**COVER PAGE**

1. “UTILIZATION OF LIQUID SMOKE TO SUPPRESSING BLOOD DISEASE ON BANANAS AND IT’S EFFECTS ON THE PLANT GROWTH”

Imas Aisyah 1), Meity Suradji Sinaga 2), Abdjad Asih Nawangsih 3), Giyanto 4\*), Gustan Pari 5)

1. **First author:**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | Name | : | Imas Aisyah |
| 2. | Affiliation | : | Bogor Agricultural University |
| 3. | E-mail | : | imas.aisyah86@yahoo.com |
| 4. | Orchid ID | : |  |
| 5. | Contribution to this Manuscript | : | Doctoral students |

1. **Second author:**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | Name | : | Meity Suradji Sinaga |
| 2. | Affiliation | : | Bogor Agricultural University |
| 3. | E-mail | : | mssinaga@yahoo.com |
| 4. | Orchid ID | : |  |
| 5. | Contribution to this Manuscript | : | Supervisor |

1. **Thrid author**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | Name | : | Abdjad Asih Nawangsih |
| 2. | Affiliation | : | Bogor Agricultural University |
| 3. | E-mail | : | asnawangsih@yahoo.com |
| 4. | Orchid ID | : |  |
| 5. | Contribution to this Manuscript | : | Supervisor |

1. **Fourth author**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | Name | : | Giyanto |
| 2. | Affiliation | : | Bogor Agricultural University |
| 3. | E-mail | : | giyanto2@yahoo.com |
| 4. | Orchid ID | : |  |
| 5. | Contribution to this Manuscript | : | Supervisor and Corresponding author |

1. **Fifth author**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | Name | : | Gustan Pari |
| 2. | Affiliation | : | Center for Research and Development on Forestry Engineering and Forest Products Processing, Bogor |
| 3. | E-mail | : | gustanp@yahoo.com |
| 4. | Orchid ID | : |  |
| 5. | Contribution to this Manuscript | : | Supervisor |

1. **Acknowledgement**

**UTILIZATION OF LIQUID SMOKE TO SUPPRESSING BLOOD DISEASE ON BANANAS AND IT’S EFFECTS ON THE PLANT GROWTH**

Imas Aisyah 1), Meity Suradji Sinaga 1), Abdjad Asih Nawangsih 1), Giyanto 1\*), Gustan Pari 2)

1) Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University

Jl. Kamper Kampus IPB Darmaga, Bogor, Indonesia, 16680

2) Center for Research and Development on Forestry Engineering and Forest Products Processing,

Bogor Jl. Gunung Batu No. 5 Bogor 16610, West Java, Indonesia

1\*) Corresponding author E-mail: [giyanto2@yahoo.com](mailto:giyanto2@yahoo.com)

**ABSTRACT**

Blood disease caused by Blood Disease Bacterium (BDB) has been reported caused loss of banana production by 20-100%. Liquid smoke (LS) has been reported act as antibacterial and can trigger the growth of plants. The study aimed to evaluate effects of coconut shell liquid smoke (CS-LS), pinecone liquid smoke (P-LS), and oil palm branch liquid smoke (OPB-LS) on the incidence of blood diseases on banana and the growth of banana seedlings. The studies showed that the CS-LS, P-LS, and OPB-LS at concentrations of 0.5, 1.0, and 2.0% can suppress the incidence of blood diseases each of them up to 100%. All of them can promote the growth of banana seedlings. The highest percentage increase plant height, stem diameter, and number of leaves was found in treatment of P-LS at concentrations of 0.5% and the lowest in treatment OPB-LS at a concentration of 2.0%. The research also showed that the CS-LS, P-LS, and OPB-LS can increased peroxidase (POD), phenylalanine ammonia lyase (PAL) enzyme activity, lignin, auxin and ethylene levels. The highest percentage increase POD, PAL enzyme activity, lignin, auxin, and ethylene levels was found in treatment of OPB-LS at concentrations of 2.0%, and the lowest at concentration of 0.5%.

Keywords: Bananas, Blood Disease, induce resistance, peroxidase, phenylalanine ammonia lyase

INTRODUCTION

Blood disease caused by Blood Disease Bacterium (BDB) is one of the important disease of banana in Indonesia. This disease has been reported caused loss of banana production by 20-100%. Infection of banana by BDB may originate from contaminated soil or water, but epidemics are usually due to nonspecific mechanical transmission by insects visiting banana flowers. Blood disease symptoms include yellowing and wilting of the mature leaves, vascular discoloration, bacterial ooze, and the eponymous typical reddish-brown fruit rot characteristic of this disease (Remenant *et al*. 2011). The pathogen infection caused systemic symptom and all part of infected banana were potential as source of infective inoculums. Controlling blood disease is complicated to do because BDB infective propagules can last up to 1- 2 years in soil without host and plant tissues infected without loss of virulence (Hadiwiyono 2011).

Treatment will be difficult to do if the disease has attacked and damaged vascular tissue of plants. Preventively control can be done, but various control methods have not been able to control pathogenic bacteria blood disease. Up to now there is no method of controlling these pathogens successful economically. Unfortunately, the emergence resistant strains of bacteria to antibiotics has limited their medical efficacy and reducing plants disease control. Therefore, it is still necessary to find another control techniques more effective.

One alternative control have hope to develop is the use of liquid smoke. The compounds in the smoke of burning wood is reported to have the ability as a preservative, so the direct evaporation technique has been widely used primarily to preserve fish, meat and seed. However, direct evaporation process has many drawbacks is potentially bad for health and cause environmental pollution (Paasonen *et al*. 2003). Therefore we need another development techniques to minimize the negative effects of smoke and to transform the smoke into other form without losing the benefits contained in the smoke.

Pyrolysis technique is a technique that can minimize the negative effects of smoke and change the development of combustion fumes into liquid smoke without eliminating the benefits contained in the smoke. In contrast to conventional charcoal making techniques, the technique pyrolysis smoke is released into the air, but it was flowed to the cooling pipes, so that the smoke condenses and transformed into a liquid, and the liquid is called liquid smoke. Liquid smoke has been reported to improve the quality of the soil, inhibiting the growth of microbes, insect pest control, accelerate seed dormancy breaking and trigger the growth of plants (Payamara 2011).

Liquid smoke from the raw material (wood waste) are different have different effects (Soldera *et al.* 2008). *Acacia nilotica* and *Cymbopogon jwarancusa* smoke stimulate on *Dacus carota* seeds germination and seedling vigour (Asaf *et.al*. 2014). Liquid smoke of coconut trees at a concentration of 4% caused mortality of nematodes 97.14%, and at 3% could inhibit growth of *F. oxysporum* 90.25% (Mugiastuti & Manan 2009). Coconut shells, pinecones, and oil palm branches are biomass wastes that have hard physical properties so difficult to be described naturally. During this time utilization only limited use as an alternative fuel substitute for oil and gas, made craft products or as charcoal with conventional techniques, or otherwise processed normally allowed to accumulate until mounting and rot or dumped into rivers that can cause new problems for the environment.

Coconut shells, pinecones, and oil palm branches are a waste of biomass whose volume is relatively abundant. But until now the management not yet optimaloptimal. Until now only used as an alternative fuel kerosene, made handicrafts, made charcoal with conventional techniques, or simply thrown into the river causing new environmental problems. Its has a hard physical properties so potentially to be used as raw material for making liquid smoke. Research on the use of liquid smoke of the three waste biomass yi. coconut shells, pinecones, and oil palm to suppress the incidence of blood diseases and trigger the growth of banana plants has not been reported. Therefore it is necessary to do further research. The study aimed to evaluate effects of coconut shell liquid smoke (CS-LS), pinecone liquid smoke (P-LS), and oil palm branch liquid smoke (OPB-LS) on the incidence of blood diseases on banana and the growth of banana seedlings.

**MATERIALS AND METHODS**

The experiments were conducted in between November 2015 to February 2016 at the Vocational Education Development Center for Agriculture Cianjur, West Java, Indonesia. Materials used are coconut shell liquid smoke, pinecone liquid smoke, and oil palm branch liquid smoke, yellow kepok banana seedlings, Blood Disease Bacterium isolate, and sterile soil in polybags. This study is divided into four stages: preparation of liquid smoke, the phytotoxic of liquid smoke on banana seedlings, the effect of liquid smoke on the incidence of Blood Diseases and the effect of of liquid smoke on the growth of banana seedlings.

**Preparation of liquid smoke**. The coconut shells, pinecones, and oil palm branches selected as a raw material for making LS. Its analyzed the levels of components of lignin, cellulose and hemicellulose.Liquid smoke (LS) made by pyrolysis technique at a temperature of 400 oC for 5 hours. LS was then distilled with a destillation temperature of 150-200 oC. Level of total phenols, acetic acid and alcohol at the three of them was analyzed. Chemical components in all kinds of liquid smoke is also identified by the method GCMS-pyr.

**The phytotoxic of liquid smoke on banana seedlings**. Yellow kepok banana seedlings of the 3 month-old tissue culture after aclimatization were treated with the CS-LS, P-LS, and OPB-LS each with a concentration of 0%, 0.5%, 1.0%, 2.0%, 3.0%, 5.0 %, 7.0%, and 9.0% (v/v). All of them are applied 1x by springkling it into the soil media as much as 100 mL/polybag. The parameters observed were the percentage of phytotoxic symptoms on banana seedlings. Phytotoxic symptoms is observed daily until the 31st day after the application of the liquid smoke. The parameters observed were percentage of phytotoxic symptoms on banana seedlings.

**The effect of liquid smoke on the incidence of Blood Diseases**. Yellow kepok banana seedlings of the 3 month-old tissue culture after acclimatization were treated with the CS-LS, P-LS, and OPB-LS each with a concentration of 0%, 0.5%, 1.0%, 2.0% (v/v). All of them are applied by pouring it into the soil media as much as 100 mL/polybag. The seedlings were then inoculated with 2 ml of BDB suspension (OD600 = 0.1) by injecting it into the stump (Rustam 2007). The development of blood diseases is observed daily until the 31st day after the application of liquid smoke and BDB inoculation. The parameters observed were percentage of blood diseases suppression. The root sample of banana seedlings taken two day after the application of liquid smoke and 14 days after the BDB inoculation to be analyzed the activity of the enzyme phenylalanine ammonia lyase (PAL), peroxidase (POD) and the lignin content.

**The effect of of liquid smoke on the growth of banana seedlings**. Yellow kepok banana seedlings of the 3 month-old tissue culture after acclimatization were treated with the CS-LS, P-LS, and OPB-LS each with a concentration of 0%, 0.5%, 1.0%, 2.0% (v/v). All of them are applied 1x by springkling it into the soil media as much as 100 mL/polybag. Plant growth was observed daily until the 30st day after application of liquid smoke. Parameters observed were the increase in plant height, stem diameter and number of leaves. The root sample of banana seedlings taken on the 30st day after the application of liquid smoke to be analyzed the level of auxin and ethylene.

The experiments were arranged in Completely Randomized Design with 3 replications. Obtained data was analyzed using Analysis of Variance (Anova) followed by Duncan’s Multiple Range Test at a level of 5%.

**RESULTS AND DISCUSSION**

**Preparation of Liquid Smoke**

Coconut shells, pinecones, and oil palm branches have different hardness levels, so have levels of lignin, cellulose and hemicellulose are varied. The highest level of of lignin contained in coconut shells, while the highest level of cellulose and hemicellulose contained in oil palm branches (Table 1). The levels of total phenols, acetic acid, alcohol, and pH in the CS-LS, P-LS, and OPB-LS can be seen in Table 2. The CS-LS showed the highest level of total phenol, because it contains lignin higher than others. The OPB-LS showed the highest level of acetic acid and alcohol, because it contains cellulose and hemicellulose higher. According to Soldera *et al*. (2008), that phenol is the result of the pyrolysis of lignin, carbonyl (alcohol) is the result of the pyrolysis of cellulose and acetic acid compound is the result of the pyrolysis of cellulose and hemicellulose. The most acidic OPB-LS (2.00), compared to CS-LS (2.21), and P-LS (2.54), but in general the pH of the three types of liquid smoke is very acidic.

Table 1. Levels of components of wood (lignin, cellulose and hemicellulose) from waste coconut shell, pinecone, and oil palm branch

|  |  |  |  |
| --- | --- | --- | --- |
| Source of liquid smoke | Level of (%) | | |
| Lignin | Cellulose | Hemicellulose |
| Coconut shell | 42.56 | 45.42 | 20.69 |
| Pinecone | 39.63 | 44.51 | 20.73 |
| Oil palm branch | 25.08 | 48.20 | 20.78 |

Table 2. Levels of total phenols, acetic acid and alcohol in liquid smoke

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source of liquid smoke | Level of compounds | | | pH |
| Total phenols (%) | Acetic acid (ppm) | Alcohol (%) |
| Coconut shell | 13.32 | 1340.46 | 8.90 | 2.21 |
| Pinecone | 7.74 | 750.51 | 6.61 | 2.54 |
| Oil palm branch | 12.89 | 1351.15 | 9.10 | 2.00 |

**Phytotoxic Effects of Liquid Smoke on Bananas Seedlings**

The research results show that the CS-LS, P-LS, and OPB-LS at a concentration ≥ 3.0% are phytotoxic on bananas seedling. The percentage of phytotoxic symptoms on banana seedlings up to 100% (Table 3). Phytotoxic symptoms found are leaf necrosis and chlorosis.

Table 3. The influence of the source and concentration of liquid smoke on the percentage of phytotoxic symptoms on banana seedlings

|  |  |  |  |
| --- | --- | --- | --- |
| Concentration  (%) | The percentage of phytotoxic symptoms on banana seedlings (%) | | |
| Source of liquid smoke | | |
| Coconut shell | Pinecone | Oil palm branch |
| 0.5 | 0 a | 0 a | 0 a |
| 1.0 | 0 a | 0 a | 0 a |
| 2.0 | 0 a | 0 a | 0 a |
| 3.0 | 100 b | 100 b | 100 b |
| 5.0 | 100 b | 100 b | 100 b |
| 7.0 | 100 b | 100 b | 100 b |
| 9.0 | 100 b | 100 b | 100 b |
| 0.0 (without liquid smoke) | 0 a | 0 a | 0 a |

Remarks: The numbers in the same column followed by the same letter are not significantly different at a test level of 5% (DMRT test)

Phytotoxic symptoms occurs because liquid smoke contains organic compounds that are toxic so the use of high concentrations is not recommended. According to Wei *et al*. (2009), at high concentrations, liquid smoke can lead to loss of membrane integrity and accelerate cell death and decrease nutrient availability. The CS-LS, P-LS, and OPB-LS contains organic compounds such as phenol, acetic acid, and alcohol and very acidic (Table 2). The three compounds can induce the accumulation of reactive oxygen species (ROS) (Giannattasio *et al*. 2005, Das & Vasudevan 2007, and Beloborodova *et al*. 2012). ROS compounds are toxic (Droge 2002). Allegedly the treatment of liquid smoke in high concentrations cause to become stressed so that can damage the plant cells. The higher the levels of ROS that formed can disrupt the balance redox system of the cell, so that eventually the oxidative damage/phytotoxic symptoms occur.

The result of GCMS-pyr analysis that the CS-LS, P-LS, and OPB-LS contains karrikin analog compounds thai comes from smoke decomposition of cellulose (Flematti *et al*. 2011). Cellulose content of pinecone was lower at 44.51%, compared with coconut shell (45.42%), and oil palm branch (48.20%) (Aisyah *et al*. unpublished). Karrikin is cytotoxic because can induce the accumulation of reactive oxygen species (ROS). Karrikin can damage cells or cause cellular necrosis, loss of membrane integrity and accelerate cell death (Guzman *et al*. 2005).

**Effects of Liquid Smoke on the Incidence of Blood Diseases**

Experimental results showed that the CS-LS, P-LS, and OPB-LS at a concentration of 0.5, 1.0, and 2.0% can suppress the incidence of blood diseases on banana seedling up to 100%. This can happen because its contain active compounds that can suppress the population density of BDB. The emphasis the population density of the BDB can inhibit of quorum sensing bacteria so that the incidence of the blood disease can be suppressed.

The CS-LS, P-LS, and OPB-LS contains lots of organic compounds such as phenol, acetic acid, and alcohol. Its can damage proteins and lipids the bacterial cell wall and membrane, so can accelerate the leakage of the cell (De Vuyst & Leroy 2007). Phenol, acetic acid, and alcohol can induce the accumulation of reactive oxygen species (ROS) also (Giannattasio *et al*. 2005, Das & Vasudevan 2007, and Beloborodova *et al*. 2012). ROS are toxic (Droge 2002), its can damage proteins and lipids the bacterial cell wall and membrane so it can kill pathogens. Scanning Electron Microscopy (SEM) results showed that the wall and membrane cell of BDB becomes ubnormal after treatment with liquid smoke that accelerate the leakage of the cell (Figure 1) (Aisyah *et al*. unpublished).

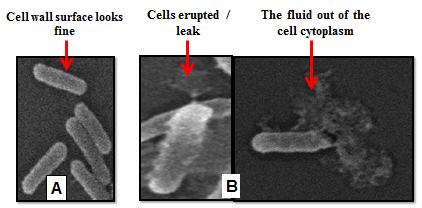


Figure 1. Condition of morphology BDB cell on (A) the control (no liquid smoke) (magnification 7500x) (B) treatment of OPB-LS 2.0% (magnification 10.000x))

The karrikins compounds in the CS-LS, P-LS, and OPB-LS also thought to suppress the incidence of blood diseases because its can degrade the bacterial *Acyl Homoserine Lactone* (AHLs) signal. AHLs is a signal quorum sensing of Gram-negative bacterial that plays a role in the regulation of important biological functions such as antibiotic production, biofilm formation, luminescence, motility, plasmid transfer, regulation of the expression of pathogen genes, and virulence (Zan *et al*. 2012). Karrikins allegedly contribute to degradation AHLs molecules bacterial because this molecules bacterial have the same lactone structure with karrikin, thus allegedly after protein α/β-hydrolase is active, this protein will degrade AHLs bacteria that thrive in the plant, resulting in quorum sensing bacteria become obstructed, and bacterial infections in plants can be suppressed. Karrikins is chemical signal for activate the receptor KAI2 (*Karrikin insensitive 2*) in plants. KAI2 is involved in the perception of karrikin. KAI2 is an alfa/beta-hydrolase enzyme that can catalyzes the hydrolysis of the ring butenolide in karrikin and the opening of this butenolide ring will speed up the process of signal transduction karrikin in plants (Guo *et al*. 2013).

Other compounds in the CS-LS, P-LS, and OPB-LS sites contributing to suppress the incidence of blood diseases are curcumin analogues yi. *2-propanone, 1 (4-hydroxy-3-methoxyphenyl)- (CAS) 1- (4-Hydroxy- 3-MET).*  Curcumin may inhibit bacterial biofilm formation, quorum sensing, inhibit bacterial AHLs synthesis, and may interfere with the binding of AHLs by the LuxR receptor protein (Rudrappa & Bais 2008). Curcumin compounds are reported to have potential as antioxidants because they can increase the activity of superoxide dismutase (SOD) enzymes and catalases that can suppress oxidative stress by converting ROS compounds into a neutral form (H2O) (Barzegar 2012). In neutral conditions the ROS compound will activate the transduction signals including activating the plant's defense signal against biotic and abiotic stress (Tripathy & Oelmuller 2012).

Treatment of the three types of liquid smoke can induces the resistance of banana seeds to the BDB. Experimental results showed that its can increased peroxidase (POD) and phenylalanine ammonia lyase (PAL) enzyme activity in the root of banana seedlings. Increased activity of POD, PAL enzyme showed that banana seed resistance system against BDB has been induced and work to suppress the incidence of blood diseases. The highest percentage increase POD and PAL enzyme activity obtained from the treatment of OPB-LS at a concentration of 2.0% are 208.75% and 111.77% and the lowest obtained from P-LS at a concentration of 0.5% are 163.13% and 68.45%. After inoculation BDB, POD and PAL enzyme activity also tend to increase compared with before inoculation BDB (Table 4).

Table 4. The influence of the source and concentrations of the liquid smoke on the increased percentage of POD and PAL enzyme activity in the roots of banana seedlings before and after BDB inoculation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source of liquid smoke | Concentration  (%) | Percentage increase in enzyme activity (%) | | | |
| POD | | PAL | |
| Before BDB inoculation | After BDB inoculation | Before BDB inoculation | After BDB  inoculation |
| Coconut shell | 0.5 | 181.25 c | 375.00 c | 68.45 a | 160.96 b |
| 1.0 | 187.50 d | 398.75 f | 90.37 e | 214.44 g |
| 2.0 | 205.00 h | 420.00 g | 101.07 f | 267.40 i |
| Pinecone | 0.5 | 163.13 a | 331.25 a | 67.38 a | 147.06 a |
| 1.0 | 178.13 b | 354.38 b | 82.89 c | 165.78 c |
| 2.0 | 187.70 e | 380.63 d | 85.03 d | 172.73 d |
| Oil palm branch | 0.5 | 194.38 f | 382.50 e | 76.47 b | 189.84 f |
| 1.0 | 201.25 g | 420.63 g | 102.14 f | 262.03 h |
| 2.0 | 208.75 i | 455.63 h | 111.77 g | 312.30 j |

Remarks: The numbers in the same column followed by the same letter are not significantly different at a test level of 5% (DMRT test)

OPB-LS contents acetic acid is highest i.e. 1351.15 ppm, so that can given the highest percentage increase POD and PAL enzyme activity. Acetic acid, fenol, and alcohol are abiotic stress that can increase activity of the enzyme POD so that induce the accumulation of ROS . According to Khan *et al*. (2015), the initial reaction of plants against biotic and abiotic stress are the increase activity of the enzyme peroxidase (POD) to induce the accumulation of ROS. Naturally the accumulation of ROS will increase enzyme activity of PAL to induce the formation of salicylic acid (SA). According to Thakur & Singh Sohal (2013), SA is signal regulator of systemic acquired resistance (SAR) in plants. SAR is a long distance signaling mechanism that provides broad spectrum and long-lasting resistance to secondary infections throughout the plant.

The data shown on Table 5 indicated also that all kinds of LS treatment at consentration of 0.5, 1.0, and 2.0% can increase lignin levels in banana seedling. Increased lignin levels showed that banana seed resistance system against BDB has been induced and work to suppress the incidence of blood diseases. The highest percentage increase lignin content obtained from the treatment of OPB-LS at a concentration of 2.0% (45.94%) and the lowest obtained from P-LS at a concentration of 0.5% (15.20%). After inoculation BDB, lignin content also tend to increase as compared with before inoculation BDB. Treatment of OPB-LS can give the highest percentage increase lignin content because given the highest percentage increase lignin content. Lignification is one plant response to biotic elicitor such as organic acid and phenolic compound. Combination among biotic and abiotic stresser can induce the lignin content in plants. Lignification is also correlated with the increase enzyme activity of POD and PAL which contribute in building lignin. Lignification can inhibit the penetration of pathogens and growth of the pathogen in host tissues, suppress the degrading cell wall enzymes activity, and block the toxin released by the pathogen, so its will affect plant defense against pathogens (Miedes *et al*. 2014).

Table 5. Effects of the source and concentrations of the liquid smoke on the increase in lignin content

|  |  |  |  |
| --- | --- | --- | --- |
| Source of liquid smoke | Concentration  (%) | Percentage increase in lignin content (%) | |
| Before BDB inoculation | After BDB inoculation |
| Coconut shell | 0.5 | 16.96 b | 36.78 b |
| 1.0 | 30.68 e | 49.36 e |
| 2.0 | 43.90 h | 57.81 h |
| Pinecone | 0.5 | 15.20 a | 32.73 a |
| 1.0 | 28.47 d | 47.53 d |
| 2.0 | 41.72 g | 55.13 g |
| Oil palm branch | 0.5 | 18.68 c | 38.83 c |
| 1.0 | 34.64 f | 52.72 f |
| 2.0 | 45.94 i | 60.73 i |

Remarks: The numbers in the same column followed by the same letter are not significantly different at a test level of 5% (DMRT test)

**Effects of Liquid Smoke on the growth of banana seedlings**

The studies showed that the CS-LS, P-LS, and OPB-LS has ability to promote the growth of banana seedlings through an increase in plant height, stem diameter and number of leaves (Table 6). This is consistent with the report Nelson *et al*. (2012), that chemical signal from smoke can regulate of seddling growth and plant growth.

Table 6. The influence of the source and concentrations of the liquid smoke on the percentage increase in plant height, stem diameter and the number of leaves of banana seedling

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source of liquid smoke | Concentration (%) | Percentage increase in plant height (%) | Percentage increase in stem diameter (%) | Percentage increase in the number of leaves (%) |
| Coconut shell | 0.5 | 153.32 g | 207.50 h | 325.00 g |
| 1.0 | 96.91 d | 111.00 e | 200.00 e |
| 2.0 | 65.77 a | 22.00 b | 100.00 b |
| Pinecone | 0.5 | 180.00 h | 233.00 i | 350.00 h |
| 1.0 | 103.09 e | 133.00 f | 300.00 f |
| 2.0 | 67.55 b | 37.00 c | 175.00 d |
| Oil palm branch | 0.5 | 148.91 f | 170.50 g | 200.00 e |
| 1.0 | 94.23 c | 74.00 d | 125.00 c |
| 2.0 | 64.45 a | 11.00 a | 25.00 a |

Remarks: The numbers in the same column followed by the same letter are not significantly different at a test level of 5% (DMRT test)

The data on Table 6 show that the highest percentage increase plant height, stem diameter, and number of leaves obtained from the treatment of P-LS at concentrations of 0.5% are 180.00%, 233.00%, and 350.00%, and the lowest obtained from the treatment of OPB-LS at a concentration of 2.0% are 64.45%, 11.00%, and 25.00%. The plant height, stem diameter and number of leaves tend to decline with increased concentrations of liquid smoke, but treatment of P-LS can give the highest percentage increase plant height, stem diameter and number of leaves on bananas seedling. Karrikins  are active compounds in smoke will affect seedling growth and vigorous growth of some seedlings, including maize and tomato, and in Arabidopsis thaliana (Nelson *et al*. 2012). Karrikins are a group of [plant growth regulators](https://en.wikipedia.org/wiki/Plant_hormone) in smoke of burning plant material. Karrikins interact with other plant hormones in regulating plant growth (Flematti *et al*. 2015). Karrikin is a positive signal for the formation of ACC (*Aminocyclopropane-1-carboxylate*), which becomes a precursor in the formation of ethylene (Kepczynski & Van Stade 2012). The higher the concentration of ACC causes levels of ethylene in the root tip increases but the auxin activity in the shoot tip decreases so elongation and root growth becomes decline (Van de Poel & Van Der Straeten 2014).

Karrikin in smoke can act as a biological signal/precursors which play a role in auxin biosynthesis (Waters *et al*. 2014). Auxin signals can induce SAM (S-Adenosylmethionine) synthase enzymes and induce ACC synthase enzymes that can catalyze the conversion of SAM to ACC. ACC formed into precursors of ethylene biosynthesis (Dodd & Cossins 1968). Data research show that the highest percentage increase levels of auxin and ethylene obtained from the treatment of OPB-LS at concentrations of 2.0% are 35.83% and 53.65%, and the lowest obtained from the treatment of P-LS at a concentration of 0.5% are 18.59% and 23:42% (Table 7). The highest percentage increase levels of ethylene and auxin occur along with the increased concentration of liquid smoke provided. The higher levels of auxin tend to increase ethylene levels. The higher levels of ethylene formed tend to decrease plant growth, inhibit the elongation of plants and can even cause plant death (Van de Poel & Van Der Straeten 2014). This is allegedly the cause plant height, stem diameter and number of leaves tend to decline with increased concentrations of liquid smoke.

Table 7. The influence of the source and concentrations of the liquid smoke on the percentage increase to level of auxin and ethylen in the root bananas seedlings

|  |  |  |  |
| --- | --- | --- | --- |
| Source of liquid smoke | Concentration (%) | Percentage increase in the level of auxin (%) | Percentage increase in the level of ethylene (%) |
| Coconut shell | 0.5 | 20.29 b | 35.14 c |
| 1.0 | 23.38 c | 43.24 f |
| 2.0 | 32.91 f | 49.55 h |
| Pinecone | 0.5 | 18.59 a | 23.42 a |
| 1.0 | 21.98 b | 33.33 b |
| 2.0 | 24.95 c | 41.54 e |
| Oil palm branch | 0.5 | 26.87 d | 39.64 d |
| 1.0 | 28.65 e | 45.95 g |
| 2.0 | 35.83 g | 53.65 j |

Remarks: The numbers in the same column followed by the same letter are not significantly different at a test level of 5% (DMRT test)

**CONCLUSION**

The CS-LS, P-LS, and OPB-LS spray treatment did show a trend towards greater values i.e can suppress the incidence of blood diseases and has ability to promote the growth of banana seedlings.

**REFERENCES**

Asaf, S., Imran, M.Q., Jan, R., Lubna, Khatoon, A., Hee-Young, J. & Shafiq-Ur-Rehman. (2014). Plant ferived smoke promotes seed germination and alleviates auxin stress in carrot. *ARPN Journal of Agricultural and Biological Science*, 9(9), 308-314.

Barzegar, A. (2012). The role of electron-transfer and H-atom donation on the superb antioxidant activity and free radical reaction of curcumin. *Food Chem*, 135,1369–1376. <http://doi.org>/10.1016/j.foodchem.2012.05.070

[Beloborodova, N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Beloborodova%20N%5BAuthor%5D&cauthor=true&cauthor_uid=23061754)., [Bairamov, I](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bairamov%20I%5BAuthor%5D&cauthor=true&cauthor_uid=23061754)., [Olenin, A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Olenin%20A%5BAuthor%5D&cauthor=true&cauthor_uid=23061754)., [Shubina, V](http://www.ncbi.nlm.nih.gov/pubmed/?term=Shubina%20V%5BAuthor%5D&cauthor=true&cauthor_uid=23061754)., [Teplova, V](http://www.ncbi.nlm.nih.gov/pubmed/?term=Teplova%20V%5BAuthor%5D&cauthor=true&cauthor_uid=23061754). & [Fedotcheva, N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fedotcheva%20N%5BAuthor%5D&cauthor=true&cauthor_uid=23061754). (2012). Effect of phenolic acids of microbial origin on production of reactive oxygen species in mitochondria and neutrophils. [*J Biomed Sci*.](http://www.ncbi.nlm.nih.gov/pubmed/23061754) 2012 Oct 12;19:89. <http://doi.org/10.1186/1423-0127-19-89>.

[Das, S.K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Das%20SK%5BAuthor%5D&cauthor=true&cauthor_uid=17570440). & [Vasudevan, D.M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Vasudevan%20DM%5BAuthor%5D&cauthor=true&cauthor_uid=17570440). (2007). Alcohol-induced oxidative stress. [*Life Sci*,](http://www.ncbi.nlm.nih.gov/pubmed/17570440) 81(3), 177-187. http://doi.org/[10.1111/j.1440-1746.2006.04589.x](https://dx.doi.org/10.1111/j.1440-1746.2006.04589.x).

De Vuyst, L. & Leroy, F. (2007). *Bakteriosins from Lactic Acid Bacteria: Production, Purification, and Food Apllication*. *Journal of Molecular Micribiology and Biotechnology*, 13, 1994-1999. http://doi.org/[10.1159/000104752](https://dx.doi.org/10.1159/000104752).

Dodd, W.A. & Cossins, E.A. (1968). Biosynthesis of S-adenosylmethionine in germinating pea seeds. *Phytochemistry*, 7, 2143-2145. <https://doi.org/10.1016/S0031-9422(00)85670-8>

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*. 82 (1): 47–95. http://doi.org/[10.1152/physrev.00018.2001](https://doi.org/10.1152/physrev.00018.2001).

Flematti, Gavin, R., Dixon, Kingsley, W., Smith. & Steven, M. (2015). ["What are karrikins and how were they 'discovered' by plants?"](http://www.biomedcentral.com/1741-7007/13/108). *BMC Biology*, 13 (1), 108. http://doi.org/[10.1186/s12915-015-0219-0](https://dx.doi.org/10.1186%2Fs12915-015-0219-0).

Giannattasio, S., Guaragnella, N., Corte-Rea,l M., Passarella, S. & Marra. E. (2005). Acid stress adaptation protects Saccharomyces cerevisiae from acetic acid-induced programmed cell death. *Gene*, 354, 93-98. http://doi.org/[10.1016/j.gene.2005.03.030](https://dx.doi.org/10.1016/j.gene.2005.03.030).

Guo, Y., Zheng, Z., La Clair, J.J., Chory, J. & Noel, P.J. (2013). Smoke-derived karrikin perception by the α/β-hydrolase KAI2 from Arabidopsis. *PNAS*, 110(20), 8284-8289. http://doi.org/[10.1073/pnas.1306265110](https://dx.doi.org/10.1073%2Fpnas.1306265110).

Guzman, M.L., Rossi, R.M., Karnischky, L., Li, X., Peterson, D.R., Howard, D.S. & Jord, C.T. (2005). The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. *Blood,* 105, 4163–4169. http://doi.org/[10.1182/blood-2004-10-4135](https://doi.org/10.1182/blood-2004-10-4135)

Hadiwiyono. (2011). Blood bacterial Wilt Disease of Banana: The Distribution of Pathogen in Infected Plant, Symptoms, and Potentiality of Diseased Tissues as Source of Infective Inoculums. *Nusantara Bioscience*, 3, 112-117. http://doi.org/10.13057/nusbiosci/n0030302.

Kepczynski, J. & Van Staden,J. (2012). Interaction of karrikinolide and ethylen in controlling germination of dormant *Avea fatua* L. Caryopsis. *Plant Growth Regul*, 67,185-190. http://doi.org/10.1007/s10725-012-9675-5

[Khan](https://www.ncbi.nlm.nih.gov/pubmed/?term=Khan%20MI%5BAuthor%5D&cauthor=true&cauthor_uid=26175738), R.I.M., [Fatma](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fatma%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26175738), M., [Per](https://www.ncbi.nlm.nih.gov/pubmed/?term=Per%20TS%5BAuthor%5D&cauthor=true&cauthor_uid=26175738),S.T., [Anjum](https://www.ncbi.nlm.nih.gov/pubmed/?term=Anjum%20NA%5BAuthor%5D&cauthor=true&cauthor_uid=26175738), A. N. & [Khan](https://www.ncbi.nlm.nih.gov/pubmed/?term=Khan%20NA%5BAuthor%5D&cauthor=true&cauthor_uid=26175738), A.N. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. [*Front Plant Sci*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4485163/), 6, 462. <http://doi.org/10.3389/fpls.2015.00462>.

[Miedes](https://www.ncbi.nlm.nih.gov/pubmed/?term=Miedes%20E%5BAuthor%5D&cauthor=true&cauthor_uid=25161657), E., [Vanholme](https://www.ncbi.nlm.nih.gov/pubmed/?term=Vanholme%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25161657), R., [Boerjan](https://www.ncbi.nlm.nih.gov/pubmed/?term=Boerjan%20W%5BAuthor%5D&cauthor=true&cauthor_uid=25161657), W. & [Molina](https://www.ncbi.nlm.nih.gov/pubmed/?term=Molina%20A%5BAuthor%5D&cauthor=true&cauthor_uid=25161657), A. (2014). The role of the secondary cell wall in plant resistance to pathogens. *Front Plant Sci,* 5, 358. <http://doi.org/10.3389/fpls.2014.00358>.

Mugiastuti, E. & Manan, A. (2009). Pemanfaatan asap cair untuk mengendalikan *Fusarium oxysporum* dan *Meloidogyne* spp. *Jurnal Pembangunan Pedesaan,* 9 (1), 43-49.

Nelson, D.C., Flematti, G.R., Ghisalberti, E.L., Dixon, K.W. & Smith, S.M. (2012). [Regulation of seed germination and seedling growth by chemical signals from burning vegetation.](http://www.ncbi.nlm.nih.gov/pubmed/22404467) *Annu Rev Plant Biol,* 63, 107-130. <http://doi.org/10.1146/annurev-arplant-042811-105545>.

Paasonen, M., Hannukkala, A., Ramo, S., Haapala, H. & Hietaniemi, V. (2003). *Smoke-a novel application of a traditional means to improve grain quality*. In: Nordic Association of Agricultural Scientists 22nd Congress, Turku, Finland.

Payamara, J. (2011). Usage of Wood Vinegar as New Organic Substance. *International Journal of ChemTech Research CODEN( USA): IJCRGG*, 3(3), 1658-1662.

Remenant, B., de Cambiaire, J.C., Cellier, G., Jacobs, J.M., Mangenot, S., Barbe, V., Lajus, A., Vallenet, D., Medigue, C., Fegan, M., Allen, C. & Prior, P. (2011). *Ralstonia syzygii*, the *Blood Disease Bacterium* and some Asian *R. solanacearum* strains form a single genomic species despite divergent lifestyles. *PLoS ONE*, 6, 1–10. <http://doi.org>/[10.1371/journal.pone.0024356](https://dx.doi.org/10.1371%2Fjournal.pone.0024356).

Rudrappa, T. & Bais, H.P. (2008). Curcumin a know phenolic from *Curcuma longa*, attenuates the virulence of *Pseudomonas aeruginosa* PAO1 in whole plant and animal pathogenicity models. *J. Agric Food Chem*, 56(6), 1955-1962. <http://doi.org>/10.1021/jf072591j

Rustam. (2007). Uji metode inokulasi dan kerapatan populasi *Blood Disease Bacterium* pada tanaman pisang. *J. Hort*. 17(4), 387-392.

Soldera, S., Sebastianutto, N. & Bortokmenzzi, R. (2008). Composition of phenolic coumpound and antioxidant activity of commercial aqueous smoke flavorings. *J. Agric Food Chem,* 56, 2727-2734. <http://doi.org/10.1021/jf072117d>.

[Thakur](http://www.hindawi.com/73602741/), M. & [Singh Sohal](http://www.hindawi.com/40346105/), B. (2013). Role of Elicitors in Inducing Resistance in Plants against Pathogen Infection: A Review. [ISRN Biochem](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4393000/), 2013, 762412. doi:  http://doi.org/[10.1155/2013/762412](https://dx.doi.org/10.1155%2F2013%2F762412)

Tripathy, C.B. & Oelmuller, R. (2012). Reactive oxygen species generation and signaling in plants. *Plant Signal Behav*, 7(12), 1621–1633. http://doi:  [10.4161/psb.22455](http://dx.doi.org/10.4161%2Fpsb.22455)

[Van de Poel, B](http://www.ncbi.nlm.nih.gov/pubmed/?term=Van%20de%20Poel%20B%5BAuthor%5D&cauthor=true&cauthor_uid=25426135). & [Van Der Straeten, D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Van%20Der%20Straeten%20D%5BAuthor%5D&cauthor=true&cauthor_uid=25426135). (2014). 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene. [*Front Plant Sci,*](http://www.ncbi.nlm.nih.gov/pubmed/25426135) 5, 640. http://doi.org/[10.3389/fpls.2014.00640](https://dx.doi.org/10.3389%2Ffpls.2014.00640)

Waters, T.M., Scaffidi, A., Sun, K.Y., Flematti, R.G. & Smith.M.S. (2014). The karrikin response system of Arabidopsis.*The Plant Journal*, 79, 623–631. <http://doi.org/10.1111/tpj.12430>.

Wei, Q.Y., Liu, G.Q., Wei, X.M., Ma, X.X.X., Dong, L. & Dong, R.J. (2009). Influence of wood vinegar as leaves fertilizer on yield and 42 quality of celery. *J. China. Agri. Univ*, 14, 89−92.

Zan J. D., Cicirelli E. M., Mohamed N. M., Sibhatu H., Kroll S., Choi O., *et al*. (2012).  A complex LuxR-LuxI type quorum sensing network in a roseobacterial marine sponge symbiont activates flagellar motility and inhibits biofilm formation. *Mol. Microbiol*. 85, 916–933. <http://doi.org/10.1111/j.1365-2958.2012.08149>.