

Utilization of Liquid Smoke to Suppress Blood Diseases on Bananas and Its Effects on the Plant Growth

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ABSTRACT

Liquid smoke is reported to be effective to inhibit some bacteria. The study aimed to evaluate the effects of liquid smoke (LS) from coconut shell (CS-LS), pinecone (P-LS), and oil palm branch (OPB-LS) on the incidences of blood disease, induced resistance to *Ralstonia syzygii* subsp. *celebesensis*, and plant growth. Two days after banana seedlings were treated with LS, ethylene, auxin, lignin, activities of the enzymes of peroxidase (POD) and phenylalanine ammonia lyase (PAL) on the root of the banana seedlings were measured. Then, the seedlings were inoculated by *R. syzygii* subsp. *celebesensis*. The plant growth and incidence of blood diseases were observed daily and the 31st day after the application of LS and this bacteria inoculation. The results showed that the CS-LS, P-LS, and OPB-LS at all tested concentration could suppress the incidence of blood diseases up to 100 %, induce resistance of banana seedlings to the *R. syzygii* subsp. *celebesensis* with increased levels of ethylene, auxin, lignin, activities of POD and PAL, and plant growth, significantly. Based on the effectiveness of this bacterial control and the ability to promote the growth of banana seedlings test, it is recommended that the most effective treatment is P-LS 0.5 %.

Keywords: bananas; blood disease; induced resistance; peroxidase; phenylalanine ammonia lyase

INTRODUCTION

Blood disease on the bananas plant (*Musa* spp.) caused by *Ralstonia syzygii* subsp. *celebesensis* (Safni, Subandiyah, & Fegan, 2018). Almost all types of bananas can be attacked by these bacteria. Kepok kuning banana with ABB genome (ABB group) has been known for being highly susceptible to this disease (Eden-Green & Sastraatmadja, 1990). The incidence of this disease and its spread is still very high in the field so that it can reduce banana production by up to 100 % (Supriadi, 2005). The incidence of the blood disease in Kepok banana was reported in Bondowoso, East Java, reaching an average of 97.70 % and Lombok, West Nusa Tenggara, reaching 86.80 % (Hadiwiyono, Subandiyah, Widada, Fegan, & Taylor, 2013). Some efforts to control blood disease have been made, but the results have not been optimal yet. The use of bactericides is not recommended because the residual impacts can potentially harm consumers' health and can lead to the emergence of new virulent pathogenic strains (Pegg, Moore, & Bentley, 1996). Therefore, the search for alternative bactericidal agents that harmless for the consumers' health (food safety) and also environmentally friendly is still ongoing.

Blood disease control in bananas using liquid smoke (LS) has never been reported. Liquid smoke is a byproduct of wood pyrolysis. According to Choi, Jung, Oh, & Kim (2014), pyrolysis is the chemical decomposition process of organic matter (lignin, cellulose, hemicellulose) through a heating process without or little oxygen. During pyrolysis, the chemical composition of the organic material will undergo a breakdown of the chemical structure into the gas phase. The phase of the gas flowed into the condenser pipe will condense and change its form into liquid. This liquid is called liquid smoke (wood vinegar). Liquid smoke has the ability as an antimicrobial (Lingbeck et al., 2014), antioxidant and antibacterial activity (Yang et al., 2016). Coconut shell from liquid smoke can inhibit *Pseudomonas aeruginosa* and *Staphylococcus aureus* in vitro (Zuraida, Sukarno, & Budijanto, 2011), so it has high potential to be used as organic pesticides and herbicides (Payamara, 2011). Liquid smoke from pelawan wood (*Tristania abavata*) can inhibit the growth of *Escherichia coli* (Panagan & Syarif, 2009). It can promote seed germination and seedling vigour too (Flematti et al., 2011).

Coconut shells (CS), pinecones (P), and oil palm branches (OPB) are a waste of biomass which volume is relatively abundant, but until now the management has not been optimal yet. The three biomass wastes were very potential to be used as raw material for making liquid smoke (LS). The ability of CS-LS, P-LS, OPB-LS to control *R. syzygii* subsp. *celebesensis* and increase the growth of bananas seedlings have not been reported, so that it still needs to be evaluated. This study conducted to evaluate the effect of three types of liquid smoke on the incidence of blood disease, the induced resistance of banana plants to this bacterium and its effects on the bananas growth.

MATERIALS AND METHODS

The experiments were conducted from November 2015 to February 2016 in the greenhouse at Vocational Education Development Center for Agriculture in Cianjur, West Java, whereas liquid smoke was made at the Research and Development on Forestry Engineering and Forest Products Processing, Bogor, Indonesia.

Preparation of Liquid Smoke

The coconut shells (CS), pinecones (P), and oil palm branches (OPB) were selected as a raw material for making Liquid Smoke (LS). The levels of the components of lignin, cellulose and hemicellulose all of them were analyzed. LS was made by pyrolysis technique at a temperature of 400 °C for 5 hours. Then, it distilled with a distillation temperature of 150-200 °C. The levels of total phenols were analyzed by spectrophotometric method, acetic acid by HPLC method and alcohol were analyzed by gas chromatography method. Chemical components in all kinds of liquid smoke were also identified by the GCMS-pyr method.

Experiment of the Effect of Giving Liquid Smoke on the Induced Resistance to *R. syzygii* subsp. *celebesensis*.

Kepok Kuning banana seedlings of the three-month-old tissue culture after acclimatization were treated with CS-LS, P-LS, and OPB-LS, each with a concentration of 0 %, 0.5 %, 1.0 %, 2.0 % (v/v). LS was applied by sprinkling it into the soil media as much as 100 mL per polybag. Two days after the application of liquid smoke, the root samples of banana seedlings were taken to analyze the activities of the enzymes of phenylalanine ammonia lyase (PAL), peroxidase (POD), the lignin content, and the levels of auxin and ethylene. The levels of phenylalanine ammonia lyase (PAL), peroxidase (POD) were analyzed by spectrophotometric method, the lignin content by SNI 0492:2008 standard method, the levels of auxin by HPLC method, and the levels of ethylene by gas chromatography method.

Experiment of the Effect of Giving Liquid Smoke on the Growth of Banana Seedlings and the Incidence of Blood Diseases

The seedlings were then inoculated with 2 mL of *R. syzygii* subsp. *celebesensis* suspension (OD₆₀₀ = 0.1) by injecting it into the stump. The plant growth and incidence of blood diseases were observed daily and the 31st day after the application of liquid smoke and *R. syzygii* subsp. *celebesensis* inoculation, the plant growth and percentage of the incidence of blood diseases observed. The plant growth parameters observed were the increase in plant height, stem diameter, and the number of leaves, while the percentage of the incidence of blood diseases was calculated by the formula, i.e. the number of plants attacked divided the total plant observed with the same treatment x 100 (Agrios, 2005).

Experimental Design and Statistical Analysis

All experiments were carried out using complete factorial random design with two factors, i.e. the concentration of liquid smoke (0 %, 0.5 %, 1.0 %, and 2.0 %) and the source of liquid smoke (CS-LS, P-LS, and OPB-LS). Every treatment was repeated three times. The data obtained were analyzed using variance (ANOVA) with Microsoft Office Excel 2010 and SPSS Statistics version 17.0 software. Significantly different treatments were further tested using the Duncan Repeat Distance Test (DMRT) at a rate of 5 %.

RESULTS AND DISCUSSION

The Liquid Smoke Effect on the Incidence of Blood Diseases

The results showed that in the control treatment (seedlings inoculated with *R. syzygii* subsp. *celebesensis* without liquid smoke), there was a latent period of 10 days, meaning that the blood disease symptoms, i.e. banana seedlings beginning to wilt and become yellow to appear on the 10th day after *R. syzygii* subsp. *celebesensis* inoculation, on the 20th day necrosis, and all of the leaves dry out on day 31st. The latent period of *R. syzygii* subsp. *celebesensis* on banana kepok in this study was in accordance with Hadiwiyono (2011), that indicated the latent period of *R. syzygii* subsp. *celebesensis* on yellow banana seedlings is between 7-10 days. Compared with the control (liquid smoke), the treatments of the three types of liquid smoke at all tested concentration could suppress the incidence of the blood disease in banana seedlings up to 100 %; this was indicated by until the 31st day, no symptoms of blood disease were found in its.

CS-LS, P-LS, and OPB-LS were considered as active compounds that could inhibit Quorum Sensing (QS). If the bacterial QS system could be disturbed/inhibited, the process of bacterial infection to the host plant tissue would be inhibited so that pathogenic bacterial infection in the host tissue did not occur. Karrikins or lactone compounds were active compounds that were suspected of suppressing the initial infection of *R. syzygii* subsp. *celebesensis* in banana seedlings through the inhibition mechanism of its QS by degrading acyl homoserine lactone bacteria to acyl-homoserine (deactivate the AHL signal). According to Dong et al. (2001), the mechanism that could interfere with the key process of the QS system of pathogenic bacteria through inhibition of N-acyl homoserine lactone (AHL) was QQ (Quorum Quenching). Because suspected liquid smoke could deactivate the AHL signal, it had potential as QQ.

Pyrolysis of the CS, P, and OPB produced the various products namely phenols, acetic acids, and alcohol (Table 1). Acetic acid and phenol were two components in liquid smoke which were indicated to inhibit QS *R. syzygii* subsp. *celebesensis* so that the incidence of blood diseases could be suppressed. Acetic acid reported could completely eradicate bacteria in mature biofilms in vitro (Bjarnsholt et al., 2015). This finding was supported by research reported by Ismael (2013), showed that the results of bacterial biofilm production testing using the microtiter-plate method, the use of vinegar from apple and grape extract respectively could inhibit the biofilm of *Streptococcus pyogenes* bacteria 95.5 % and 90.9 % compared to the controls. The use of vinegar had also been shown to eliminate the biofilm of *Candida albicans* bacteria (Jafari, Falah-Tafti, Lotfi-Kamran, Zahraei, & Kazemi, 2012). The antibiofilm activity of the phenol compound had been tested using the microtiter-plater method, and the results indicated that the natural phenolic compound showed a significant decrease in biofilm formation by *P. aeruginosa* bacteria (Jagani, Chelikani, & Kim, 2009). Phenol compounds that indicating the ability to inhibit QS were vanillin (Kappachery, Paul, Yoon, & Kweon, 2010; Ponnusamy, Kappachery, Thekeettle, Song, & Kweon, 2013), eugenol (Niu & Gilbert, 2004), analog curcumin (Rudrappa & Bais, 2008), and furanone (Ponnusamy, Paul, Kim, & Kweon, 2010). The Karrikins from plant smoke could inhibit bacterial quorum sensing in *Pseudomonas aeruginosa* (Mandabi, Ganin, Krief, Rayo, & Meijler, 2014).

Table 1. Levels of total phenols, acetic acid and alcohol in liquid smoke

Source of liquid smoke	Compound Level		
	Total phenols (%)	Acetic acid (ppm)	Alcohol (%)
Coconut shell	13.32	1340.46	8.90
Pinecone	7.74	750.51	6.61
Oil palm branch	12.89	1351.15	9.10

The Potential of Liquid Smoke as An Agent for Inducing Plant Resistance to *R. syzygii* subsp. *celebesensis*

The treatments of the three types of liquid smoke were expected to be able to induce the resistance of Kepok Kuning banana seedlings to the *R. syzygii* subsp. *celebesensis* and work to suppress the incidence of blood diseases. Experimental results showed that it could increase levels of ethylene, auxin, lignin, enzyme activities of peroxidase (POD) and phenylalanine ammonia lyase (PAL) in the roots of banana seedlings. The average enhancement levels of ethylene, auxin, lignin, enzyme activities of POD

and PAL at the roots of banana seedlings on the 2nd day after the application of various types and concentrations of liquid smoke were presented in Table 2.

Table 2. The average increase of ethylene, auxin, lignin, POD and PAL enzyme activity at the roots of banana seedlings on the 2nd day after the application of various types and concentrations of liquid smoke

Source of liquid smoke	Concentration (%)	Average increase				
		Ethylene (ppm)	Auxin (ppm)	Lignin (%)	POD (UAE mg ⁻¹)	PAL (UAE mg ⁻¹)
Control (without LS)		1.130 a	48.190 a	8.070 a	0.0160 a	0.0105 a
CS-LS	0.5	1.527 cd	58.170 d	9.439 bc	0.0450 d	0.0177 c
P-LS		1.395 b	57.150 b	9.297 b	0.0421 b	0.0176 bc
OPB-LS		1.578 de	61.140 g	9.578 bc	0.0471 g	0.0185 d
CS-LS	1.0	1.619 efg	59.460 e	10.546 de	0.0460 ef	0.0200 g
P-LS		1.507 c	58.780 d	10.368 d	0.0445 c	0.0192 e
OPB-LS		1.649 fgh	61.990 h	10.865 def	0.0482 h	0.0212 hi
CS-LS	2.0	1.689 fgh	64.050 i	11.613 gh	0.0488 i	0.0211 h
P-LS		1.599 ef	60.210 f	11.437 g	0.0460 e	0.0194 ef
OPB-LS		1.736 i	65.460 j	11.777 ghi	0.0494 j	0.0222 j

Remarks: According to Duncan's Multiple Range Test, numbers with different letters in the same column indicate a significant difference at the 5 % level.

The result of the data indicated that CS-LS, P-LS, and OPB-LS significantly increased levels of ethylene and auxin. The mechanism of increasing ethylene and auxin levels by liquid smoke in the plant tissue was due to the liquid smoke containing karrikins compounds (lactone). Karrikins were a group of plant growth regulators in smoke that interacts with other plant hormones in regulating plant growth (Flematti, Dixon, & Smith, 2015). Karrikins were a positive signal for the formation of ACC (Aminocyclopropane-1-carboxylate) (Kępczyński & Van Staden, 2012). Waters, Scaffidi, Sun, Flematti, & Smith (2014) stated that karrikins in smoke could act as a biological signal/precursor which played a role in auxin biosynthesis that could catalyze the conversion of SAM to ACC, and it was a precursor of ethylene biosynthesis (Van de Poel & Van Der Straeten, 2014).

The increased level of ethylene and auxin was thought to have increased the lignin levels and the activities of POD and PAL enzymes in the root tissue of Kepok Kuning banana seedlings. Growth hormone could also play a role in regulating plant resistance to pathogens through increased activities of POD and PAL. The ethylene hormone was reported to mediate the increased activity of PAL enzyme (Liang et al., 2013). The auxin hormone was also reported to mediate the increased activity of PAL enzyme (Correa-Aragunde, Foresi, Delledonne, & Lamattina, 2013). Gailite, Samsone, & levinsh (2005) noted that the increased POD enzyme activity was preceded by the increased levels of peanut ethylene 4 hours after the *Trichoderma harzianum* treatment so that the bean plant was resistant to pathogen infection of *P. syringae*. An increase in lignin level (lignification) in the root tissue of Kepok Kuning banana seedlings was also correlated with an increase in POD and PAL enzyme activities two days after the application of liquid smoke. Lignification was correlated with the increased enzyme activities of POD and PAL which contributed to the building of lignin. Lignification can inhibit the penetration of pathogens, suppress the degrading cell wall enzyme activities, and block the toxin released by the pathogen so that it will affect plant defense against pathogens (Miedes, Vanholme, Boerjan, & Molina, 2014).

Increased POD and PAL enzyme activities were thought to occur because the liquid smoke triggered stress in plants. According to Khan, Fatma, Per, Anjum, & Khan (2015), the initial reaction of the plants against biotic and abiotic stress was the increased activity of POD enzyme to induce the accumulation of Reactive Oxygen Species (ROS) that could increase the activity of PAL enzyme to induce the formation of salicylic acid (SA). SA was a signal regulator of Systemic Acquired Resistance (SAR) in plants. SAR was an induced plant resistance mechanism that protects plants from various pathogens with a broad spectrum and a relatively long time (Thakur & Sohal, 2013).

The role of ethylene in improving SAR had been reported, and the results showed that SAR was mediated by salicylic acid compounds, not by ethylene it was suspected that ethylene could induce SAR

by increasing the sensitivity of plant tissue to the action of salicylic acid (Lawton, Potter, Uknes, & Ryals, 1994). The presence of ethylene regulates the accumulation of salicylic acid in the infected tissue, further synergizing in reducing the symptoms of necrosis in tomatoes caused by *Xanthomonas campestris* pv *vesicatoria*. These results indicated that ethylene signals were involved in improving plant defense responses to pathogens (O'Donnell, Jones, Antoine, Ciardi, & Klee, 2001).

The Effects of Liquid Smoke on the Growth of Banana Seedlings

The treatment of P-LS 0.5 % provided the highest increase in plant height, stem diameter and number of leaves while the lowest occurred in the treatment of OPB-LS 0.5 % (Table 3). The mechanism of regulating the plant growth by liquid smoke was thought to be the most effective because it contained karrikins compound (lactone). Karrikins compounds had similar structural and biological properties to strigolactone so that karrikins was also incorporated into growth regulators from the butenolide class (Flematti, Ghisalberti, Dixon, & Trengove, 2004). Karrikins-like compounds such as butyrolacton derivatives also had been reported to be produced from the fungus *Aspergillus terreus* Thorn var. *terreus* and was considered to have an activity to stimulate germination of seeds and seedling growth (Cazar, Schmeda-Hirschmann, & Astudillo, 2005). It was in accordance with the report by Nelson, Flematti, Ghisalberti, Dixon, & Smith (2012) that karrikins was a chemical signal from smoke which could regulate seedling and plant growth of some seedlings, including maize and tomato, and in *Arabidopsis thaliana*.

Research data showed that the highest level increase of auxin and ethylene was obtained from the treatment of OPB-LS at a concentration of 2.0 %, and the lowest was obtained from the treatment of P-LS at a concentration of 0.5 % (Table 2). The highest level increase of ethylene and auxin occurred along with the increased concentration of the liquid smoke provided. The higher auxin level tended to increase the ethylene level and the higher ethylene content tended to decrease plant growth. It was allegedly what made plant height, stem diameter and number of leaves tended to decline with the increased concentration of liquid smoke.

Table 3. The growth of banana seedling on the 31st day after the application of various types and concentrations of liquid smoke

Source of liquid smoke	Concentration (%)	Average increase		
		Plant height (cm)	Stem diameter (cm)	Number of leaves (sheet)
Control (without LS)		2.200 a	0.200 a	1.00 a
CS-LS	0.5	5.573 f	0.615 h	4.25 gh
P-LS		6.160 g	0.666 i	4.50 h
OPB-LS		5.476 f	0.541 g	3.00 e
CS-LS	1.0	4.332 d	0.422 ef	3.00 ef
P-LS		4.468 de	0.466 f	4.00 g
OPB-LS		4.273 d	0.348 d	2.25 cd
CS-LS	2.0	3.647 b	0.244 bc	2.00 c
P-LS		3.686 bc	0.274 c	2.75 e
OPB-LS		3.618 b	0.222 b	1.25 ab

Remarks: According to Duncan's Multiple Range Test, numbers with different letters in the same column indicate a significant difference at the 5 % level.

CONCLUSION

The CS-LS, P-LS, and OPB-LS at all tested concentration could suppress the incidence of blood diseases up to 100 %, induce resistance of Kepok Kuning banana seedlings to the *R. syzygii* subsp. *celebesensis* with increased levels of ethylene, auxin, lignin, and activities of POD and PAL, and promote the growth of its, significantly. The treatment of P-LS 0.5 % provided the highest increase in plant height, stem diameter and number of leaves, while the lowest occurred in the treatment of OPB-LS 0.5 %. Based on the effectiveness of *R. syzygii* subsp. *celebesensis* control and the ability to promote the growth of banana seedlings test, it is recommended that the most effective treatment is P-LS 0.5 %.

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