JPPIPA 8(SpecialIssue) (2022)

JurnalPenelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

In Vitro Effectiveness Test of Sagoo Cortex Liquid Smoke to Inhibit the Growth of *Ganoderma orbiforme* (Fr.) Ryvarden

Yusmar M[1,2*], Antama Surwadinata[1], Irwan Tasla[1], Oksana[1], Syukria Ikhsan Zam[1]

[1] Program Studi Agroteknologi, Fakultas Pertanian dan Peternakan, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru, Indonesia,
[2] Program Studi Ilmu Lingkungan Program S3 Pascasarjana Universitas Riau Pekanbaru, Indonesia

Received: October 28, 2022
Revised: November 25, 2022
Accepted: December 5, 2022
Published: December 10, 2022

Corresponding Author:
Yusmar M.
yusmar@uin-suska.ac.id

DOI:
10.29303/jppipa.v8iSpecialIssue.2480

Abstract: *Ganoderma orbiforme* is a pathogenic fungus that cause basal stem rot especially in oil palm, so it needs to be controlled. One of the alternative controls used is liquid smoke. This research aims to examine several concentrations of sagoo cortex liquid smoke to inhibit the growth of *G. orbiforme* in vitro. This research was conducted in October untill Desember 2021 at the Pathology, Entomology, Microbiology and Soil Science Laboratory, Faculty of Agriculture and Animal Science, Islamic State University Sultan Syarif Kasim Riau. Research was test of 6 concentrations of sagoo cortex liquid smoke (0, 1, 2, 3, 4, and 5%) with 4 replications. Liquid smoke had a total phenol ± 7.44%. Sagoo cortex liquid smoke with 1% concentration has already very effective to inhibit in the macroscopic, microscopic, growth rate, wet mass and dry mass.

Keywords: *Ganoderma orbiforme*; liquid smoke; sagoo cortex

Introduction

Ganoderma orbiforme is a pathogen that causes stem rot disease which is commonly found in oil palm (Widiastuti et al., 2016), currently stem rot disease in oil palm plants is the most destructive disease so it must be watched out for especially in oil palm plantations (Angraini, 2017). Stem rot disease is the most deadly disease, especially in oil palm plantations that have undergone rejuvenation. The more often a garden undergoes rejuvenation, the higher the percentage of stem rot disease. This happens because after *G. orbiforme* infects the plants, the planting area will continue to be contaminated and the pathogenic inoculum will accumulate as oil palm is planted more frequently (Midot et al., 2019). *G. orbiforme* is a soil-borne (soil born) pathogenic fungus which infects through roots and spreads the disease by producing basidiospores as an inoculum source for stem rot disease infection (Chong et al., 2017). Stem rot disease caused by *G. orbiforme* is one of the most lethal main diseases in oil palm plantations in Southeast Asia, in Indonesia this disease is a factor causing a decrease in oil palm production per unit area in several oil palm plantations (Chong et al., 2017).

One of the efforts that farmers often make is to use synthetic chemical fungicides as the main control (Angraini, 2017) because of the convenience and the results shown are relatively short. However, the use of synthetic fungicides is considered to be less effective in controlling *G. orbiforme* (Widiastuti et al., 2016). The use of synthetic fungicides in the long term will cause resistance, resurgence and leave residues that are harmful to environmental sustainability (Irfan, 2016).

Considering the negative impacts caused by the use of synthetic fungicides, it is necessary to have other alternatives that are more environmentally friendly. One way is to utilize several wastes such as sago bark, coconut shells and empty oil palm bunches as liquid smoke to control pathogens that cause stem rot disease in oil palm plantations (Sari et al., 2018).

How to Cite:
Yusmar, M., Surwadinata, A., Tasla, I., Oksana, O., & Zam, S. I. (2022). In Vitro Effectiveness Test of Sagoo Cortex Liquid Smoke to Inhibit the Growth of Ganoderma orbiforme (Fr.) Ryvarden. *Jurnal Penelitian Pendidikan IPA*, *8*(SpecialIssue), 53–57. https://doi.org/10.29303/jppipa.v8iSpecialIssue.2480

Liquid smoke is an active ingredient that has the ability to inhibit fungal growth obtained from the condensation of vapor or gas fractions formed during the pyrolysis process from materials containing lignin, cellulose and hemicellulose (Sarwendah et al., 2019). Liquid smoke can be used as an antimicrobial because it contains phenolic compounds and organic acids (Pradhana and Trivana, 2018). This can be used as an alternative to reducing the use of chemical fungicides whose long-term use has an impact on the environment and farming communities.

The chemical components of liquid smoke production are influenced by the chemical components of the raw materials such as cellulose, hemicellulose and lignin. One of the ingredients that can be used in the manufacture of liquid smoke is sago bark because sago bark is composed of 22.8% lignin, 47.8% cellulose and 15.5% hemicellulose (Gultom et al., 2018).

The ability of liquid smoke to inhibit fungal growth is influenced by the main chemical components that make up liquid smoke, namely phenol, carbonyl and organic acids which function as antimicrobials and antioxidants (Mahmud et al., 2016). Liquid smoke from sago bark contains 10.906% phenol, 2.117% carbonyl and 10.112% organic acids so it has potential as an alternative fungicide (Gultom et al., 2018).

Based on the description above, liquid smoke from sago bark needs to be tested for its potential to inhibit the growth of *G. orbiforme* in vitro.

Method

Effectiveness Test of Sago Bark Liquid Smoke

This research was carried out in a laboratory experiment with a completely randomized design (CRD) consisting of 6 concentrations of liquid smoke (0%, 1%, 2%, 3%, 4% and 5). Each treatment was repeated 4 times to obtain 24 experimental units.

Liquid Smoke Manufacturing

The process of making liquid smoke begins with cleaning the raw material from impurities and drying it in the sun for 5 days (Gultom et al., 2018). Furthermore, as much as 3 kg of each material is cut into small pieces with a size of 5–7 cm and put into the pyrolysator reactor then closed tightly and burned at ± 265 °C for approximately 3 hours. The smoke produced from combustion will flow into the condenser and a condensation process will occur resulting in liquid smoke. Liquid smoke is collected and allowed to stand for 48 hours. After settling, the liquid smoke is filtered using Whatman No. filter paper. 40, filtered again to reduce the tar content contained in liquid smoke using a 0.2 µm filter membrane, so that liquid smoke was obtained for analysis of phenol content (Wardoyo et al., 2020).

Quantitative Analysis of Total Phenol in Liquid Smoke

Quantitative analysis of total phenolic compounds was carried out using the Folin-Ciocalteau method. Gallic acid solutions (in distilled water) were prepared in concentrations (0, 20, 40, 60, 80, and 100 mg/L). Take 0.5 ml of gallic acid solution and blank, then react with 2.5 ml of 10% *Folin-Ciocalteau* reagent and leave for 4 minutes. After that, 2 ml of 7.5% Na2CO3 solution was added and incubated for 30 minutes at room temperature. After that, the absorption was determined at a wavelength (λ) of 765 nm with a UV-Vis spectrophotometer. The same treatment was also carried out on sago bark liquid smoke with a concentration of 100 mg/L (Rungruang and Suwanne, 2010).

Cultivation of the fungus G. orbiforme cc

The *G. orbiforme* isolates used came from the collection of the Laboratory of Pathology, Entomology, Microbiology and Soil Science, Faculty of Agriculture and Animal Husbandry, Sultan Syarif Kasim Riau State Islamic University, then propagated by means of *G. orbiforme* isolates in tubes transferred using an ose needle, then inoculated into Petri dishes containing PDA media aseptically in laminar air flow. The Petri dish is then closed and sealed on the sides using plastic wrap. Cultivation of *G. orbiforme* in Petri dishes was carried out 5 times, this was done as a precaution in case of contamination of the isolates. The culture was then incubated at room temperature incubator with a temperature of 30 °C until the fungus filled the Petri dish (Agustina, 2020).

Testing Liquid Smoke against G. orbiforme

In vitro inhibition testing of liquid smoke against *G. orbiforme* was carried out based on the food poisoned technique. The food poisoning method is the method used by poisoning the growth of the fungus *G. orbiforme* through PDA growing media mixed with liquid smoke. This test was carried out by pouring liquid PDA media that had been homogenized with liquid smoke according to the concentration of the treatment into a Petri dish with a final volume of 20 ml and allowed to stand until the liquid media became solid. The pure culture of the fungus *G. orbiforme* was cut using a cork borer, and then inoculated in the middle of the PDA media that had been treated with the material. After the inoculation was carried out, the Petri dishes were then closed and sealed with plastic wrap, then incubated at room temperature for further observation (Wardoyo et al., 2020).

This research was carried out in a laboratory experiment with a completely randomized design (CRD) consisting of 6 concentrations of liquid smoke (0%, 1%, 2%, 3%, 4%, and 5%). Each treatment was repeated 4 times to obtain 24 experimental units.

Observation

Parameters observed in this study included macroscopic and microscopic characteristics of *G. orbiforme*, growth rate and inhibition of sago bark liquid smoke against *G. orbiforme*. Observation of the growth rate of *G. orbiforme* colonies was measured from the start of growth until the *G. orbiforme* colonies in the control treatment filled the Petri dishes. The growth rate is calculated by the formula:

$$\mu = \frac{X}{T} \quad (1)$$

with μ is growth rate (cm/day); X is colony diameter on the last day of observation (cm); T is number of observation days (days).

Observation of the inhibition of liquid smoke on the growth of *G. orbiforme* was carried out after the petri dishes in the control treatment were filled with fungi. The percentage of inhibition of *G. orbiforme* colony growth was calculated by the formula:

$$\text{Inhibition} = \frac{DC - DP}{DC} \times 100\% \quad (2)$$

with DC is control colony diameter (cm); DP is treated colony diameter (cm).

The research data were analyzed by analysis of variance and if it had a significant effect, then it was continued with Duncan's multiple range test at the confidence level α 0.05 using the SPSS ver. 23

Result and Discussion

Result

Liquid Smoke Total Phenol

The results of total phenol analysis using the Folin-Ciocalteu method using a UV-Vis spectrophotometer showed that sago bark liquid smoke had a phenol content of 74.40 mg GAE/g sample (7.44%) (Table 1).

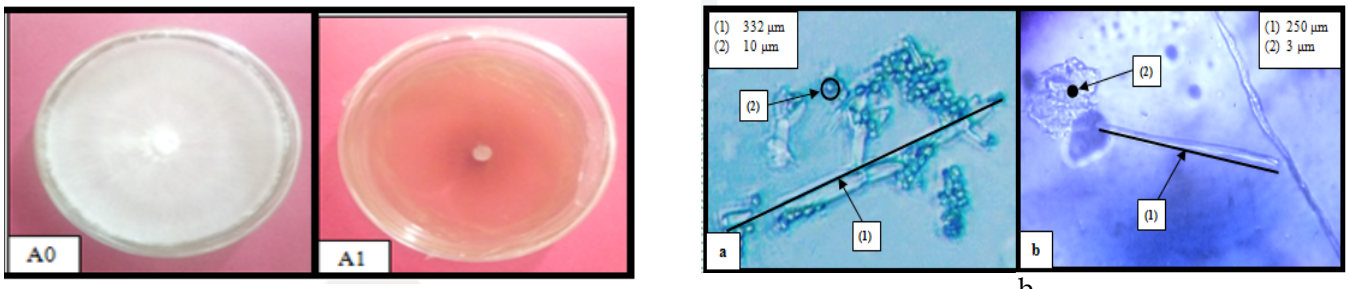
Macroscopic and Microscopic Characteristics of G. orbiforme

Based on the research that has been carried out, it appears that the application of liquid smoke from sago bark causes changes in the macroscopic characteristics of *G. orbiforme* colonies (Figure 1a) and microscopic characteristics of *G. orbiforme* (Figure 1b). Microscopic observations were carried out using a microscope at 1000x magnification.

Table 1. Total Phenolic Liquid Smoke Sago Bark

Test Sample	Total Phenol (%)*
KBS Liquid Smoke Grade 2	7.44

*Results are the average of three repetitions.



a b

Figure 1. Inhibition of *G. orbiforme* colony growth in sago bark liquid smoke test. a, macroscopic control (A0) and treatment (A1) colonies; b, microscopy on control (a) and treatment (b).

Table 2. Average growth rate of *G. orbiforme* for 14 days after incubation

Treatment	Growth Rate (cm/day)*
0% liquid smoke	0.64b
1% liquid smoke	0a
2% liquid smoke	0a
3% liquid smoke	0a
4% liquid smoke	0a
5% liquid smoke	0a

*Numbers followed by the same letter in the same column are not significantly different at α .0.05.

Growth Rate of G. orbiforme

Administration of sago bark liquid smoke had a significant effect on inhibiting the growth rate of *G. orbiforme* pathogenic colonies in Petri dishes. This is based on the research that has been done, in the treatment of liquid smoke with a concentration of 1% has shown inhibition of *G. orbiforme*. This is because the phenolic compounds contained in liquid smoke interfere with the metabolic processes of *G. orbiforme* and impair the ability of hyphae as food absorbers and reproductive organs (Aditria et al., 2013). Mahmud et al. (2016) stated that liquid smoke contains organic acid compounds such as carbonyl acid and phenolic derivatives such as 1.6-dimethoxy phenol which can interfere with the process of forming reproductive structures and metabolic processes in pathogenic fungi (Table 2).

Inhibitory Power of Liquid Smoke on the Growth of G. orbiforme

Liquid smoke from sago bark can inhibit the growth of *G. orbiforme*. In the control treatment there was

no growth inhibition of *G. orbiforme*, while in the liquid smoke treatment with a concentration of 1% to 5% it was able to inhibit the growth of *G. orbiforme* by 100% which was indicated by the absence of growth of *G. orbiforme* (Table 3). This proves that liquid smoke from sago bark is antimicrobial for *G. orbiforme*.

Effectiveness of Wet Weight and Dry Weight Effectiveness of G. orbiforme Colonies

The results showed that the concentration of liquid smoke had a significantly different effect on the effectiveness of wet weight loss and the effectiveness of dry weight loss of *G. orbiforme* colonies (Table 4).

The results showed that the higher the concentration of liquid smoke in the treatment, the effectiveness of the wet weight and the effectiveness of the dry weight of *G. orbiforme* colonies was higher. It is suspected that in the control treatment, *G. orbiforme* had a higher biomass because *G. orbiforme* grew well without any obstacles. Meanwhile, *G. orbiforme* colonies in liquid smoke treatment experienced growth inhibition resulting in lower biomass.

Table 3. Inhibition of sago bark liquid smoke against *G. orbiforme*

Treatment	Inhibition Percentage (%)
0% liquid smoke	0
1% liquid smoke	100
2% liquid smoke	100
3% liquid smoke	100
4% liquid smoke	100
5% liquid smoke	100

Table 4. Effectiveness of wet weight and dry weight of colonies *G. orbiforme* with various treatment concentrations.

Treatment	Wet Weight Effectiveness (%)*	Dry Weight Effectiveness (%)*
0% liquid smoke	0e	0e
1% liquid smoke	98.00d	80.50d
2% liquid smoke	98.23c	92.95c
3% liquid smoke	98.32b	96.76b
4% liquid smoke	98.33b	97.92a
5% liquid smoke	98.54a	98.01a

*Numbers followed by the same letter in the same column are not significantly different at α .0.05.

Discussion

Figure 1a. showed macroscopic differences in *G. orbiforme* in control Petri dishes and Petri dishes with liquid smoke treatment. Macroscopically, *G. orbiforme* on control Petri dishes had round-shaped colonies, concentric growth directions, the surface of the colonies was fibrous, the mycelium was white, the edges were filamentous and the elevations were raised due to the thickening of the mycelium. This is due to the absence of competition and inhibition for *G. orbiforme* to grow and develop (Merciere et al., 2017). Whereas in the liquid smoke treatment with a concentration of 1%, the mycelium of *G. orbiforme* did not experience growth. The same thing happened in the 2% to 5% treatment where the hyphae's ability to grow and form mycelium was difficult to occur, this was indicated by the absence of an increase in colony diameter. The inability of the *G. orbiforme* mycelium to grow properly is due to the active compounds contained in the liquid smoke. One of the active compounds that play an important role as antimicrobial, antifungal and antioxidant is phenolic compounds (Mahmud et al., 2016).

Figure 1b shows that the administration of sago bark liquid smoke resulted in microscopic changes to *G. orbiforme*. In the control treatment without liquid smoke administration, the microscopic characteristics of *G. orbiforme* were fine thread-shaped hyphae with a length of 332 µm and spherical conidia with a diameter of 10 µm (Figure 1b(a)). Whereas in the liquid smoke treatment, *G. orbiforme* had smaller hyphae with a length of 250 µm and a spore diameter of 3 µm (Figure 1b (b)). The mechanism of activity of phenol antimicrobial compounds in liquid smoke includes the deposition of pathogenic fungal proteins, inactivation of essential enzymes (isoleucine and tryptophan) and functional inactivation of genetic material (DNA and RNA) (Bivi et al., 2010; Apituley and Darmadji, 2013).

Observation of the wet weight and dry weight of *G. orbiforme* mushroom colonies showed that liquid smoke from sago bark had the ability to suppress the wet weight and dry weight of *G. orbiforme* colonies. The wet weight and dry weight of the fungal colonies were related to the colony area of *G. orbiforme*. Where large colony area shows high wet weight and dry weight of *G. orbiforme* colonies and vice versa with small colony area shows low wet weight and dry weight of colonies. This difference occurs because of the suppression of fungal growth and development. This indicates that the phenolic compounds contained in liquid smoke from sago bark have antifungal properties and inhibit hyphal growth, thereby affecting the small colony biomass. Liquid smoke is able to suppress growth and control the biomass of fungal colonies (Thamrin, 2007)

Conclusion

Based on the results of the study it was concluded that liquid smoke from sago bark at a concentration of 1% was very effective in suppressing the growth of *G. orbiforme*. This proves that sago bark liquid smoke has potential as an alternative fungicide in controlling fungal pathogens.

References

Aditria RB, Cahyono, & Swatawati F. (2013). Identifikasi komponen asap cair dari ampas sagu dan kulit

batang sagu (Metroxylon sagoo Rottb.) serta penentuan senyawa fenolat total dan aktivitas antioksidan. *Jurnal Chemical Info*, 1(1): 240-246. Retrieved from http://ejournal-s1.undip.ac.id/index.php/kimia/article/view/1884/1882

Agustina, N.A. (2020). Efektivitas Daya Hambat Asap Cair Tempurung Kelapa (Coccus nucifera) terhadap Pertumbuhan Jamur Ganoderma orbiforme, *Agroprimatech,* 3(2): 79-88. https://doi.org/10.34012/agroprimatech.v3i2.914

Apituley D. A. N dan Darmadji P. 2013. Daya hambat asap cair kulit batang sagu terhadap kerusakan oksidatif lemak ikan tuna (Thunnus sp.). *Agritech,* 33(2): 162-167. https://doi.org/10.22146/agritech.9797

Angraini E. 2017. Uji antagonisme Lentinus cladopus LC4 terhadap Ganoderma orbiforme penyebab penyakit busuk pangkal batang kelapa sawit. *Jurnal Biosfera,* 34: 144-149. https://doi.org/10.20884/1.mib.2017.34.3.512

Bivi MR, Farhana, S.N, Khairulmazmi, A., & Idris A. (2010). Control of Ganoderma orbiforme: a causal agent of basal stem rot disease in oil palm with endophyte bacteria and cernel palm liquid smoke. *International Journal of Agriculture and Biology,* 12(6): 833-839. Retrieved from http://www.fspublishers.org/published_papers/53592_..pdf

Chong, K.P, Dayou, J, &Alexander A. (2017). Detection and control of Ganoderma boninese in oil palm corp. *Journal Springer Briefs in Agriculture,* 8: 5-12. Retrieved from https://link.springer.com/book/10.1007/978-3-319-54969-9

Gultom, S.O, Silamba, I, Darmadji, P., & Pranoto Y. (2018). Produksi Asap Cair Berbahan Dasar Kulit Sagu (Metroxylon) sebagai Bahan Pengawet Alami Menggunakan Teknologi Pirolisis. Dalam: *Prosiding SNST ke-9 Tahun 2018 Fakultas Teknik Universitas Wahid Hasyi*m : 64-68. Retrieved from https://publikasiilmiah.unwahas.ac.id/index.php/PROSIDING_SNST_FT/article/view/2284/2272

Irfan, M. (2016). Uji pestisida nabati terhadap hama dan penyakit tanaman. *Jurnal Agroteknologi,* 6(2): 39-45. Retrieved from https://ejournal.uin-suska.ac.id/index.php/agroteknologi/article/download/2239/1451

Mahmud, K.N., Yahayu, M,. Sarip, S.H.M, Rizan, N.H, Min, C.B., Mustafa, N.F., Ngadiran, S,. Ujang, S., & Zakaria, Z.A. (2016). Evaluation on efficiency of pyroligneous acid from palm kernel shell as antifungal and solid pineapple biomass as antimicrobe and plant growth promoter. Sains Malaysiana, 45(10): 1423-1434. Retrieved from https://core.ac.uk/download/pdf/84306792.pdf

Merciere, M., Boulord, R., Lacombe, C.C., Klopp C, Lee YP, De franqueville H, Breton F, and Kulandaivelu LC. (2017). About Ganoderma orbiforme in oil palm plantations of sumatra and penisular Malaysia: ancient population expansion, exterme gene flow and large scale dispersion ability. *Journal Fungal Biology,* 1: 529-540. DOI: 10.1016/j.funbio.2017.01.001

Midot F, Lau SYL, Wong WC, Tung HJ, Yap ML, Lo ML, Jee MS, Dom SP & Melling L. (2019). Genetic diversity and demographic history of Ganoderma orbiforme in oil palm plantations of Sarawak, *Malaysia inferred from ITS Region*s. Microorganisms, 7(464): 1-17. https://doi.org/10.3390/microorganisms7100464

Pradhana, A.Y. & Trivana. L. (2018). Proses Pembuatan Asap Cair Tempurung Kelapa dan Pemanfaaannya. *Warta Penelitian dan Pengembangan Tanaman Industri*, 24(3): 21-25. Retrieved from https://www.litbang.pertanian.go.id/info-teknologi/3604/file/ProsesPembuatanAsapCairTem.pdf

Rungruang, P., & Suwanne, J. (2010). Antioxidative activity of phenolic compounds in pyroligneous acid produced from eucalyptus wood. *The 8th International Symposium on Biocontrol and Biotechnology,* 102-106

Sari, Y.P, Samharianto, & Langai, B.F. (2018). Penggunaan asap cair tandan kosong kelapa sawit (TKKS) sebagai pestisida nabati untuk mengendalikan hama perusak daun tanaman sawi (Brassica juncea L.). *Jurnal EnviroScienteae*, 14: 272-284. http://dx.doi.org/10.20527/es.v14i3.5699

Sarwendah, M., Feriadi, T. Wahyuni dan T.N. Arisanti. (2019). Pemanfaatan Limbah Komoditas Perkebunan untuk Pembuatan Asap Cair. *Jurnal Littri,* 25(1): 22 - 30. http://dx.doi.org/10.21082/littri.v25n1.2019.22-30

Thamrin. (2007). Efek Asap Cair Cangkang Kelapa Sawit terhadap Jamur Ganoderma sp. pada Kayu Kelapa Sawit. *Jurnal Sains Kimia*, 11 (1): 9-14. Retrieved from https://docplayer.info/59347221-Efek-asap-cair-cangkang-kelapa-sawit-terhadap-jamur-ganoderma-sp-pada-kayu-kelapa-sawit.html

Wardoyo, E.R.P., W. Anggraeni, Rahmawati dan H.A. Oramahi. (2020). Aktivitas Antifungi Asap Cair Tandan Kosong Elaeis guineensis Jacq. Terhadap Colletotrichum sp. (WA2). Jurnal Bioteknologi dan Biosains Indonesia, 7(2): 271-27. https://doi.org/10.29122/jbbi.v7i2.3582

Widiastuti, H., D.D. Eris & D. Santoso. (2016). Potensi Fungisida Organik untuk Pengendalian Ganoderma pada Tanaman Kelapa Sawit. *Jurnal Menara Perkebunan*, 84: 98-106. http://dx.doi.org/10.22302/iribb.jur.mp.v84i2.223