Submit ke Agrivita tgl November 08, 2019 Received: November 18, 2019 Accepted: December 14, 2020 Review I pada tanggal 18 Desember 2020 Revisi 19 Desember 2020 Copy editing 23 Desember 2020 Publish 01 Februari 2021 Accepted: December 14, 2020

Fwd: [AGRIVITA] Editor Decision Inbox ×



Witiyasti Imaningsih <witiyastiimaningsih@ulm.ac.id> to me 💌

------ Forwarded message ------From: AGRIVITA <agrivita@ub.ac.id> Date: Mon, 14 Dec 2020, 17:14 Subject: [AGRIVITA] Editor Decision To: Witiyasti Imaningsih <<u>witiyastiimaningsih@ulm.ac.id</u>>

Dear Dr. Witiyasti Imaningsih,

We have reached a decision regarding your submission to AGRIVITA, Journal of Agricultural Science, "Antifungal Activities of Combination Ulin Liquid Smoke Wood and Endophytic Fungi Isolated from Cayyene Pepper (Capsicum frustescens L) Hiyung Root Againts Colletotrichum capsici".

Our decision is to: accepted

Sincerely, Dr. Akhmad Rizali Editor

Agrivita Editorial Team Faculty of Agriculture University of Brawijaya JI. Veteran Malang 65145 East Java Indonesia E-mail : agrivita@ub.ac.id agrivitafaperta@yahoo.com website http://www.agrivita.ub.ac.id

Copy editing 23 Desember 2020



Antifungal Activities of Combination Ulin Liquid Smoke Wood and Endophytic Fungi Isolated from Cayenne Pepper (*Capsicum frustescens* L) Hiyung Root Against Colletotrichum capsici (Reduce titile and make not more than 20 words) (Running title: contain of 3-5 words or phrases and should be arranged as a sentence)

Silahkan di cek komentar di dalam naskah.

Witiyasti Imaningsih^{1,2*)}, Mariana³⁾, Ahmad Budi Junaedi⁴⁾ and Rasyidah²⁾

¹⁾ Biology Study Program, Mathematics and Science Faculty, Lambung Mangkurat University, South Kalimantan, Indonesia

²⁾ Microbiology Laboratory, Mathematics and Science Faculty, Lambung Mangkurat University, South Kalimantan, Indonesia

³⁾ Plant Protection Study Program, Faculty of Agriculture, Lambung Mangkurat University, South Kalimantan, Indonesia

⁴⁾ Chemistry Studi Program, Mathematics and Science Faculty, Lambung Mangkurat University, South Kalimantan, Indonesia

 $^{\ast)}$ Corresponding author E-mail: witiyastiimaningsih@ulm.ac.id

Received: November 18, 2019 /Accepted: December 14, 2020

ABSTRACT (max. 200 words)

Chili plants cultivated on the farmland face some constraints, one of them is a pathogenic fungus such as *Colletotrichum capsici*. Therefore, to overcome the obstacles of chili plant cultivation, indigenous endophytic fungi which has potency as antimicrobial can be used. At the same time, the usage of liquid smoke from the plant wood, such as the liquid smoke of the *Ulin* wood (*Eusideroxylon zwageri* Teijsm. & Binn.) which has an attributed of fungicidal. This research aimed to quantify and measure the effectiveness of an antimicrobial liquid smoke, endophytic filtrate, and the combination to suppress *C. capsici* growth. Subsequently, the research was conducted to apply the liquid smoke, endophytic fungi, and the two combinations of treatments on the growth of *C. capsici*. Thus, the results of this research showed that liquid smoke with a concentration of 0.085-1.75% can inhibit 3.56-62.17% in range. Meanwhile, the endophytic fungi filtrate, of 2% concentration can inhibit 91.69% *C. capsici*. Two of the combination liquid smoke in a concentration of 0.68%, 1.36% and the endophytic fungi filtrate in 2% have a demonstrated to inhibit the growth of *C. capsici* with the highest inhibition into 88.08%. Based on the analysis results, liquid smoke, endophytic fungi filtrate, and a combination of both showed significantly different inhibitory effects between treatments. This indicates that all those three treatments have antimicrobial potential.

Keywords: chili plant; endophytic fungi filtrate; the inhibitory effect

INTRODUCTION

Chilli plant is one of the horticultural commodities that has economical value in Indonesia. The demand for chili is increasing, encouraging farmers to cultivate chili plants. One of the chilies that are cultivated by farmers in the Tapin area of South Kalimantan is a Hiyung cayenne. The year 2012 Hiyung chili pepper is listed on the Center for Crop varieties Protection and Agriculture Licensing Ministry of Agriculture Republic of Indonesia No. 09/PLV/2012 dated 12 April 2012 as a local variety with the name of cayenne pepper (Pramudiani & Hasbianto, 2014), and in June 2012 the Ministry of Agriculture of the Republic of Indonesia established Cayenne pepper as a national variety. Cayenne Pepper has several advantages, such as high production amount, fruit number per plant height has a shelf life durability that is long enough about 10-16 days after harvesting at room temperature and has a high degree of robustness With capsaicin levels 802,95 ppm (Hamdani, Salawati, & Nuryadin, 2016).

Chili plants have various obstacles in the process of cultivation, the cause is the presence of plant destruction organisms (PEST), that can attack and disrupt the health of plants, one of which is a pathogenic fungus (Duriat, Gunaeni, & Wulandari, 2007). Species of fungi that can infect chili plants include *Colletotricum capsici, Cercospora capsici, Fusarium oxysporum, Stemphylum solani,* and *Leveilluls taurica* (Tsitsigiannis, Antoniou, Tjarnos, & Paplomatas, 2008). Fungi that cause disease in plants often cause structural and physical damage so that a lot of harm to agriculture (Marques, Soares, & Appezzato-Da-Gloria, 2013). The control is done with the use of fungicides because it is considered easy and quick to eradicate plant destruction organisms. The presence of excessive levels of fungicides that accumulates in the soil can be harmful to humans and the environment (Wightwick, Walters,

Allinson, Reichman, & Menzies, 2010). Safe and environmentally friendly control of pathogenic fungi can be done by utilizing microorganisms derived from the plant itself, such as endophyte microbes (Köhl, Postma, Nicot, Ruocco, & Blum, 2011).

Endophytic microbes can derive from bacteria and fungi groups that have the ability to create a colony, part or all of its life cycle on a plant network without harming its host (Köhl, Postma, Nicot, Ruocco, & Blum, 2011; Selim, El-Beih, AbdEl-Rahman, & El-Diwany, 2012; Strobel & Daisy, 2003). Endophytic fungi are found in the plant tissue system, including flowers, twigs, leaves, and plant roots. Endophytic microorganisms grow and take food from the plant, and can infect crops. Endophyte microorganisms grow and take food from the plant, and can infect trops in certain tissues as well as able to produce mycotoxin, enzymes, and antibiotics (Stone, Polishook, & White Jr, 2004). Endophyte fungi can inhibit pathogenic microbes that cause plant disease through mechanisms i.e. space and nutrition competitions and producing bioactive compounds such as antibacterial and antifungal (Gao, Dai, & Liu, 2010; Strobel & Daisy, 2003).

Compounds that have biological activity are produced by fungi such as Auxin (Imaningsih, Kadarsah, & Rusmannurrachmad, 2019) and especially for endophytic fungi such as alkaloids, terpenoids, and phenolic. Endophytic microbial metabolites exhibit antibacterial, antifungal, growth hormone, and insecticide activity. The activity of endophytic metabolite is a defense mechanism against bacterial and pathogenic attack on the stem (Tan & Zou, 2001).

Control of pathogenic mold as a plant destruction organism can also be done by utilizing liquid smoke. Liquid smoke is a vapor condensate of pyrolysis of wood containing the main compounds of acids, phenols, and carbonyl (Katja, Suryanto, Momuat, & Tambunan, 2008; Lee et al., 2011). The constituent components of the liquid smoke compound have the ability to inhibit the growth of fungi and bacteria (Mohan et al., 2008; Nami Kartal, Terzi, Kose, Hofmeyr, & Imamura, 2011; Okutucu, Duman, Ucar, Yasa, & Yanik, 2011; Sunarsih, Pratiwi, & Sunarto, 2012). Basri (2010) mentioned that liquid smoke in agriculture can be utilized, among others, to improve soil quality, neutralize soil acid, kill pests, and insect repellents. The liquid smoke of pine and oak wood can inhibit the growth of the mold Gloeophyllum trabeum and Trametes versicolor (Mohan et al., 2008). *Ulin* wood (*Eusideroxylon zwageri* Teijsm. & Binn.) liquid smoke contains acids and phenol compounds with acidity levels up to pH 2.08 (Junaidi, Apriyani, Abdullah, & Santoso, 2019) so that it has the potential to inhibit the growth of

Cayenne pepper originated from the village of Hiyung Tapin Regency of South Kalimantan is a natural resource that must be guarded, therefore it is necessary to do an exploration of endophytes potential as an antimicrobial agent for pathogenic fungi. The potency of endophytes as an antimicrobial to pathogenic mold is known for its mechanisms including bioactive compounds as antibacterial and antifungal. Acetic acid and phenol which are the main constituent compounds of liquid smoke have the ability as antimicrobial. Endophytic molds and liquid smoke have the two. This research was intended to study the ability of endophytic fungi and liquid smoke to inhibit the growth of pathogenic fungi and examine the ability of endophytic fungi added by a combination of liquid smoke at different concentrations to the growth of pathogenic fungi.

MATERIALS AND METHODS

Isolation and Purification of Endophytic Fungi

This research was conducted from November 2018 to April 2019 at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat, Banjarbaru, South Kalimantan. Samples of chili cayenne pepper plants were taken from the village of Hiyung, Tapin District, South Kalimantan in November 2018. The chili plants that were taken were put in polybags and coded as sampling information. Isolation and purification of endophytic fungus were carried out to obtain pure isolates by direct planting methods. The procedure is performed based on the method used by Nurzannah, Lisnawita, & Bakti (2014).

Identification and Screening of Endophytic Fungi

The fungi Identification includes macroscopic and microscopic observation. The macroscopic observation of pure isolates is performed by observing the shape, color, and diameter of the colony for 7 days, the color and presence of the Hypha region determined on the 7th day (Budi R., Santoso, & Wahyudi, 2010; Gusnawaty, Taufik, Triana, & Asniah, 2014). Microscopic observation was done by the method of slide culture (Budi R., Santoso, & Wahyudi, 2010; Rosana, Matsuzawa, Gonoi, & Karuniawati, 2014). Isolation identification refers to the Fungi identification book: Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 1998) and The Genera of Hyphomycetes (Seifert & Gams, 2011).

Screening of endophytic mold is conducted through the test of pathogenicity and antagonism. The pathogenicity test was carried out against the endophyte isolates that were derived from the roots

Commented [L1]: Or Lambung Mangkurat University or Universitas Lambung Mangkurat? Please be consistent and check the institution in the authors identities as well of healthy chili plants based on the methods done by Irawati et al. (2017). Percent germination is obtained by dividing the number of germinated seeds (normal/ abnormal / not growing) by the total number of seeds multiplied by 100% (Kasmiyati, Santosa, Priyambada, Dewi, & Sandradewi, 2015). The antagonism test refers to the dual culture method in the PDA medium (Rahman, Begum, & Alam, 2009). The inhibitory percentage was obtained by dividing the difference from the diameter of the control pathogen colony and the diameter of the treatment pathogen colony by the diameter of the control pathogen multiplied by 100% (Mawardika & Suharjono, 2015).

Production of Endophytic Fungus Filtrate

The endophytic fungus was prepared using the method of Hasanah, Riwayati, & Idramsa (2015) and Suciatmih, Yuliar, & Supriyati (2011) that has been modified. The endophytic fungus was inoculated in a slanted PDA medium incubated for 7 days, then harvested by adding 9 ml of sterile distilled water to a tube containing endophytic molds. The culture was homogeneous using a soft brush to obtain a spore suspension, then the suspension was transferred into another sterile test tube. Fungus suspension is centrifuged at a speed of 3000 rpm for 20 minutes to get the filtrate supernatant. The results of the process were separated using a 0.45 μ m syringe filter and used as a crude biocontrol agent for further testing.

Inhibition Ability Test of Liquid Smoke Endophytic Fungi on C. capsici

Test the ability to inhibit liquid smoke is carried out by the agar dilution method (Pangestu, Suswanto, & Supriyanto, 2014). The liquid smoke used in this study came from ulin wood (*Eusideroxylon zwageri* Teijsm. & Binn.) obtained from the result of condensation of ulin charcoal smoke production in Ranggang Village, Takisung District, Tanah Laut Regency, South Kalimantan. This method is carried out by mixing the media with liquid smoke, and the pathogen *C. capsici* is inoculated on the media that has been mixed with liquid smoke. The concentration used in this test was 0.00%; 0.17%; 0.34%; 0.68%; 1.36%; 1.75%. Observations were made from day 1 to day 7 after inoculation. The minimum inhibitory concentration was determined by the dilution method, using the method of Malinda, Soekarno, & Yuliani (2015) that has been modified. The concentration of endophytic fungus used in this test was 0%; 2%; 4%; 6%; 8%; 10% Positive control treatment using ketoconazole 200 mg at a concentration of 2%. Pathogenic mold pieces 6 mm inoculated in the middle of the PDA medium positive control treatment and concentration treatment of endophytic fungus filtrate, then incubated at 28°C for 7 days, and observations were made every day. The minimum inhibitory concentration is determined from the presence or absence of pathogenic fungus growth at the lowest concentration of endophytic fungus filtrate.

Inhibitory test of the combination of endophytic fungus and liquid smoke was carried out based on the method carried out by Pangestu, Suswanto, & Supriyanto (2014), the combination of endophytic fungus filtrate (EFF) and liquid smoke (LS) was 2% for filtrate and 0.68%, 1.36% for liquid smoke. So the first combination is 2% (EFF) + 0.68% (LS) and 2% (EFF) + 1.36% (EFF).

A 6 mm pathogenic fungus is inoculated in the middle of the PDA medium in a Petri dish. Observation of inhibition of pathogenic fungus was carried out from day 1 to day 7 after inoculation. The inhibitory effect was obtained by dividing the difference from the diameter of the control pathogen colony and the diameter of the treatment pathogen colony by the diameter of the control pathogen multiplied by 100% (Noveriza & Tombe, 2003).

Data Analysis

Analysis of the data using One-Way ANOVA at alpha 0.05 was followed by Duncan's test, or when the data is not homogeneous, the Kruskal Wallis test is used. All data were analyzed using IBM SPSS Statistic Version 22, 2013.

RESULTS AND DISCUSSION

Diversity of Endophytic Fungi from Roots of The Cayyane Pepper Hiyung

Isolation and purification of endophytic fungi derived from chili cayenne root were obtained by 8 isolates and given a code name using a sample source that is chili root (ACH). Isolates obtained from chili cayenne roots were characterized macroscopically and microscopically. Pure isolates obtained were *Trichoderma* sp. ACH1.1, *Trichoderma* sp. ACH1.6, *Trichoderma* sp. ACH2.2, *Botrytis* sp. ACH2.3, *Gliocladium* sp. ACH2.4, *Harmoniella* sp. ACH2.5, *Humicola* sp. ACH2.6, *Cunninghamella* sp. ACH2.7.

The factors affecting the presence of endophytic fungi include the environment and the plant tissue used (Maheswari & Rajagopal, 2013). The Habitat of plant origin is one of the environmental factors affecting the structure and type of microbes that colonize the plant tissue such as roots, stems, leaves, and branches (Araújo et al., 2002). Sieber & Grünig (2006) mentions that affecting the diversity of endophytes derived from plants is environmental factors, vegetation, and interactions with other types

of microbes. Isolates obtained from the roots of cayenne pepper are characterized by macroscopic and microscopic. Microscopic characteristics refer to the books Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 1998) and The Genera of Hyphomycetes (Seifert & Gams, 2011).

Pathogenicity and Antagonism of Endophytic Fungi from the Roots of Hiyung Cayenne Pepper

Screening of endophytic fungi was carried out by pathogenicity and antagonistic tests. The pathogenicity test was carried out on pure isolates resulting from the isolation of endophytic fungi. The percentage rate of pepper seed germination on the 7 and 14th day after inoculation (DAI) in the pathogenicity test is presented in Table 1. The fungus penetrates its host through mechanical and enzymatic mechanisms (Ashry & Mohamed, 2012). Fungi penetrate the epidermis, the cuticles, and cell walls (Underwood & Somerville, 2008). Enzymes that act as degenerating cell walls are pectinase and cellulase, where these enzymes are used for fungus for the process of penetration and colonization of host plants (Ashry & Mohamed, 2012; Kikot, Hours, & Alconada, 2009).

The antagonistic test was carried out on 3 selected isolates from the results of the pathogenicity test using the dual culture method. The results of antagonistic tests based on diameter measurements of pathogenic fungi colonies and inhibitory effect can be seen in Table 2. This antagonistic nature is consistent with the statements of Melysa, Suharjono, & Dwiastuti (2013) that fungus is grown side by side and has the ability to grow faster, then these fungi are able to occupy space and suppress the growth of their opponent's fungus. This antagonistic nature occurs because of the same needs as each fungus, nutrition, and growing needs.

Endophytic screening results showed isolates of *Cunninghamella* sp. ACH2.7 was selected as an isolate used for testing endophytic fungus filtrate and in combination using liquid smoke. This was obtained from the Kruskal Wallis test where the isolate had the highest normal growing percentage of sprouts, the lowest abnormal sprouts, and the lowest ungrown sprouts as well as the highest percent inhibition of pathogens (Table 1 and Table 2).

En de abadía	Germination (%)*						
fungi	7 th -DAI			14 th -DAI			
	Normal	Abnormal	Not grow	Normal	Abnormal	Not grow	
Without	100±0c	0±0a	0±0a	100±0c	0±0a	0±0a	
endophytic fungi addition							
Trichoderma sp. ACH1.1	0±0ab	0±0a	100±0ab	30±51.96ab	6.67±5.77a	63.33±55.07ab	
Trichoderma sp.	0±0ab	0±0a	100±0ab	33.33±41.63ab	10±10a	56.67±45.09ab	
Trichoderma sp.	0±0a	23.33±20.82ab	76.66±20.81ab	0±0a	60±30ab	40±30ab	
ACH2.2		40.07.00.00.1				70 00 15 1	
Botrytis sp. ACH2.3	0±0a	16.67±28.86ab	83.33±28.86ab	0±0a	30±26.46ab	70±26.45ab	
Gliocladium sp.	0±0a	0±0a	100±0b	0±0a	26.67±30.55a	73.33±30.55b	
Harmoniella	0±0abc	3.33±5.77a	96.66±5.77ab	90±0abc	3.33±5.77a	6.67±5.77ab	
Humicola sp.	10±17.32abc	0±0a	90±17.32ab	73.33±23.1abc	13.33±11.55a	13.33±11.54ab	
Cunninghamella sp. ACH2.7	66.67±25.16bc	0±0a	33.33±25.16ab	90±10bc	0±0a	10±10ab	

Table 1. The percentage rate of pepper seed germination on the 7 and 14^{th} day after inoculation (DAI) in the pathogenicity test

Remarks: * The number followed by the same letter is not significantly different based on Duncan (α=0.05)

 Table 2. Pathogenic fungi colony diameter and inhibitory effect of endophytic fungi against *C. capsici*

 7th day after inoculation (antagonisms test result)

Endophytic fungi	Colletotrichum capsici Diameter (mm)	Inhibitory effect (%)*

Harmoniella sp. ACH2.5	43.33±2.82	10.71±4.79 b
Humicola sp. ACH2.6	46.50±2.55	5.11±0.18 c
Cunninghamella sp. ACH2.7	32.23±1.50	33.26+2.54 a

Remarks: * The number followed by the same letter is not significantly different based on the Kruskal Wallis Test $(\alpha\text{=}0.05)$

The Ability of Liquid Smoke and Endophytic Fungi Inhibits C. capsici

Based on the test results of liquid smoke ability inhibit the growth of *C. capsici*, obtained at all concentrations of liquid smoke is able to inhibit growth. The concentration of ironwood liquid smoke from 0.085-1.75% can inhibit *C. capsici* by 3.56-62.17%. But only at concentrations of 0.34%, 0.68%, 1.36%, and 1.75% showed significant inhibition compared to control (0.00% liquid smoke concentration). Inhibitory effect (%) of liquid smoke against *C. capsici* on the 1-7th day after inoculation is shown in Fig. 1. The inhibition effect was significantly different between treatments (F=11.053, P=0.000). Ironwood liquid smoke inhibits *C. capsici* causing changes in the diameter of the colony. The increasing concentration of liquid smoke the smaller the colony that forms (Fig. 2). The condition occurs because the content of liquid smoke affects the growth of fungi. Phenol and acid compounds in liquid smoke can damage the structure of the fungus. The acid content of liquid smoke causes acid conditions in the cytoplasm, therefore causing damage to membrane surface tension and loss of active transport, resulting in unstable function and structure of cell components (Ray, 1996; Ray & Sandine, 1992).

The best concentration of liquid smoke is then selected for the combination test. Endophytic fungal filtrate tests selected from previous tests were also conducted to determine the minimum inhibitory concentration (MIC) to be used to test the combination of endophytic fungal filtrate and liquid smoke. Based on the test results, endophyte fungi filtrate can inhibit the growth of *C. capsici*. The results of the inhibition of endophilic fungus filtrate can be seen in Fig. 3. Inhibitory effects differ significantly between treatments (F = 41.634, P = 0.000). All concentration treatments differ significantly from control (ketoconazole 2%). Even at a concentration of 2%, *Cunninghamella* sp. ACH2.7 filtrate is already able to inhibit *C. capsici* (91.69%), even better when compared to ketoconazole 2% 200 mg as a positive control.



Fig. 1. Inhibitory effect (%) of liquid smoke against *C. capsici* on the $1-7^{th}$ day after inoculation the bar indicates standard deviation between replicates. The concentration of liquid smoke followed by the same letter is not significantly different based on Duncan test (α =0.05).



Commented [L2]: Please check again all the graphic, make sure the decimal numbers are separated by dot not comma, figyre should not be framed, and all the numbers should be readable





Fig. 3. Inhibitory effect (%) of *Cunninghamella* sp. ACH2.7 filtrate against *C. capsici* on the 1-7th day after inoculation the bar indicates standard deviation between replicates. The concentration of crude extract followed by the same letter is not significantly different based on Duncan test (α =0.05)

In addition to inhibiting growth, isolate *Cunninghamella* sp. ACH2.7, also causes the morphology of *C. capsici* to change. One of them is a colony that was originally blackish gray to be lighter gray-white on PDA medium. These discoloration and morphology are likely caused by the ability of compounds produced by endophytic fungi to damage the structure of *C. capsici*. Sunarwati & Yoza (2010) explained that the interaction of endophyte and pathogenic fungi causes the occurrence of lysis mechanisms that are characterized by changing the color of pathogenic hyphae because the contents of cells are utilized for endophyte fungi nutrients.

The Synergistic of Endophytic Mold and Liquid Smoke Filtrate Inhibits C. capsici

Based on the previous test results, 2 concentrations of ironwood liquid smoke (LS) were used (0.68% and 1.36%) and MIC concentration of filtrate *Cunninghamella* sp. ACH27(2%) (EFF) to be combined so that the synergy can be known. The combination of 0.68% LS and 2% EFF, as well as 1.36% LS and 2% EFF is able to inhibit the growth of *C. capsici* differs significantly with 2% ketoconazole as control (F=14.676, P=0.000) (Fig. 4). The utilization of a combination of liquid smoke and endophytic fungus filtrate as an antimicrobial can be developed with the use of the right

Commented [L3]: Please send to us the fig files in HD, 4-6 MB. The font in image should balance with the image

concentration because both of these ingredients have active compounds that can be utilized. Liquid smoke and endophyte fungi have the same benefits that act as antibacterial and antifungal. In line with Aisyah, Juli, & Pari (2013) that liquid smoke is able to inhibit the growth of fungi, in his research on coconut shell liquid smoke. Gunatilaka (2006) also stated that endophyte fungi can produce secondary metabolites and compounds that act as an antifungal and antibacterial agent.





The combination of liquid smoke and the endophytic filtrate is able to inhibit growth and cause the morphology of *C. capsici* to change. Morphological changes in *C. capsici* due to the presence of active compounds that can damage cell membranes. Phenols from liquid smoke and flavonoids from endophytic fungi can improve the permeability of cell membranes, as well as inhibit the activation of essential enzymes, and the functioning of genetic materials (Davidson & Branden, 1981; Karseno, Darmadji, & Rahayu, 2001; Konaté et al., 2012).

The treatment of a combination of liquid smoke and endophytic fungi filtrate in vitro is able to inhibit the growth of *C. capsici*, so from these results, there is the potential that the combination of those two compounds can be antimicrobial agents that can be used to control disease-causing pathogens in plants. Utilization of a combination of liquid smoke and endophytic fungi filtrate as antimicrobial agents can be developed with the use of appropriate concentrations because both of these materials have active compounds.

CONCLUSION

The combination of liquid smoke and endophytic fungi filtrate has the ability to inhibit *C. capsici*, and has the potential to be antimicrobial. The highest inhibitory power was generated in a combination of 1.36% liquid smoke and 2% endophytic fungi filtrate at 88.08%. The benefits of the combination of liquid smoke and endophytic fungi filtrate need further research, especially its potential as antimicrobial and appropriate concentration so that it can be utilized to control pathogens that can damage crops.

ACKNOWLEDGEMENT

This study was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia in 2018-2019. The authors would like to thank the Mr. Junaidi Secretary of Hiyung Village and farmer group "Karya Baru" Hiyung Village, Tapin Tengah District, Tapin Regency, South

Kalimantan. And also sincere thanks conveyed to the charcoal production business group "TALASIANA" at Ranggang Village, Takisung District, Tanah Laut Regency, South Kalimantan.

REFERENCES

Mohon utk dpt melakukan updating references dengan artikel jurnal scopus yang terbit pada tahun 2018-2020 dan juga mohon utk mengganti references dari jurnal yang belum terrindex sinta dengan artikel yang setidaknya terbit di jurnal sinta 2 karena ada beberapa pustaka yang websitenya tidak dpt kami akses. Setiap publikasi artikel wajib dilengkapi dengan DOI atau url artikel. Penambahan atau perubahan daftar pustaka mohon untuk diberi warna lain baik di text maupun di references list.

Pramudiani, L., & A. Hasbianto. 2014. *Cabai Hiyung, si Kecil* Yang Rasanya Sangat Pedas. http://kalsel.litbang.pertanian.go.id/ind/index.html

- Aisyah, I., Juli, N., & Pari, G. (2013). Pemanfaatan asap cair tempurung kelapa untuk mengendalikan cendawan penyebab penyakit antraknosa dan layu fusarium pada ketimun. Jurnal Penelitian Hasil Hutan, 31(2), 170–178. https://doi.org/10.20886/jphh.2013.31.2.170-178
- Araújo, W. L., Marcon, J., Maccheroni Jr, W., van Elsas, J. D., van Vuurde, J. W. L., & Azevedo, J. L. (2002). Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Applied and Environmental Microbiology*, *68*(10), 4906–4914. https://doi.org/10.1128/aem.68.10.4906-4914.2002
- Ashry, N. A., & Mohamed, H. I. (2012). Impact of secondary metabolites and related enzymes in flax resistance and/or susceptibility to powdery mildew. *African Journal of Biotechnology*, *11*(5), 1073–1077. Retrieved from https://www.ajol.info/index.php/ajb/article/view/100252
- Barnett, H. L., & Hunter, B. B. (1998). Illustrated genera of imperfect fungi (4th ed.). Amer Phytopathological Society. Retrieved from https://my.apsnet.org/ItemDetail?iProductCode=41922
- Basri, A. (2010). Manfaat asap cair untuk tanaman. Serambi Pertanian, 4(5), 1–2. Retrieved from http://nad.litbang.pertanian.go.id/ind/images/dokumen/SerambiPertani/01-Cabe-asapcair.pdf
- Budi R., S. W., Santoso, E., & Wahyudi, A. (2010). Identifikasi jenis-jenis fungi yang potensial terhadap pembentukan gaharu dari batang Aquilaria spp. Jurnal Silvikultur Tropika, 1(1), 1–5. Retrieved from https://repository.ipb.ac.id/handle/123456789/54435
- Davidson, P. M., & Branden, A. L. (1981). Antimicrobial activity of non-halogenated phenolic compounds. Journal of Food Protection, 44(8), 623–632. https://doi.org/10.4315/0362-028x-44.8.623
- Duriat, A. S., Gunaeni, N., & Wulandari, A. W. (2007). Penyakit penting tanaman cabai dan pengendaliannya. Monografi No. 31. Bandung, Indonesia: Balai Penelitian Tanaman Sayuran. Retrieved from http://balitsa.litbang.pertanian.go.id/ind/images/isi_monografi/M-31 Penyakit Penting pada Tanaman Cabai dan Pengendaliannya.pdf
- Gao, F., Dai, C., & Liu, X. (2010). Mechanisms of fungal endophytes in plant protection against pathogens. *African Journal of Microbiology Research*, *4*(13), 1346–1351. Retrieved from https://academicjournals.org/article/article1380280021_Gao et al.pdf
- Gunatilaka, A. A. L. (2006). Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity, and implications of their occurrence. *Journal of Natural Products*, 69(3), 509–526. https://doi.org/10.1021/np058128n
- Gusnawaty, H., Taufik, M., Triana, L., & Asniah. (2014). Karakterisasi morfologis Trichoderma spp. indigenus Sulawesi Tenggara. Jurnal Agroteknos, 4(2), 88–94. DOI?? Jurnal link???
- Hamdani, Salawati, U., & Nuryadin, R. (2016). Daya saing agribisnis dan potensi pengembangan cabe hiyung di Kabupaten Tapin, Kalimantan Selatan. In *Prosiding Seminar Nasional Lahan BasahTahun 2016 Jilid 1* (pp. 156–163). Lambung Mangkurat University Press. Retrieved from http://eprints.ulm.ac.id/2778/
- Hasanah, U., Riwayati, & Idramsa. (2015). Uji antijamur patogen ekstrak metabolit sekunder jamur endofit tumbuhan raru (*Cotylelobium melanoxylon*). Jurnal Biosains, 1(2), 6–12. Mohon diganti dengan artikel dari scopus indexed jurnal.
- Imaningsih, W., Kadarsah, A., & Rusmannurrachmad, R. D. T. (2019). The capability of consortium phosphate solubilizing bacteria and IAA producing fungi on promoting elephant grass growth. *Jurnal Biodjati*, 4(1), 138–148. https://doi.org/10.15575/biodjati.v4i1.4284
- Irawati, A. F. C., Mutaqin, K. H., Suhartono, M. T., Sastro, Y., Sulastri, & Widodo. (2017). Eksplorasi dan pengaruh cendawan endofit yang berasal dari akar tanaman cabai terhadap pertumbuhan benih cabai merah. Jurnal Hortikultura, 27(1), 105–112. https://doi.org/10.21082/jhort.v27n1.2017.p105-112

Commented [K4]: The URL can not be accessed. Please change the reference or update the URL

- Junaidi, A. B., Apriyani, H., Abdullah, & Santoso, U. T. (2019). Fraksinasi dan karakterisasi asap cair dari kayu ulin (*Eusideroxylon zwageri* Teijsm. & Binn.) sebagai pelarut kitosan. *Jurnal Riset Industri Hasil Hutan*, 11(2), 53–64. https://doi.org/10.24111/jrihh.v11i2.4861
- Karseno, Darmadji, P., & Rahayu, K. (2001). Daya hambat asap cair kayu karet terhadap bakteri pengkontaminan lateks dan ribbed smoke sheet. *Agritech*, *21*(1), 10–15. Retrieved from https://jurnal.ugm.ac.id/agritech/article/view/13639
- Kasmiyati, S., Santosa, Priyambada, I. D., Dewi, K., & Sandradewi, R. (2015). Perkecambahan biji dan pertumbuhan kecambah varietas sorgum (*Sorghum bicolor* L.) pada cekaman krom heksavalen. *Bioma: Berkala Ilmiah Biologi, 17*(1), 41–54. https://doi.org/10.14710/bioma.17.1.41-54
- Katja, D. G., Suryanto, E., Momuat, L. I., & Tambunan, Y. (2008). Pengaruh adsorben terhadap aktivitas antioksidan dari asap cair kayu cempaka (*Michelia champaka* Linn). *Chemistry Progress*, 1(1), 54–59. Retrieved from https://ejournal.unsrat.ac.id/index.php/chemprog/article/view/27
- Kikot, G. E., Hours, R. A., & Alconada, T. M. (2009). Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: A review. *Journal of Basic Microbiology*, 49(3), 231–241. https://doi.org/10.1002/jobm.200800231
- Köhl, J., Postma, J., Nicot, P., Ruocco, M., & Blum, B. (2011). Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. *Biological Control*, 57(1), 1– 12. https://doi.org/10.1016/j.biocontrol.2010.12.004
- Konaté, K., Hilou, A., Mavoungou, J. F., Lepengué, A. N., Souza, A., Barro, N., ... Nacoulma, O. G. (2012). Antimicrobial activity of polyphenol-rich fractions from Sida alba L. (Malvaceae) against co-trimoxazolresistant bacteria strains. Annals of Clinical Microbiology and Antimicrobials, 11, 5. https://doi.org/10.1186/1476-0711-11-5
- Lee, S. H., H'ng, P. S., Chow, M. J., Sajap, A. S., Tey, B. T., Salmiah, U., & Sun, Y. L. (2011). Effectiveness of pyroligneous acids from vapour released in charcoal industry against biodegradable agent under laboratory condition. *Journal of Applied Sciences*, 11(24), 3848–3853. https://doi.org/10.3923/jas.2011.3848.3853
- Maheswari, S., & Rajagopal, K. (2013). Biodiversity of endophytic fungi in *Kigelia pinnata* during two different seasons. *Current Science*, *104*(4), 515–518. Retrieved from https://www.jstor.org/stable/24089753?seq=1
- Malinda, N., Soekarno, B. P. W., & Yuliani, T. S. (2015). Penghambatan Fusarium oxysporum oleh kultur filtrat bakteri endofit dari tanaman kedelai secara in vitro. Jurnal Fitopatologi Indonesia, 11(6), 196. https://doi.org/10.14692/jfi.11.6.196
- Marques, J. P. R., Soares, M. K. M., & Appezzato-Da-Gloria, B. (2013). New staining technique for fungal-infected plant tissues. *Turkish Journal of Botany*, 37, 1–4. https://doi.org/10.3906/bot-1204-9
- Mawardika, H., & Suharjono. (2015). Isolasi dan uji antagonis kapang tanah terhadap fusarium patogen pada tanaman tomat (*Lycopersicum esculentum* Mill.) di lahan pertanian Bocek, Jawa Timur. *Biotropika: Journal of Tropical Biology*, 3(3), 142–145. Retrieved from https://biotropika.ub.ac.id/index.php/biotropika/article/view/375
- Melysa, N. F., Suharjono, & Dwiastuti, M. E. (2013). Potensi Trichoderma sp. sebagai agen pengendali Fusarium sp. patogen tanaman strawberry (Fragaria sp.). Biotropika: Journal of Tropical Biology, 1(4), 177–181. Retrieved from https://biotropika.ub.ac.id/index.php/biotropika/article/view/173
- Mohan, D., Shi, J., Nicholas, D. D., Pittman Jr, C. U., Steele, P. H., & Cooper, J. E. (2008). Fungicidal values of bio-oils and their lignin-rich fractions obtained from wood/bark fast pyrolysis. *Chemosphere*, 71(3), 456– 465. https://doi.org/10.1016/j.chemosphere.2007.10.049
- Nami Kartal, S., Terzi, E., Kose, C., Hofmeyr, J., & Imamura, Y. (2011). Efficacy of tar oil recovered during slow pyrolysis of macadamia nut shells. *International Biodeterioration and Biodegradation*, 65(2), 369–373. https://doi.org/10.1016/j.ibiod.2010.08.011
- Noveriza, R., & Tombe, M. (2003). Uji in vitro limbah pabrik rokok terhadap beberapa jamur patogenik tanaman. Buletin Penelitian Tanaman Rempah Dan Obat, 14(2), 1–10. Retrieved from http://ejurnal.litbang.pertanian.go.id/index.php/bultro/article/view/1983
- Nurzannah, S., Lisnawita, & Bakti, D. (2014). Potensi jamur endofit asal cabai sebagai agens hayati untuk mengendalikan layu fusarium (*Fusarium oxysporum*) pada cabai dan interaksinya. *Jurnal Agroekoteknologi*, 2(3), 1230–1238. Retrieved from https://jurnal.usu.ac.id/index.php/agroekoteknologi/article/view/7543
- Okutucu, C., Duman, G., Ucar, S., Yasa, I., & Yanik, J. (2011). Production of fungicidal oil and activated carbon from pistachio shell. *Journal of Analytical and Applied Pyrolysis*, 91(1), 140–146. https://doi.org/10.1016/j.jaap.2011.02.002

- Pangestu, E., Suswanto, I., & Supriyanto. (2014). Uji penggunaan asap cair tempurung kelapa dalam mengendalikan *Phytophthora* sp. penyebab penyakit busuk buah kakao secara in vitro. *Jurnal Perkebunan Dan Lahan Tropika*, 4(2), 39–44. Retrieved from https://jurnal.untan.ac.id/index.php/perkebunan/article/view/9375
- Rahman, M. A., Begum, M. F., & Alam, M. F. (2009). Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. *Mycobiology*, 37(4), 277– 285. https://doi.org/10.4489/myco.2009.37.4.277
- Ray, B. (1996). Fundamental food microbiology. CRC Press. Retrieved from https://agris.fao.org/agrissearch/search.do?recordID=US201300298911
- Ray, B., & Sandine, W. E. (1992). Acetic, propionic, and lactic acid of starter culture bacteria as biopreservatives. In B. Ray & M. Daeschel (Eds.), *Food Biopreservatives of Microbial Origin* (1st ed., pp. 103–136). Boca Raton, FL: CRC Press. Retrieved from https://books.google.co.id/books?hl=en&Ir=&id=0zSoDwAAQBAJ&oi=fnd&pg=PP1&dq=Food+Biopreser vatives+of+Microbial+Origin&ots=5Z3_bl2rZ0&sig=4yCTtGduHaN7pR2foHLcG1uTRpE&redir_esc=y#v= onepage&q=Food Biopreservatives of Microbial Origin&f=false
- Rosana, Y., Matsuzawa, T., Gonoi, T., & Karuniawati, A. (2014). Modified slide culture method for faster and easier identification of dermatophytes. *Microbiology Indonesia*, 8(3), 7. https://doi.org/10.5454/mi.8.3.7
- Seifert, K. A., & Gams, W. (2011). The genera of Hyphomycetes 2011 update. Persoonia: Molecular Phylogeny and Evolution of Fungi, 27, 119–129. https://doi.org/10.3767/003158511X617435
- Selim, K., El-Beih, A., AbdEl-Rahman, T., & El-Diwany, A. (2012). Biology of endophytic fungi. Current Research in Environmental & Applied Mycology, 2(1), 31–82. https://doi.org/10.5943/cream/2/1/3
- Sieber, T. N., & Grünig, C. R. (2006). Biodiversity of fungal root-endophyte communities and populations, in particular of the dark septate endophyte *Phialocephala fortinii* s. I. In B. J. E. Schulz, C. J. C. Boyle, & T. N. Sieber (Eds.), *Microbial Root Endophytes* (pp. 107–132). Berlin, Heidelberg: Springer. https://doi.org/10.1007/3-540-33526-9_7
- Stone, J. K., Polishook, J. D., & White Jr, J. F. (2004). Endophytic fungi. In M. Foster & G. Bills (Eds.), *Biodiversity of Fungi: Inventory and Monitoring Methods* (1st ed., pp. 241–270). Elsevier Academic Press. https://doi.org/10.13140/RG.2.1.2497.0726
- Strobel, G., & Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, 67(4), 491–502. https://doi.org/10.1128/mmbr.67.4.491-502.2003
- Suciatmih, Yuliar, & Supriyati, D. (2011). Isolasi, identifikasi, dan skrining jamur endofit penghasil agen biokontrol dari tanaman di lahan pertanian dan hutan penunjang Gunung Salak. Jurnal Teknologi Lingkungan, 12(2), 171–186. https://doi.org/10.29122/jtl.v12i2.1249
- Sunarsih, S., Pratiwi, Y., & Sunarto, Y. (2012). Pengaruh suhu, waktu dan kadar air pada pembuatan asap cair dari limbah padat pati aren (Studi kasus pada Sentra Industri Sohun Dukuh Bendo, Daleman, Tulung, Klaten). In Prosiding Seminar Nasional Aplikasi Sains & Teknologi (SNAST) Periode III (pp. A290–A297). Yogyakarta, ID: Institut Sains & Teknologi AKPRIND Yogyakarta. Retrieved from http://repository.akprind.ac.id/sites/files/conference-proceedings/2012/sunarsih_14377.pdf
- Sunarwati, D., & Yoza, R. (2010). Kemampuan *Trichoderma* dan *Penicillium* dalam menghambat pertumbuhan cendawan penyebab penyakit busuk akar durian (*Phytophthora palmivora*) secara in vitro. In *Seminar Nasional Program dan Strategi Pengembangan Buah Nusantara* (pp. 176–189). solok: Balai Penelitian Tanaman Buah Tropika. Retrieved from http://balibu.litbang.pertanian.go.id/index.php/publikasimainmenu-47/94-karya-ilmiah/prosiding/213-kemampuan-trichoderma-dan-penicillium-dalammenghambat-pertumbuhan-cendawan-penyebab-penyakit-busuk-akar-durian-phytophthora-palmivorasecara-in-vitro
- Tan, R. X., & Zou, W. X. (2001). Endophytes: A rich source of functional metabolites. Natural Product Reports, 18, 448–459. https://doi.org/10.1039/b1009180
- Tsitsigiannis, D., Antoniou, P., Tjamos, S., & Paplomatas, E. (2008). Major diseases of tomato, pepper and egg plant in green houses. *The European Journal of Plant Science and Biotechnology*, 2(Special Issue 1), 106–124. Retrieved from http://www.globalsciencebooks.info/Online/GSBOnline/images/0812/EJPSB_2(SI1)/EJPSB_2(SI1)106-1240.pdf
- Underwood, W., & Somerville, S. C. (2008). Focal accumulation of defences at sites of fungal pathogen attack. Journal of Experimental Botany, 59(13), 3501–3508. https://doi.org/10.1093/jxb/ern205
- Wightwick, A., Walters, R., Allinson, G., Reichman, S., & Menzies, N. (2010). Environmental risks of fungicides used in horticultural production systems. In O. Carisse (Ed.), *Fungicides* (pp. 273–304). IntechOpen. https://doi.org/10.5772/13032

Publish 01 Februari 2021

https://agrivita.ub.ac.id/index.php/agrivita/article/view/2458