of synthetic pesticides. The prolonged use of synthetic pesticides may lead to the initiation of microbial resistance to pesticides despite their other detrimental effects on the environment and human health. Biopesticides are safer bioproducts that can be applied to hinder *F. oxysporum* growth with varying efficacy and mechanism of action. This study deals with the investigation of volatile organic compounds (VOCs) produced by an endophytic manglicolous fungus as one of the potential biopesticides to control *F. oxysporum*. *Lasiodiplodia avicenniae* P2P4 was isolated from the pneumatophore of *Avicennia alba* growing in the mangrove forest near Belawan City, North Sumatra, Indonesia, and was tested for its antifungal activity against *F. oxysporum* using poison food technique. The highest inhibition (>80%) was observed from 1000 ppm treatment with an effective concentration (EC_{50}) value of 888.228 ppm after 7 d of incubation. GC-MS analysis of the EtOAc extract of *L. avicenniae* P2P4 revealed a mixture of volatile and non-volatile organic compounds (27 in total). Acetic acid, glycerol, and 1,3/2,3-Butanediol were identified as the major portion of compounds in the extract that could be responsible for its antifungal activity against *F. oxysporum*.

Keywords: Acetic Acid, *Avicennia alba*, Butanediol, *Fusarium oxysporum,* GC-MS, *Lasiodiplodia avicenniae*.
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INTRODUCTION

The *Fusarium* genus is important in agriculture because it causes at least one disease in most crops throughout the world. Depending on the variety of hosts and the colonized sites (leaves, stems, roots, seeds, or fruits), these diseases can range in severity.[1] The invasive phytopathogen, *Fusarium oxysporum* (*FOX*), which causes cortical rot and vascular wilt in more than a hundred commercially valuable crops, is classified as one of the most significant species economically. A sizable portion of financial losses in the global agriculture industry is attributed to the presence of *FOX* in the agricultural field and postharvest sectors.[2,3] *FOX*-related plant diseases are mitigated by synthetic agrochemicals, but there are still inevitable adverse consequences on the environment and human health, including the development of microbial resistance to agrochemicals.[4] The utilization of biopesticides is a promising solution to prevent fungal diseases in commercially significant crops.[5] One class of known biopesticides is volatile organic compounds (VOCs), which can be synthesized by endophytic fungi and have substantial antimicrobial properties such as alcohols, carboxylic acids, esters, ketones, and terpenes.[6,7] VOCs and other secondary metabolites have been reported from mangrove-associated or manglicolous fungi, such as *Aspergillus* sp from *Rhizophora mucronata*,[8] *Aspergillus flavus* from *Sonneratia alba*,[9] *Diaporthe amygdali* from *Sonneratia griffithii*,[10] etc. In this study, we reported the potential antifungal of VOCs produced by an endophytic fungus,

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Lasiodiplodia avicenniae P2P4 from the pneumatophore of *Avicennia alba* that could be potential as a future biopesticide-producing agent.

EXPERIMENTAL

Molecular Identification of Endophytic Fungus

An endophytic fungus, *Lasiodiplodia avicenniae* P2P4, was isolated from the pneumatophore of *Avicennia alba* collected at a mangrove forest near Belawan City, North Sumatra, Indonesia. The identification of fungal isolate was performed based on its ITS-rDNA region sequenced by Macrogen, Inc. (Singapore). The consensus sequence combined from ITS1 and ITS4 region was searched for its similarity using BLASTn. A phylogenetic tree was then constructed using MEGA11 to designate the identity of *L. avicenniae* among other species within the same family.[11]

Submerged Fermentation

The fungal strain was cultivated in liquid Potato Dextrose Broth (PDB) medium supplemented with yeast extract (2 g/L) and $CaCO_3$ (5 g/L) with a final pH of 5.3.[12] A 250-mL flask was filled with 50 mL of PDB medium and inoculated with 2 fresh fungal agar plugs and incubated for 14 d at room temperature. The liquid portion was filtered using a syringe filter (pore size: 0.22 μm) and extracted with EtOAc (1:1, v/v). The organic portion was dehydrated using Na_2SO_4 and dissolved in one mL of MeOH and then stored for further analysis.

Antifungal Test

The poison food technique was used as a method to determine the antifungal activity of crude extract of *L. avicenniae* P2P4 against *Fusarium oxysporum*.[13] Fifteen mL of poisoned PDA medium with varying concentrations (50, 100, 200, 500, 1000 ppm, v/v) was allowed to solidify and inoculated with an agar plug of the active-growing colony of *F. oxysporum* at the center of the plate. PDA medium without crude extract was used as a control plate for *F. oxysporum* growth. The plates were incubated for 7 d at room temperature. Radial growth (mm) of *F. oxysporum* mycelium was expressed in the growth inhibition (%): $[(D_1 - D_2)/D_1]\times100\%$. Where D_1 is the radial growth (mm) of the control plate and D_2 is the radial growth of poison plates. The test was performed in three replicates. Effective concentration (EC_{50}) was measured using a Hill's equation retrieved from an online tool, AAT Bioquest (httpss://www.aatbio.com/tools/ec50-calculator).

GC-MS Analysis

Identification of putative volatile organic compounds (VOCs) from *L. avicenniae* P2P4 was investigated using a GC-MS QP-2010 (Shimadzu, Germany). The column used was RTX-5MS (30×0.25×0.10 m) with one μL of splitless sample injection. The oven temperature was maintained at 60ºC for 3 min until 220ºC with a flow rate of 5ºC/min. The total flow was 37.5 mL/min under 60 kPa. Helium was used as a carrier gas and set with a flow rate of 1.03 mL/min and linear velocity of 37.1 cm/sec. Compounds were identified automatically based on the available database of NIST along with the retention time (min) and the relative percentage of area.

RESULTS AND DISCUSSION

Identity of Fungal Strain

Based on the phylogenetic construction of our fungal sequence with other accessions in the ITS-rDNA region, P2P4 was positioned in clade 1, later identified as *Lasiodiplodia avicenniae* (Fig.-1). Members of *Lasiodiplodia* (Ascomycota, Dothideomycetes, Botryosphaeriaceae) are cosmopolitan fungal taxa distributed in the tropical and subtropical regions with a wide range of substrate or host colonization and displaying diverse lifestyles, such as endophytes, saprobes, and pathogens.[14] The fungal species, *L. avicenniae* was first described as new fungal taxa that were isolated from the branches of *Avicennia marina* in South Africa.[15] Here, our finding of *L. avicenniae* from *Avicennia alba* in North Sumatra may be regarded as a new record that will add information regarding the biodiversity of manglicolous fungal species from the region.

Antifungal Activity of *L. avicenniae* P2P4

Anti-*FOX* properties of *L. avicenniae* P2P4 were evaluated by testing the fungal EtOAc extract obtained from fermentation broth in liquid PDA medium against *F. oxysporum* based on the poison food technique.

The poison food technique was used to determine the antifungal properties as expressed in the reduction/ percentage of radial growth as well as the minimum inhibitory and fungicidal concentration of an extract.[16]

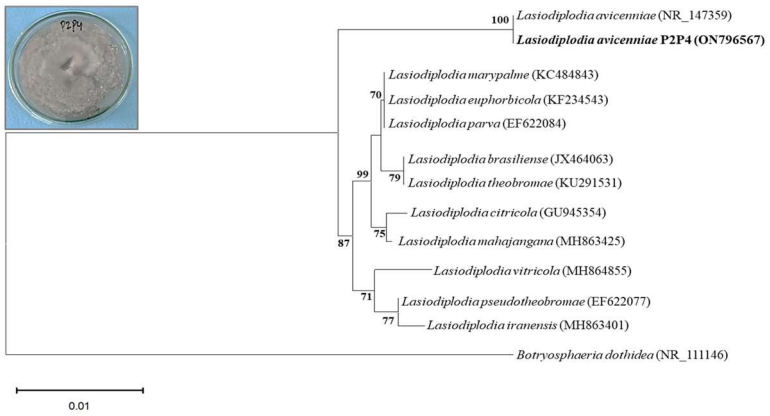

Fig.-1: Unrooted Neighbor-Joining Tree for an Endophytic Fungal Isolate, P2P4 within *Lasiodiplodia* Species *Botryosphaeria dothidea* is Used as an Outgroup. Numbers at Each Node Indicate bootstrap values (% of 1000 replications). Scale bar = 0.01 Substitutions Per Nucleotide Position

The reduction of *F. oxysporum* radial growth (in mm) was monitored after 7-d of incubation which resulted in a concentration-dependent manner (Fig.-2). The greatest inhibition (>80%) was observed in a 1000-ppm treatment then gradually decreased until 50 ppm. The effective concentration (EC_{50}) value of *L. avicenniae* P2P4 extract against radial growth of *F. oxysporum* was obtained at 888.228 ppm (Fig.-3). Compared to synthetic fungicides, the antifungal properties of *L. avicenniae* extract were less effective than Carbendazim at 200 ppm, yet more effective than Dithane M-45 which suppressed the growth of *F. oxysporum* by 37% at 200 ppm.[17] In addition, the radial growth of pathogenic *Fusarium* sp. was reduced by 71% after 7 days of incubation using a poison agar technique impregnated with the crude extract of endophytic *Fusarium solani* from shallot.[18] Information on *L. avicenniae* including members of *Lasiodiplodia* as antagonistic fungi is still limited and may be elaborated further as biocontrol agents.

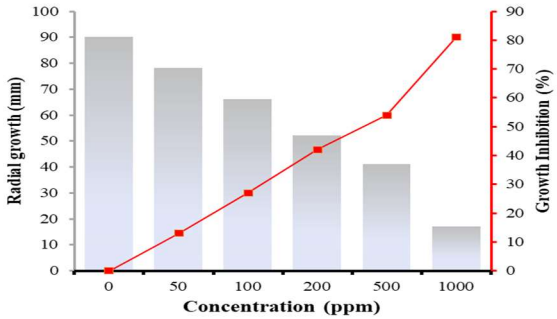

Fig.-2: Results of Antifungal Test of Crude Extract of *L. avicenniae* P2P4 against *F. oxysporum* Using Poison Food Method after 7-d of Incubation

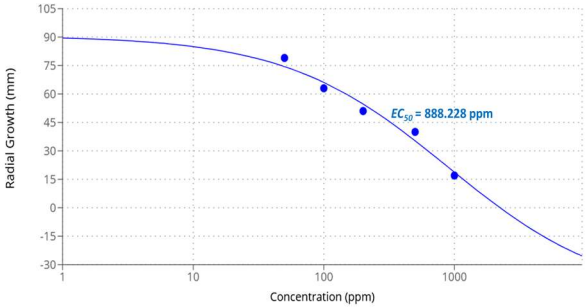

Fig.-3: Results of Antifungal Test of Crude Extract of *L. avicenniae* P2P4 against *F. oxysporum* using Poison Food Method after 7-d of Incubation

VOCs Profile by GC-MS Analysis

The GC spectrum of the EtOAc extract of *L. avicenniae* P2P4 (Fig.-4) confirmed the presence of 27 VOCs with different retention times and relative percentages of area. Three major VOCs and one non-volatile compound (based on % area) were identified namely glycerol, acetic acid, 1,3-butanediol, and 2,3-butanediol, and were discussed in the following.

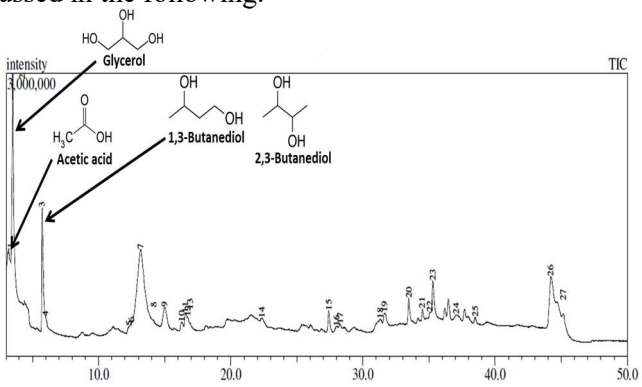

Fig.-4: GC-MS Spectrum of Crude Extract of an endophytic fungus, *L. avicenniae* P2P4 from *Avicennia alba* Showing the Major Compound Based on Relative Percentage of Area

Glycerol is a colorless and odorless polyol compound synthesized in the cell interior or secreted to the environment by microorganisms as an adaptation to the limited water availability and for osmotic adjustment.[19] The presence of glycerol may be considered a sign of strain adaptation towards medium conditions though it was not clearly understood from this study. Another study also reported that glycerol (glycerin) was one of the chemical compounds found in the EtOAc extract of *Cladosporium tenuissimum* from a native Indian medicinal plant, *Memecylon edule* that displayed antimicrobial and antiseptic properties.[20] Acetic acid is a carboxylic acid that exhibits antimicrobial properties including various fungi on practical applications. The acidic compound was proven to delay the conidial germination of postharvest decay fungi such as *Botrytis, Colletotrichum, Monilinia,* and *Penicillium*.[21] An endophytic fungus, *Trichoderma asperelloides* PSU-P1 also emitted acetic acid as one of the major VOCs involved in the inhibition of the growth of *Ganoderma* sp, a causal agent of basal stem rot.[22] The fungicidal activity of acetic acid involved the lowering of pH in the target cellular protoplasm, thus killing the conidia of exposed fungi.[23] In addition, an application using a combination of liquid smoke with high acetic acid content and cayenne leaf was also proven to reduce the *in vitro* growth of *F. oxysporum*, showing the flexibility of the application.[5] Two alcohol compounds namely 1,3-Butanediol and 2,3-Butanediol were already reported to exert antifungal activity against postharvest fungal pathogens such as *B. cinerea, M. fruticola, M. laxa, P. Digitatum,* and *P. expansum*.[24] In addition, a rhizospheric bacterial strain, *Bacillus amyloliquefaciens* L3 also emitted 2,3-Butanediol that was able to delay the growth and development of *Fusarium oxysporum* f.sp. *niveum* (*FON*) and also promoted the growth of its host, *Arabidopsis thaliana*.[25] The prospective inhibition of *F. oxysporum* growth by *L. avicenniae* P2P4 extract still requires deeper investigation since the crude extract is a mixture of VOCs which may display different results when tested in pure condition (single compound).

CONCLUSION

The presence of diverse VOCs in the EtOAc extract of *Lasiodiplodia avicenniae* P2P4 recovered from the pneumatophore of *Avicennia alba* was confirmed through GC-MS analysis. A total of 27 compounds with four major VOCs namely acetic acid, glycerol, and 1,3/2,3-Butanediol may be responsible for the antifungal activity against *Fusarium oxysporum* although not fully understood of its mechanism. The finding may promote the field trial of *L. avicenniae* P2P4 to obtain a better understanding of its efficacy to mitigate the disease caused by *F. oxysporum* in the future.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. Y.M. Medina-Romero, G. Roque-Flores and M.L. Macias-Rubalcava, *Applied Microbiology and Biotechnology*, **101**, 8209(2017), https://doi.org/10.1007/s00253-017-8542-8
2. L.J. Ma, D.M. Geiser, R.H. Proctor, A.P. Rooney, K. O'Donnell, F. Trail, D.M. Gardiner, J.M. Manners and K. Kazan, *Annual Review of Microbiology,* **67**, 399(2013), https://doi.org/10.1146/annurev-micro-092412-155650
3. R. Dean, J.A.L.V. Kan, Z.A. Pretorius, K.E. Hammond-Kosack, A.D. Pietro, P.D. Spanu, J.J. Rudd, M. Dickman, R. Kahmann, J. Ellis and G.D. Foster, *Molecular Plant Pathology,* **13(4)**, 414(2012), https://doi.org/10.1111/j.1364-3703.2011.00783.x
4. P. Nicolopoulou-Stamati, S. Maipas, C. Kotampasi, P. Stamatis and L. Hens, *Frontiers in Public Health,* **4(148)**, 1(2016), https://doi.org/10.3389/fpubh.2016.00148
5. M. Faisal, A. Gani, A.R.N. Hidayati, P. Mardhatillah and H. Husni, *Rasayan Journal of Chemistry*, **14(2)**, 705(2021), https://doi.org/10.31788/RJC.2021.1426239
6. M.C. Lemfack, B.O. Gohlke, S.M.T. Toguem, S. Preissner, B. Piechulla and R. Preissner, *Nucleic Acids Research*, **46(D1)**, D1261(2018), https://doi.org/10.1093/nar/gkx1016
7. A. Kaddes, M.L. Fauconnier, K. Sassi, B. Nasraoui and M.H. Jijakli, *Molecules,* **24(6)**, 1(2019), https://doi.org/10.3390/molecules24061065
8. O. Fadriyanti, I.D. Nasution, D. Handayani and W. Siswomiharjo, *Rasayan Journal of Chemistry*, **12(4)**, 2284(2019), https://doi.org/10.31788/RJC.2019.1245415
9. A.R.B. Ola, C.A.P. Soa, Y. Sugi, T.D. Cunha, H.L.L. Belli and H.J.D. Lalel, *Rasayan Journal of Chemistry*, **13(1)**, 377(2020), https://doi.org/10.31788/RJC.2020.1315585
10. D. Handayani, T. Wahyuningsih, Rustini, M.A. Artasastra, A.E. Putra and P. Proksch, *Rasayan Journal of Chemistry*, **13(1)**, 327(2020), https://doi.org/10.31788/RJC.2020.1315589
11. K. Tamura, G. Stetcher and S. Kumar, *Molecular Biology and Evolution,* **38(7)**, 3022(2021), https://doi.org/10.1093/molbev/msab120
12. S. Kumala, R. Utji, P. Sudarmono and L.B.S. Kardono, *Research Journal of Microbiology,* **2(8)**, 625(2007), https://doi.org/10.17311/jm.2007.625.631
13. P.K. Mukherjee and K. Raghu, *Mycopathologia,* **139(3)**, 151(1997), https://doi.org/10.1023/A:1006868009184

14. N.I. de Silva, A.J.L. Phillips, J.K. Liu, S. Lumyong and K.D. Hyde, *Scientific Reports,* **9(14355)**, 1(2019), https://doi.org/10.1038/s41598-019-50804-x
15. J.A. Osorio, C.J. Crous, Z.W.D. Beer, M.J. Wingfield and J. Roux, *Fungal Biology,* **121(4)**, 361(2017), https://doi.org/10.1016/j.funbio.2016.09.004
16. M.M. Gakuubi, A.W. Maina and J.M. Wagacha, *International Journal of Microbiology*, **2017**, 1(2017), https://doi.org/10.1155/2017/8761610
17. N. Dahal and R.K. Shrestha, *Journal of the Institute of Agriculture and Animal Science,* **35(1)**, 105(2018), https://doi.org/10.3126/jiaas.v35i1.22520
18. A. Rizali, N.L. Aziza and N. Sari, *Pakistan Journal of Biological Sciences*, **24(10)**, 1015(2021), https://doi.org/10.3923/pjbs.2021.1015.102
19. A. Stevenson, P.G. Hamill, A. Medina, G. Kminek, J.D. Rummel, J. Dijksterhuis, D.J. Timson, N. Magan, S.L.L. Leong and J.E. Hallsworth, *Environmental Microbiology*, **19(3)**, 947(2017), https://doi.org/10.1111/1462-2920.13530
20. M. Kanjana, G. Kanimozhi, R. Udayakumar and A. Panneerselvam, *Journal of Biomedical and Pharmaceutical Sciences*, **2(1)**, 1(2019)
21. S. Rogawansamy, S. Gaskin, M. Taylor and D. Pisaniello, *International Journal of Environmental Research and Public Health,* **12(6)**, 6319(2015), https:doi.org/10.3390/ijerph120606319
22. N. Phoka, N. Suwannarach, S. Lumyong, S. Ito, K. Matsui, S. Arikit and A. Sunpapao, *Journal of Fungi,* **6(341)**, 1(2020), https://doi.org/10.3390/jof6040341
23. M. Kuroki, Y. Shiga, M.N. Nara, T. Azazoe and T. Kamakura, *iScience*, **23(1)**, 1-27(2019), https://doi.org/10.1016/j.isci.2019.100786
24. H. Calvo, I. Mendiara, E. Arias, A.P. Gracia, D. Blanco and M.E. Venturini, *Postharvest Biology and Technology,* **166**, 1(2020), https://doi.org/10.1016/j.postharvbio.2020.111208
25. Y. Wu, J. Zhou, C. Li and Y. Ma, *MicrobiologyOpen,* **8(8)**, 1(2019), https://doi.org/10.1002/mbo3.813

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