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Microbial activities and minimum liquid smoke killing concentration made of cacao pod toward *Lasiodiplodia theobromae* growth

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Abstract. Cocoa has become the leading export commodity from the plantation sector. The Increasing of cocoa production results in the increase of the cocoa shell waste amount. Cocoa shell utilization is still very limited. The benefit of cocoa shell is that this waste contains secondary metabolites that can be used as antimicrobial agents. One of the products that can be produced from cocoa shell waste is liquid smoke. This study was focused in determining the antimicrobial activity and the minimum kill concentration from liquid smoke on the growth of the Lasiodiplodia theobremae fungi from different amounts of raw materials moisture content and pyrolysis temperatures. This research indicated that cocoa shells liquid smoke from different amounts of water content and temperature of pyrolysis had antimicrobial activity and the application of different concentrations in the minimum kill concentration test can inhibit Lasiodiplodia theobremae fungi growth. Liquid smoke antimicrobial activity at 10% water content and temperatures at 200, 300, and 400°C obtained inhibition zone values of 10.40; 16.75 and 17.80 mm. At the 15% moisture content and temperatures of 200, 300and 400°C, the inhibition zone value is 10.15; 15.70 and 16.15 mm. At 20% water content and temperatures of 200, 300and 400°C, it obtained inhibition zone values of 4.25; 11,45 and 12.30 mm. At 25% water content and temperatures of 200, 300, and 400°C, it obtained inhibition zone values of 3.70; 7.65 and 8.65 mm. The value of minimum kill concentration and minimum inhibitory concentration of liquid smoke at 10, 15, 20, and 25 % water content at temperatures of 200, 300, and 400°C obtained the values of 1% and 9%.

Keywords: Cocoa Shell, Antimicrobial Activity, Lasiodiplodia Theobremae fungi

1. Introduction

Cocoa or Theobroma Cacao L has high economic value as the plantation crop with considerable market opportunities [1]. On smallholder plantations, cocoa fruit often has rotten fruit because of pests attack and plant diseases resulting in the decline in cocoa fruit production. One of the diseases that cause great losses in cocoa is fruit rot disease caused by Phytophthora sp [2]. In cocoa cultivation, cocoa shell waste has not been handled properly and has a great chance of spreading the disease that causes inoculum. The efforts to use cocoa shells need to be developed.

Cocoa fruit skin has lots of advantages if it is developed into the manufacture of smoke liquid. Some reports mention the content of cocoa shells has beneficial active compounds to humans. Cocoa shell contains flavonoids and phenolic compounds [3]. This polyphenol content includes cinnamic acid, pyrogallol, tannin, resorcinol, quercetin, and epicatechin-3-galat [4]. The main compounds found from cocoa shell are (-)- epicatechin and phidroxybenzoic acid [5], these compounds both have functions as antimicrobials [6][7].

Prior studies have reported that liquid smoke has the antimicrobial compound that can inhibit the growth of several fungi types [2][8][9][10]. This liquid smoke is observed as the fungicide to Lasiodiplodia theobremae fungi as the result of isolated cacao fruit that has been infested by pests diseases and also to determine minimum kill concentration (KBM) of Lasiodiplodia theobremae fungi growth during incubation. Based on this description, in order to maximize the use of cocoa shells as the liquid smoke raw material that has antimicrobial activity, the researchers have studied antimicrobial activity and minimum kill concentration of cocoa shell liquid smoke on Lasiodiplodia theobromae fungi growth.

2. Material And Methods

The research on cocoa shell has been conducted in the Agricultural Products Technology laboratory at Universitas Ekasakti and the Microbiology and Biotechnology of Agricultural Products laboratory at Universitas Andalas from April 2019 to May 2019. The research design was an exploratory study by using Lasiodiplodia theobremae fungi isolates that have been isolated from cocoa shells attacked by pests (Figure 1b). Liquid smoke can be produced from using pyrolysis tool from cocoa shells raw material with different levels of water content.

The materials in this study were cocoa shells from the village of Lubuk Minturun, Padang, it had been regulated according to the water content, and the process of pyrolysis was performed until cocoa shell liquid smoke was obtained. Potato Dextrose Agar (PDA) is used as the growth media and test media, Amoxycillin, aquades.

The equipment is the series of pyrolysis devices, autoclaves, ovens, Petri dishes, ose needles, Bunsen lamps, 250 ml Erlenmeyer, micropipettes, vortices, hot plate stirrers, incubators, plastic wrap, calipers, tweezers, and aluminum foil.

2.1 Cacao shell liquid smoke production [11]

Cocoa shell is weighed 2-5 kg and then the size is reduced. Drying under the sun to reach the water content following the treatment that is 10%, 15%, 20%, and 25%. Then it is poured into liquid smoke maker with the initial temperature of 27oC and sealed up airtight. The pyrolysis process is carried out using the appropriate treatment temperature of 200, 300, and 400oC. During the pyrolysis process, it is a necessity to make sure that enough water flows and inundate the condenser spiral pipe so that the condensation process is complete.

2.2 Isolation of the Lasiodiplodia theobremae fungi [12]

The symptomatic cocoa fruit is cut into a size of about 1 cm x 1 cm. The symptomatic fruit cut is then sterilized using Sodium Hypochlorite 1% for 2 minutes followed by distilled water for 2 minutes. This is done to avoid microbial contaminants that carry over so as not to grow when insulating. Pieces of fruit to be isolated were transferred to a Petri dish which had been filled with PDA for incubation. Fungi that have grown in a Petri then purified, by cutting the fungi isolated using cork borer size 0,7 cm and then transferred to another Petri which is already filled with PDA media and placed amid a Petri, then it is wrapped and incubated.

2.3 Test for antimicrobial activity and minimum kill concentration [13] [14]

An antifungal activity test was performed through the diffusion method of wells. The diffusion method is usually called media poisoning. Liquid PDA is poured a little on a petri dish as a base layer and after it is solid. The proponent is placed to make sumuran. The semi-solid PDA media which was still liquid

was then added to the test fungi suspension of 1 mL. The semi-solid PDA media is poured on top of the base layer which has been placed by the proponents and allowed to solidify. Once solid, the proponent sare removed using sterile tweezers. Sumuran that have been formed are then filled with the solution of smoke liquid pyrolysis from the cocoa shell as much as 50 mL, sterile distilled water as a negative control and M45 Dhitane brand synthetic pesticides as a positive control.

Test of minimum kill concentrations is done by making variations concentration of liquid smoke which is made into 6 concentrations by way of each of the liquid smoke pipette a volume of 1, 3, 5, 7, and 9 ml were diluted with 100 ml of water in order to obtain various concentrations of 1, 3, 5, 7, and 9% (v/v). Tests were carried out using the diffusion method using the Lasiodiplodia theobremae fungi planted on solid PDA media which had been mixed with liquid smoke from pyrolysis with concentrations of 1, 3, 5, 7, and 9% and water as a negative control. Observations were made on the growth inhibition of the Lasiodiplodia theobremae fungi from the first day after isolation until the growth of the Lasiodiplodia theobremae fungi in the control of meeting the petri dish, then measured the diameter of the fungal colonies. Inhibition of fungal growth Lasiodiplodia theobremae can be calculated using the formula.

$$P = \frac{(a - b)}{a} \times 100\%$$

Information: P is inhibition, a is fungal colonies diameter on control, and b is fungal colonies diameter in treatment

3. Results and Discussion

Cocoa shells infected with pests and diseases are isolated and observed in the laboratory. The results of the isolation of symptomatic cocoa are obtained as shown below:

Figure 1. Cocoa shell attacked by pests

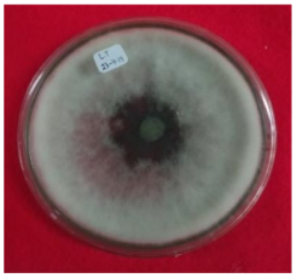

Figure 2. Lasiodiplodia theobromae fungi from isolation

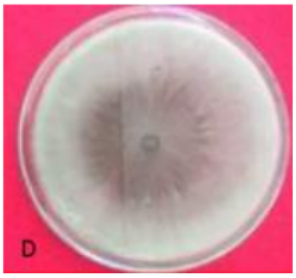

Figure 3. Lasiodiplodia theobromae isolates [12]

The form of macroscopic fungal isolates isolated from cacao has similarities with Lasiodiplodia theobromae (syn. Botryodiplodia theobromae) as shown in Figure 1, 2, and 3. Microscopically only insulated hyphae are only seen, while the conidia and their pituitary have not yet been seen. According to [15] on PDA media, L. theobromae from cocoa has not seen the formation of pycnidia, young conidia, and mature conidia, this is because the PDA media is one of the nutrient-rich media such as glucose and carbohydrates. According to Shivas and Beasley in [15] environment that is not natural, such as jelly medium rich in nutrients, can be less suitable conditions for sporulation of plant pathogenic fungi.

Antifungal activity test from liquid smoke extracts from the cacao shells pyrolysis on the pathogenic fungi growth in plants was performed using the diffusion method of wells. The method of Sumuran diffusion is the method to determine activity of liquid smoke extract from the pyrolysis of cocoa shell in inhibiting the growth of the fungi Lasiodiplodia theobromae by observing the forming of clear zone. The results of antimicrobial activity tests for cocoa shells liquid smoke with different levels of water content and temperature of pyrolysis can be seen from Table 1.

Table 1. Results of antimicrobial test for cocoa liquid smoke from Lasiodiplodia theobremae fungi

Treatment	Temp. (ºC)	Inhibition zone (mm)	Inhibition Category
10% of water content	200	10,40	Is
15% of moisture content		10,15	Is
20% of moisture content		4.25	Weak
25% of moisture content		3.70	Weak
10% of water content	300	16.75	Strong
15% of moisture content		15.70	Strong
20% of moisture content		11.45	Is
25% of moisture content		7.65	Is
10% of water content	400	17.8	Strong
15% of moisture content		16,15	Strong
20% of moisture content		12.3	Is
25% of moisture content		8.65	Is
Positive Control		21.3	Very strong
Negative Control		0	No

From the previous table, the antimicrobial activity of liquid cocoa shell smoke on the growth of Lasiodiplodia theobremae fungi shows that the cocoa shell liquid smoke with different variations in pyrolysis temperature and water content has antimicrobial compounds. At the pyrolysis temperature of 200oC liquid smoke of cocoa shell has moderate category antimicrobial activity at 10 and 15% of moisture contents, while on 20 and 25% of moisture contents have the weak category. At the pyrolysis temperature of 300oC liquid smoke of cocoa shell has a strong category of antimicrobial activity at 10 and 15% water content, while at 20 and 25% water content the medium category. At the pyrolysis temperature of 400oC liquid smoke of cocoa shell has strong category antimicrobial activity at 10 and 15% water content, while at 20 and 25% water content the medium category. Negative control in the form of sterile distilled water does not have antimicrobial activity and positive control has a very strong category of antimicrobial activity.

From the Table 1, the water content and pyrolysis temperature affect the liquid smoke antimicrobial activity from the cocoa shell produced. The lower of raw materials water content for making liquid smoke, the greater the antimicrobial activity produced. Similarly, from the temperature of pyrolysis in the liquid smoke manufacture, the higher of liquid smoke pyrolysis temperature, then the greater the antimicrobial activity produced. This is consistent with the results of the study [8], where higher pyrolysis temperatures produce liquid smoke with higher antimicrobial properties in inhibiting the growth of Aspergillus niger fungi. High pyrolysis temperatures produce higher levels of acids and phenols. The results of the study [16] revealed that liquid smoke has the ability as a material to control fungi, the higher of liquid smoke pyrolysis temperature, then the higher the inhibitory power against the growth of Trametes Versicolor and Fomitopsis palustris fungi. [17] states that the acids and phenols contained in liquid smoke act as antifungal agents.

Liquid smoke has antimicrobial (fungicide) activity so that it can act as a natural fungicide instead of chemical fungicides. According to [16], liquid smoke can inhibit Fomitopsis palustris and Trametes Versicolor growths. Recent research shows that it can also inhibit the fungi growth of Ophiostoma polonicum, O. narcissi, O. tetropii, and O. flexuosum [18] [19]. Phenolic compounds are known to have antibacterial and antifungal properties [20]. These compounds are secondary metabolites that are abundantly found in plants.

Table 2. Results of KHM and KBM tests of cocoa shell liquid smoke against the *Lasiodiplodia theobremae* fungi

Treatment	Temp. (ºC)	Concentration (%)	Diameter (mm)	Inhibitor (%)
10% water content	200	0	78.6	0
		1	58.7	25.32
		3	35.6	54.71

		5	17.1	78.24
		7	9.2	88.30
		9	0	100
		K + 5	0	100
15% moisture content	300	0	78.6	0
		1	69.3	11.83
		3	40.1	48.98
		5	16.6	78.88
		7	11	86.01
		9	0	100
		K + 5	0	100
20% of water content	300	0	78.6	0
		1	72.6	7.63
		3	61.1	22.26
		5	40.3	48.73
		7	17.2	78.12
		9	0	100
		K + 5	0	100
25% of water content	300	0	78.6	0
		1	78.4	0.25
		3	50.1	36.26
		5	29.8	62.09
		7	20.2	74.30
		9	0	100
		K + 5	0	100
10% water content	300	0	78.6	0
		1	54.5	30.66
		3	37.8	51.91
		5	14.7	81.30
		7	4.9	93.77
		9	0	100
		K + 5	0	100
15% moisture content	300	0	78.6	0
		1	57.6	26.72
		3	33.5	57.38
		5	15	80.92
		7	6.7	91.48
		9	0	100
		K + 5	0	100
20% of water content	300	0	78.6	0
		1	44.7	43.13
		3	31	60.56
		5	13.9	82.32
		7	10.2	87.02
		9	0	100
		K + 5	0	100
25% of water content	300	0	78.6	0
		1	62.8	20.10
		3	47.5	39.57
		5	22.6	71.25
		7	13	83.46
		9	0	100
		K + 5	0	100
10% water content	400	0	78.6	0
		1	38.9	50.51
		3	30	61.83
		5	13.4	82.95

	7	0.1	99.87
	9	0	100
	K + 5	0	100
15% moisture content	0	78.6	0
	1	47.5	39.57
	3	33.6	57.25
	5	11.1	85.88
	7	4.5	94.27
	9	0	100
	K + 5	0	100
20% of water content	0	78.6	0
	1	57.9	26.34
	3	36.6	53.44
	5	20.2	74.30
	7	8.6	89.06
	9	0	100
	K + 5	0	100
25% of water content	0	78.6	0
	1	59.2	24.68
	3	31.5	59.92
	5	17.9	77.23
	7	11.7	85.11
	9	0	100
	K + 5	0	100

From the table 2, minimum kill concentration (KBM) and the minimum inhibitory concentration (KHM) of liquid smoke with different levels of pyrolysis temperature and water content. This result indicated that the use of cocoa shells liquid smoke could inhibit the growth of Lasiodiplodia theobromae fungi. At 1% concentration of liquid smoke, it was able to inhibit Lasiodiplodia theobromae fungi growth, it indicates that these concentrations may be regarded as the minimum concentration of inhibitory (KHM) where the concentration of liquid smoke 9% growth of Lasiodiplodia theobromae fungi have been unable to grow on PDA, it means that the concentration is the minimum kill concentration (KBM).

Also from the table 2, at 9% concentration of liquid smoke from the skin of the Lasiodiplodia theobromae fungi cannot grow in all treatments namely at different water levels and pyrolysis temperatures. The results showed the treatment of pyrolysis temperature of 400oC and water content at 10% was the optimal treatment that was characterized by the amount of inhibition of liquid smoke on the growth of Lasiodiplodia theobromae fungi. The value of the percentage of inhibition in this treatment that is at 1% concentration of liquid smoke can inhibit 50.51% growth of Lasiodiplodia theobromae fungi, at a concentration of 7% can inhibit the growth of Lasiodiplodia theobromae fungi by 99.87% and at a concentration of 9% can inhibit the growth of Lasiodiplodia theobromae fungi by 100%. From this study, the researchers conclude that at a concentration of 7% the liquid smoke of cocoa shells can already be used as a minimum kill concentration because its value is not different from the 9% concentration.

Liquid smoke is known to contain secondary metabolites which can inhibit the bacteria and fungi growths. The main components are phenolic compounds. Phenolic compounds contained in liquid smoke can produce coagulation of microbial cell protein that further causes the microbial growth inhibition. Proteins coagulation can disrupt the peptidoglycan constituent components in microbial cells. This has caused the cell wall layer cannot be formed, and finally causing the death of cell. Phenolic compounds interact with membrane proteins of microbial cell from the adsorption process through binding the cell membrane hydrophilic part. Phenolic compounds enter the cell membrane and later it causes precipitation of cell protein. This disrupts permeability of cell membrane where cell membranes can undertake lysis [21]. The study results of minimum kill concentration (KBM) and minimum inhibitory concentration (KHM) are in the following.

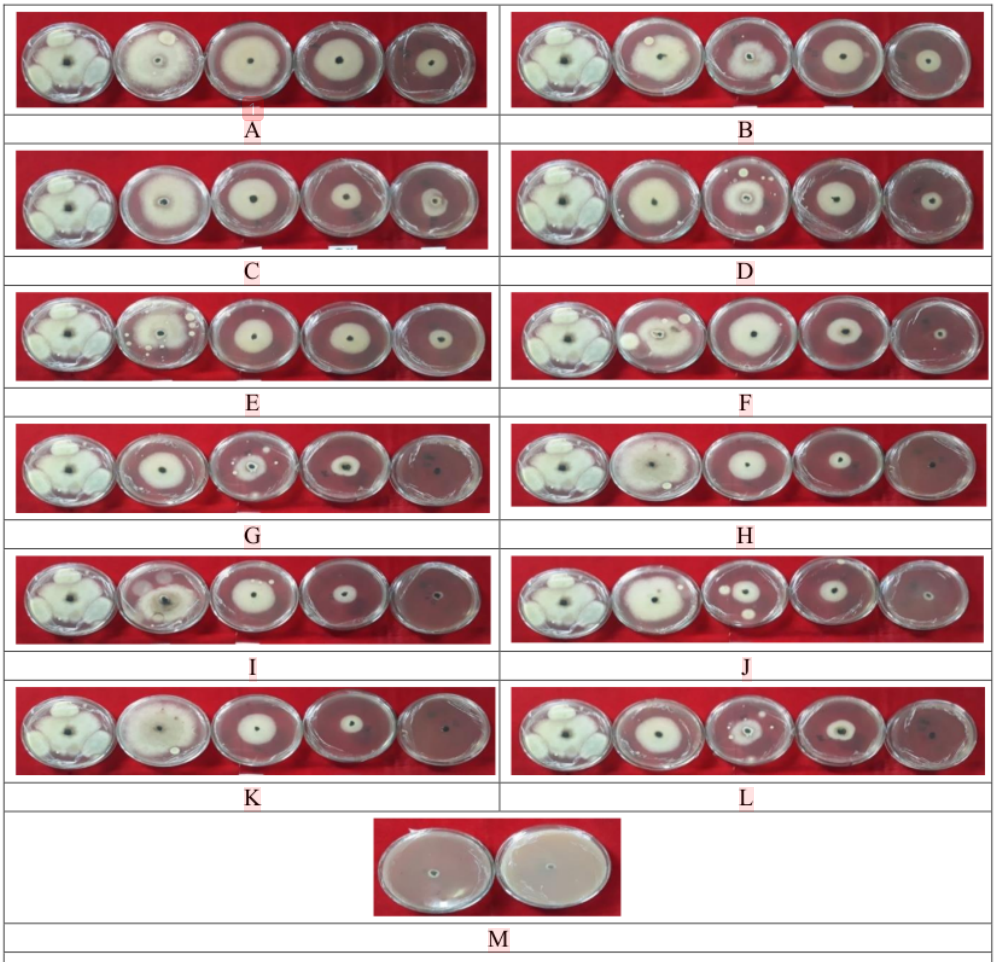

Figure 4. Minimum Kill Concentration (KBM) and Minimum Inhibitory Concentration (KHM) liquid smoke of cocoa shell. A (200;25), B (200;20), C (200;15), D (200;10) E (300;25), F (300;20), G (300;15), H (300;10), I (400;25), J (400;20), K (400;15), L (400;10), and M (Positive control 1 and 5%)

4. Conclusion

Liquid smoke antimicrobial activity on the water content of 10% at a temperature of 200, 300, and 400°C values obtained inhibitory zone at 10.40; 16.75 and 17.80 mm. At 15% water content at temperatures of 200, 300, and 400°C obtained inhibition zone values of 10.15; 15.70 and 16.15 mm. At 20% water content at temperatures of 200, 300, and 400°C obtained inhibition zone values of 4.25; 11, 45 and 12.30 mm. At 25% water content at temperatures of 200, 300, and 400°C obtained inhibition zone values of 3.70; 7.65 and 8.65 mm. The value of minimum kill concentration and minimum inhibitory concentration of liquid smoke at 10, 15, 20, and 25% water content at temperatures of 200, 300 and 400°C obtained values of 1% and 9%.

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References
[1] A. Amran, "Sudi Evaluasi Gerakan Nasional Peningkatan Produksi dan Mutu Kakao (Gernas Kakao) Di Kabupaten," 2009.
[2] E. Pangestu, I. Suswanto, and Supryanto, "Uji Penggunaan Asap Cair Tempurung Kelapa Dalam Pengendalian Phytopthora sp. Penyebab Penyakit Busuk Buah Kakao Secara In Vitro," J. Perkeb. dan Lahan Trop., vol. 4, no. 2, pp. 39–44, 2014.
[3] A. Jusmiati, R. Rusli, and L. Rijai, "Aktivitas antioksidan kulit buah kakao masak dan kulit buah kako muda," J. Sains dan Kesehat., vol. 1, no. 2, pp. 34–39, 2016.
[4] S. Fapohunda and A. Afolayan, "Fermentation of Cocoa Beans and Antimicrobial Potentials of the pod Husk Phytochemicals," J. Physiol. Pharmacol. Adv., 2012.
[5] M. Arlorio, J. D. Coıison, F. Travaglia, F. Varsaldi, G. Miglio, G. Lombardi, and A. Martelli, "Antioxidant and biological activity of phenolic pigments from Theobroma cacao hulls extracted with supercritical CO2," Food Res. Int., vol. 38, pp. 1009–1014, 2005.
[6] K. Osawa, K. Miyazaki, S. Shimura, J. Okuda, M. Matsumoto, and T. Ooshima, "Identification of cariostatic substances in the cacao bean husk : their anti-glucosyltransferase and antibacterial activities," J. Dent. Res., vol. 80, no. 11, pp. 2000–2004, 2001.
[7] R. M. Lamuela-raventós and C. Andres-la Cueva, "Review : Health effects of cocoa flavonoids," Food Sci. Technol. Int., vol. 11, no. June, pp. 159–176, 2005.
[8] H. A. Oramahi, F. Diba, and Wahdina, "Efikasi Asap Cair Dari Tandan Kosong Kelapa Sawit (TKKS) Dalam Penekanan Perkembangan Jmaur Aspergillus niger," J. HPT Trop., vol. 10, no. 2, pp. 146–153, 2010.
[9] H. A. Oramahi, F. Diba, and Wahdina, "Aktivitas Antijamur Asap Cair Dari Serbuk Gergaji Kayu Akasia (Acacia mangium WILLD) dan Kayu Laban (Vitex pubescent VAHL)," Bionatura - J. Ilmu-ilmu Hayati dan Fis., vol. 13, no. 1, pp. 79–84, 2011.
[10] M. Pallawagau, N. A. Yanti, M. Jahiding, O. La Kadidae, A. W. Asis, and F. H. Hamid, "Penentuan Kandungan Fenolik Total Liquid Volatile Matter dari Pirolisis Kulit Buah Kakao dan Uji Aktivitas Antifungi terhadap Fusarium oxysporum," Alchemy J. Penelit. Kim., vol. 15, no. 1, pp. 165–176, 2019.
[11] I. K. Budaraga, Arnim, Y. Marlida, and U. Bulanin, "Liquid Smoke Production Quality from Raw Materials Variation and Different Pyrolysis Temperature," Int. J. Adv. Sci. Eng. Inf. Technol., vol. 6, no. 3, pp. 306–315, 2016.
[12] T. R. Febbiyanti, A. P. J. Kusdiana, S. Wiyono, S. Yahya, and Widodo, "Pathogenicity Test Of Lasiodiplodia theobromae Isolates From Six Host Plants On Rubber and Their Phylogeny Analysis," Proc. Int. Rubber Conf., pp. 477–491, 2017.
[13] G. Sharma and R. R. Pandey, "Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes," J. Yeast Fungal Res., vol. 1, no. 8, pp. 157–164, 2010.
[14] E. Pangestu, I. Suswanto, and Supryanto, "Uji Penggunaan Asap Cair Tempurung Kelapa Dalam Pengendalian Phytophthora sp. Penyebab Penyakit Busuk Buah Kakao Secara In Vitro," J. Perkeb. dan Lahan Trop., vol. 2, no. 2, pp. 39–44, 2014.

[15] Y. S. Nurhasanah, “Karakterisasi Cendawan Botryodiplodia theobromae dan Rhizoctonia solani Dari Berbagai Tanaman Inang Berdasarkan Morfologi dan Pola RAPD-PCR,” 2012.
[16] S. Inoue, T. Hata, Y. Imamura, and D. Meier, “Components and Anti-fungal Efficiency of Wood vinegar liquor Prepared under Different Carbonization Conditions,” Wood Res., vol. 87, pp. 34–36, 2000.
[17] Y. Kimura, S. Suto, and M. Tatsuka, "Evaluation of Carcinogenic / Co-carcinogenic Activity of Chikusaku-eki , a Bamboo Charcoal By-product Used as a Folk Remedy, in BALB / c 3T3 Cells," Biol. Pharm. Bull, vol. 25, no. 8, pp. 1026–1029, 2002.
[18] N. Velmurugan, S. S. Han, and Y. Lee, "Antifungal Activity of Neutralized Wood Vinegar with Water Extracts of Pinus densiflora and Quercus Serrata Saw Dusts," Int. J. Environ. Res, vol. 3, no. 2, pp. 167–176, 2009.
[19] N. Velmurugan and Y. Lee, “Characterization of chikusaku-eki and mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory-scale Characterization of chikusaku-eki and mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory scale," Int. J. Environ. Sci. Tech, vol. 6, no. 1, pp. 13–22, 2009.
[20] R. M. Lamuela-raventós and C. Andres-la Cueva, "Review : Health Effects of Cocoa Flavonoids," Food Sci. Technol. Int., vol. 11, pp. 159–176, 2005.
[21] A. S. Mulyatni, A. Budiani, and D. Taniwiryono, "Aktivitas antibakteri ekstrak kulit buah kakao (Theobroma cacao L .) terhadap Escherichia coli, Bacillus subtilis , dan Staphylococcus aureus," Menara Perkeb., vol. 80, no. 2, pp. 77–84, 2012.

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