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Antifungal Activities of the Combination of *Ulin* Wood Liquid Smoke and *Hiyung* Cayenne Pepper Root Endophyte Fungi Against *Colletothricum capsici*

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ABSTRACT

Chili farming faces several constraints, one of which is the pathogenic fungus *Colletotrichum capsici*. To overcome it can be used indigenous endophytic fungus and liquid smoke wood Ulin (*Eusideroxylon zwageri* Teijsm. & Binn.) which has the potential as antimicrobial can be used. 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.

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 namely *Hiyung* cayenne pepper. 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 *Hiyung* cayenne pepper (Balai Penelitian dan Pengembangan Pertanian, 2018), and in June 2012 the Ministry of Agriculture of the Republic of Indonesia established *Hiyung* cayenne pepper as a national variety. This cayenne pepper can support national chili production because of its high productivity with good market prospects, growing both on swampy and dry land. Compared to the three commercial varieties (Sonar, Bara, and Santika) *Hiyung* cayenne pepper has the highest dry weight, and higher productivity with a longer harvest duration, with the highest capsaicin levels about 699 ppm (Pramudyani, Sabran, & Noor, 2019).

Plant disease is one of the factors of failure in harvesting, including in chili plants (Islam, Schreinemachers, & Kumar, 2020). Species of fungi that can infect chili plants include *Colletotrichum scovillei* (Caires et al., 2014), *Aspergillus spp.*,

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Fusarium spp., *Colletotrichum spp.* (Frimpong et al., 2019)8 genera, and 17 species were identified on the basis of morphology, culture characteristics, and DNA sequencing of the internal transcribed spacer (ITS, and many more fungi have been reported. Fungi that cause disease in plants often cause structural and physical damage (Marques, Soares, & Appezzato-Da-Gloria, 2013). 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). Endophytic fungi are found in the plant tissue system, including flowers, twigs, leaves, and plant roots. These microorganisms grow and take food from the plant, and can infect healthy crops in certain tissues as well as able to produce mycotoxin, enzymes, and antibiotics (Stone, Polishook, & White Jr, 2004). Endophyte fungi also 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). Fungi also have the ability to produce plant growth hormones such as auxin (Imaningsih, Kadarsah, & Rusmannurrachmad, 2019) and herbicidal activity (de Souza et al., 2017).

Control of pathogenic fungi as a plant destruction organism can also be done by utilizing liquid smoke, a vapor condensate of pyrolysis of wood containing the main compounds of acids, phenols, and carbonyl (Lee et al., 2011). The constituent components of the liquid smoke compound have the ability to inhibit the growth of fungi and bacteria (Nami Kartal, Terzi, Kose, Hofmeyr, & Imamura, 2011; Okutucu, Duman, Ucar, Yasa, & Yanik, 2011). *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 microbes.

Hiyung 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 same ability to act as antimicrobials, but their potential is unknown when combined between 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, Lambung Mangkurat University, 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 Septiana, Sukarno, Sukarno, & Simanjuntak (2017) with modification.

Identification and Screening of Endophytic Fungi

The fungi Identification includes macroscopic and microscopic observation. The macroscopic observation of pure isolates was done by modification of the method of Gerardo-Lugo et al. (2020) 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 7^{th} day. Microscopic observation was done by the method of slide culture (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 fungi 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 of healthy chili plants based on the methods done

by Soesanto, Hartono, Mugiastuti, & Widarta (2020). Percent germination is obtained by dividing the number of germinated seeds (normal/abnormal/not growing) by the total number of seeds multiplied by 100%. The antagonism test refers to the dual culture method in the PDA medium (Tomah, Abd Alamer, Li, & Zhang, 2020). 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% (Kunova et al., 2016).

Production of Endophytic Fungus Filtrate

The endophytic fungus was prepared using the method of Qiao, Ling, Yu, Huang, & Wang (2017) 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 fungi. 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 µm 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 (Balouiri, Sadiki, & Ibnsouda, 2016). 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%; 0.085%; 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 Venkateswarulu et al. (2018) 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.

An inhibitory test of the combination of endophytic fungus and liquid smoke was carried out based on the method carried out by Balouiri, Sadiki, & Ibnsouda (2016) 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 between 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% (Kunova et al., 2016).

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 *Hiyung* Cayenne Pepper

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 by 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.

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 De la Cruz-Quiroz, Roussos, Rodríguez-Herrera, Hernandez-Castillo, & Aguilar (2018) 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).

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

Endophytic fungi	Germination (%)*					
	7th-DAI			14th-DAI		
	Normal	Abnormal	Not grow	Normal	Abnormal	Not grow
Without endophytic fungi addition	100±0c	0±0a	0±0a	100±0c	0±0a	0±0a
Trichoderma sp. ACH1.1	0±0ab	0±0a	100±0ab	30±51.96ab	6.67±5.77a	63.33±55.07ab
Trichoderma sp. ACH1.6	0±0ab	0±0a	100±0ab	33.33±41.63ab	10±10a	56.67±45.09ab
Trichoderma sp. ACH2.2	0±0a	23.33±20.82ab	76.66±20.81ab	0±0a	60±30ab	40±30ab
Botrytis sp. ACH2.3	0±0a	16.67±28.86ab	83.33±28.86ab	0±0a	30±26.46ab	70±26.45ab
Gliocladium sp. ACH2.4	0±0a	0±0a	100±0b	0±0a	26.67±30.55a	73.33±30.55b
Harmoniella sp.ACH2.5	0±0abc	3.33±5.77a	96.66±5.77ab	90±0abc	3.33±5.77a	6.67±5.77ab
Humicola sp. ACH2.6	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

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 (α=0.05)

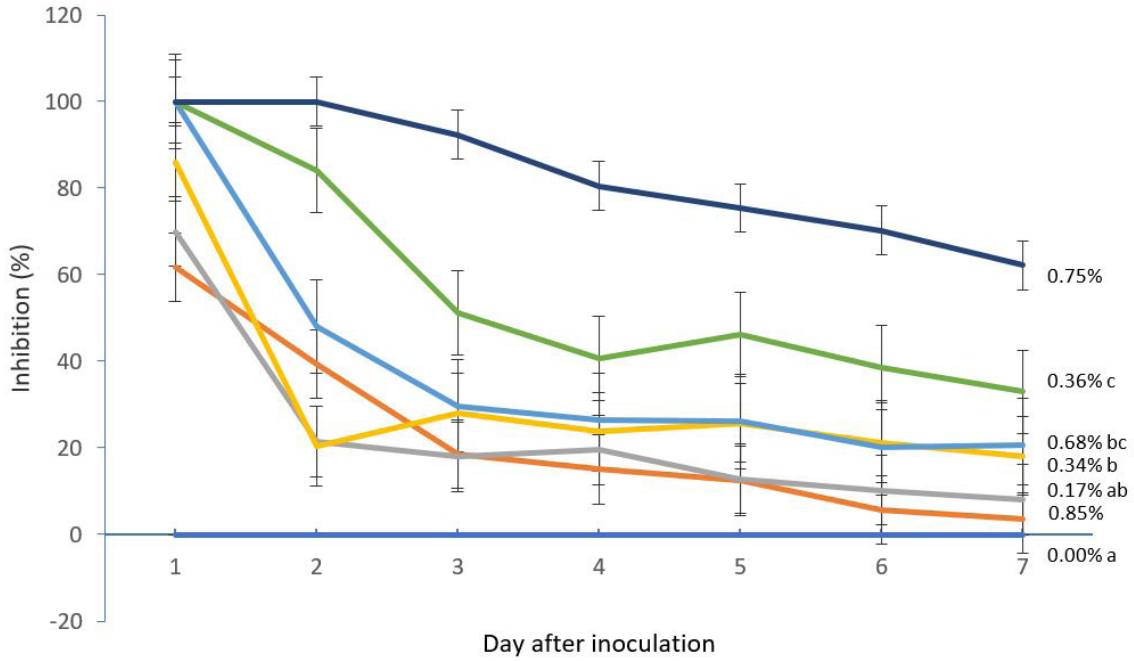

Fig. 1. Inhibitory effect (%) of liquid smoke against *C. capsici* on the 1-7th day after inoculation the bar indicates standard deviation between replicates. The concentration of liquid smoke followed by the same letter is not significantly different based on Duncan test (α=0.05).

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. This is in line with research of Suresh et al. (2019) which uses pyroligneous acid from a mixture of several kinds of wood that has the ability to inhibit the growth of fungi. 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 (Hassan, Sand, & El-Kadi, 2012).

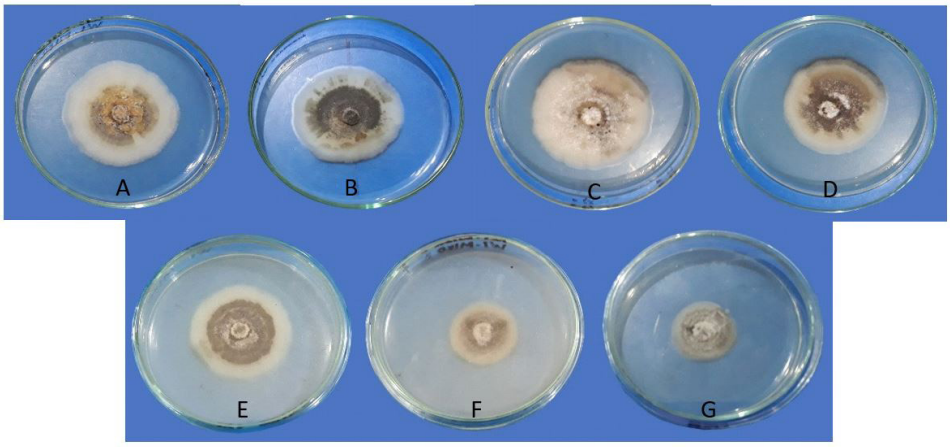

Fig. 2. Visual comparison of the growth of *C. capsici* in the addition of liquid smoke with a concentration of (A) 0.00% (B) 0.085% (C) 0.17% (D) 0.34% (E) 0.68% (F) 1.36% (G) 1.75% of the 7th day of incubation.

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.

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,* this requires further research. De la Cruz-Quiroz, Roussos, Rodríguez-Herrera, Hernandez-Castillo, & Aguilar (2018) in research on the ability of *Trichoderma* to inhibit *P.capsici* states that the pathogen cell part is used as a source of nutrition for the growth of *Trichoderma*, the same is likely to happen in this study.

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 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, Sinaga, Nawangsih, Giyanto, & Pari (2018) that liquid smoke has the ability as an antibacterial agent and stimulates plant growth, in research on liquid smoke from some wood to overcome the banana disease. 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 (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.

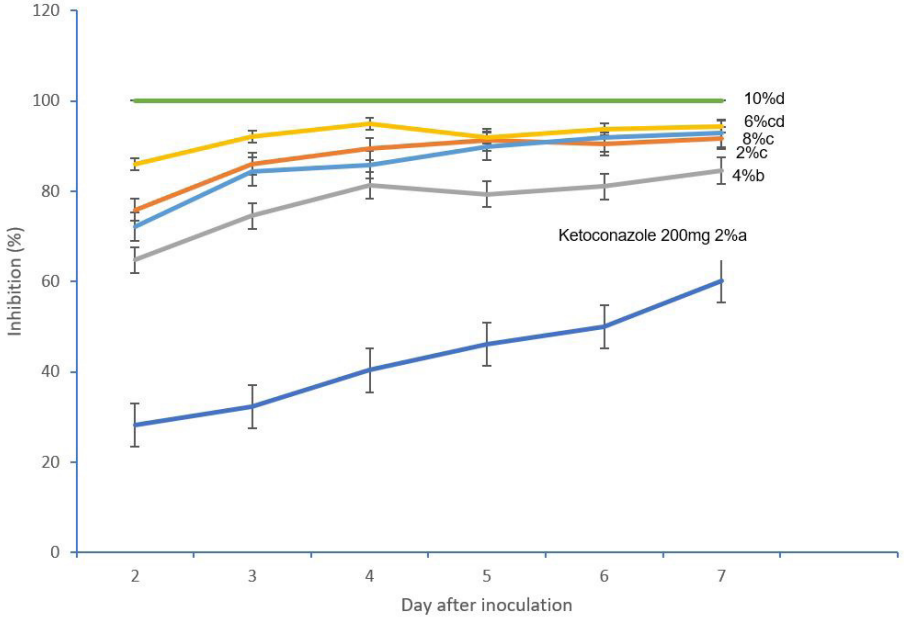

Fig. 3. Inhibitory effect (%) of *Cunninghamella* sp. ACH2.7 filtrate against *C. capsici* on the 1-7th day after inoculation the bar indicates standard deviation between replicates. The concentration of crude extract followed by the same letter is not significantly different based on Duncan test (α=0.05)

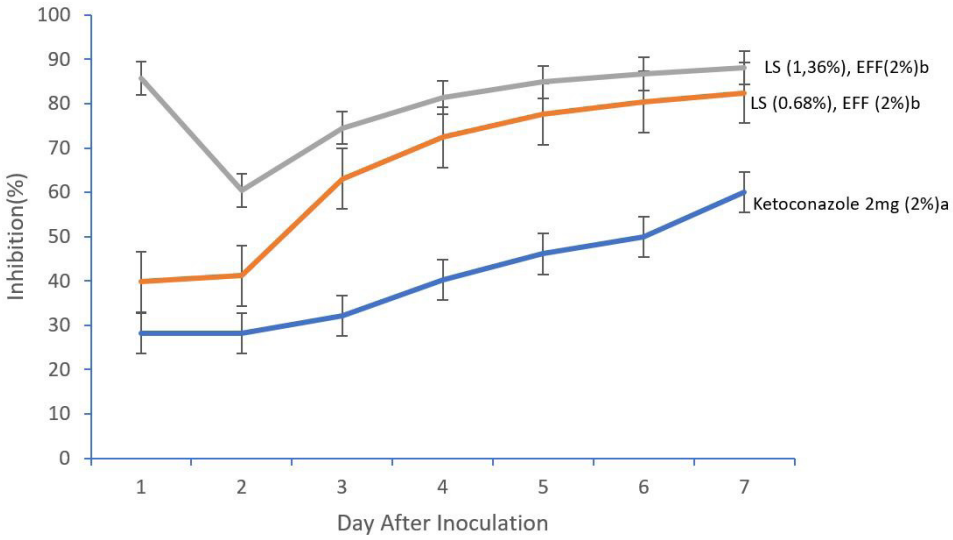

Fig. 4. Inhibitory effect (%) of the combination of Liquid Smoke (LS) and *Cunninghamella* sp. ACH2.7 filtrate/Endophyte Fungi Filtrate (EEF) against *C. capsici* on the 1-7th day after inoculation the bar indicates standard deviation between replicates. The concentration of crude extract followed by the same letter is not significantly different based on Duncan test (α=0.05)

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 especially *Hiyung* cayenne pepper.

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REFERENCES

Aisyah, I., Sinaga, M. S., Nawangsih, A. A., Giyanto, & Pari, G. (2018). Utilization of liquid smoke to suppress blood diseases on bananas and its effects on the plant growth. *AGRIVITA Journal of Agricultural Science*, *40*(3), 453–460. https://doi.org/10.17503/agrivita.v40i3.1390

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

Balai Penelitian dan Pengembangan Pertanian. (2018). *Cabai Hiyung, SDG Lokal Kalimantan Selatan*. Retrieved from http://www.litbang.pertanian.go.id/info-teknologi/3152/

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Barnett, H. L., & Hunter, B. B. (1998). Illustrated genera of imperfect fungi (4th ed.). St. Paul. USA: American Phytopathological Society (APS Press). Retrieved from https://www.academia.edu/35499449/Illustrated_genera_of_imperfect_fungi_fourth_edition_Barnett_y_Hunter_pdf_pdf

Caires, N. P., Pinho, D. B., Souza, J., Silva, M. A., Lisboa, D. O., Pereira, O. L., & Furtado, G. Q. (2014). First report of anthracnose on pepper fruit caused by *Colletotrichum scovillei* in Brazil. *Plant Disease*, *98*(10), 1437. https://doi.org/10.1094/PDIS-04-14-0426-PDN

De la Cruz-Quiroz, R., Roussos, S., Rodríguez-Herrera, R., Hernandez-Castillo, D., & Aguilar, C. N. (2018). Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala International Journal of Modern Science, 4*(2), 237–243. https://doi.org/10.1016/j.kijoms.2018.03.002

de Souza, A. R. C., Baldoni, D. B., Lima, J., Porto, V., Marcuz, C., Machado, C., … Mazutti, M. A. (2017). Selection, isolation, and identification of fungi for bioherbicide production. *Brazilian Journal of Microbiology*, *48*(1), 101–108. https://doi.org/10.1016/j.bjm.2016.09.004

Frimpong, G. K., Adekunle, A. A., Ogundipe, O. T., Solanki, M. K., Sadhasivam, S., & Sionov, E. (2019). Identification and toxigenic potential of fungi isolated from capsicum peppers. *Microorganisms*, *7*(9), 1–10. https://doi.org/10.3390/microorganisms7090303

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

Gerardo-Lugo, S. S., Tovar-Pedraza, J. M., Maharachchikumbura, S. S. N., Apodaca-Sánchez, M. A., Correia, K. C., Sauceda-Acosta, C. P., … Beltrán-Peña, H. (2020). Characterization of *Neopestalotiopsis* species associated with mango grey leaf spot disease in Sinaloa, Mexico. *Pathogens*, *9*(10), 788. https://doi.org/10.3390/pathogens9100788

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

Hassan, R. A., Sand, M. I., & El-Kadi, S. M. (2012). Effect of some organic acids on fungal growth and their toxins production. *Journal of Agricultural Chemistry and Biotechnology*, *3*(9), 391–397. https://doi.org/10.21608/jacb.2012.55011

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

Islam, A. H. M. S., Schreinemachers, P., & Kumar, S. (2020). Farmers' knowledge, perceptions and management of chili pepper anthracnose disease in Bangladesh. *Crop Protection*, *133*, 105139. https://doi.org/10.1016/j.cropro.2020.105139

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

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-trimoxazol-resistant bacteria strains. *Annals of Clinical Microbiology and Antimicrobials*, *11*, 5. https://doi.org/10.1186/1476-0711-11-5

Kunova, A., Bonaldi, M., Saracchi, M., Pizzatti, C., Chen, X., & Cortesi, P. (2016). Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. *BMC Microbiology*, *16*(1), 272. https://doi.org/10.1186/s12866-016-0886-1

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

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

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

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

Pramudyani, L., Sabran, M., & Noor, A. (2019). Agronomic performance and nutrition content of hiyung as local variety of cayenne pepper (*Capsicum frutescens*) at dry land and swamp land of South Kalimantan Province. *Buletin Plasma Nutfah*, *25*(1), 43-52. https://doi.org/10.21082/blpn.v25n1.2019.p43-52

Qiao, W., Ling, F., Yu, L., Huang, Y., & Wang, T. (2017). Enhancing taxol production in a novel endophytic fungus, *Aspergillus aculeatinus* Tax-6, isolated from *Taxus chinensis* var. *mairei*. *Fungal Biology*, *121*(12), 1037–1044. https://doi.org/10.1016/j.funbio.2017.08.011

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

Septiana, E., Sukarno, N., Sukarno, & Simanjuntak, P. (2017). Endophytic fungi associated with turmeric (*Curcuma longa* L.) can inhibit histamine-forming bacteria in fish. *HAYATI Journal of Biosciences, 24*(1), 46–52. https://doi.org/10.1016/j.hjb.2017.05.004

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. l. 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

Soesanto, L., Hartono, A. R. R., Mugiastuti, E., & Widarta, H. (2020). Seed-borne pathogenic fungi on some soybean varieties. *Biodiversitas, 21*(9), 4010–4015. https://doi.org/10.13057/biodiv/d210911

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

Suresh, G., Pakdel, H., Rouissi, T., Brar, S. K., Fliss, I., & Roy, C. (2019). *In vitro* evaluation of antimicrobial efficacy of pyroligneous acid from softwood mixture. *Biotechnology Research and Innovation*, *3*(1), 47–53. https://doi.org/10.1016/j.biori.2019.02.004

Tomah, A. A., Abd Alamer, I. S., Li, B., & Zhang, J. Z. (2020). A new species of *Trichoderma* and gliotoxin role: A new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper. *Biological Control, 145*, 104261. https://doi.org/10.1016/j.biocontrol.2020.104261

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

Venkateswarulu, N., Shameer, S., Bramhachari, P. V., Basha, S. K. T., Nagaraju, C., & Vijaya, T. (2018). Isolation and characterization of plumbagin (5- hydroxyl- 2- methylnaptalene-1,4-dione) producing endophytic fungi *Cladosporium delicatulum* from endemic medicinal plants: Isolation and characterization of plumbagin producing endophytic fungi from endemic medicinal plants. *Biotechnology Reports*, *20*, e00282. https://doi.org/10.1016/j.btre.2018.e00282