

Fungicidal Action of Coconut Waste Liquid-Smoke on Citrus Fruit-Rot Pathogens (*Penicillium digitatum* and *Penicillium italicum*)

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Submission date: 09-Aug-2021 09:50AM (UTC+0700)

Submission ID: 1629338712

File name: ot_Pathogens_Penicillium_digitatum_and_Penicillium_italicum.pdf (269.83K)

Word count: 4165

Character count: 21922

Fungicidal Action of Coconut Waste Liquid-Smoke on Citrus Fruit-Rot Pathogens (*Penicillium digitatum* and *Penicillium italicum*)

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To cite this article:

Budy Rahmat, Alaik Zhilalul Haq Albaki. Fungicidal Action of Coconut Waste Liquid-Smoke on Citrus Fruit-Rot Pathogens (*Penicillium digitatum* and *Penicillium italicum*). *International Journal of Microbiology and Biotechnology*. Vol. 6, No. 2, 2021, pp. 53-58. doi: 10.11648/j.ijmb.20210602.14

Received: April 24, 2021; Accepted: May 13, 2021; Published: May 26, 2021

Abstract: The purpose of this study was to determine the fungicidal action of liquid smoke generated from young coconut waste for infection of green rot (*Penicillium digitatum*) and blue rot (*Penicillium italicum*) pathogens in postharvest citrus fruit. The pyrolysis of 1 kg of young-coconut resulted in 409 mL of crude liquid smoke, and 300 mL of distilled liquid smoke. The resulting distilled liquid smoke has the following characteristics: brownish-yellow in color, pH of 2, specific density of 1.02 g. mL⁻¹, and a total acid content of 10.7. Liquid smoke produced from young coconut waste was of good quality, in accordance with international quality standards. The overall characteristics of liquid smoke from coconut waste meet international liquid smoke standards, which include specific gravity, color, acidity, and pH in the required range and the absence of dispersed substances. The results showed that in *in vitro* testing, liquid smoke treatment at a concentration of 2.5% was able to retard the mycelium of *Penicillium digitatum* and *Penicillium italicum* with 100% inhibition. Whereas in *in vivo* testing, liquid smoke treatment at a concentration of 75% was able to retard the increase in the lesion diameter of the *P. italicum* fungal infection by 76.1%. However, all concentrations of liquid smoke treatments had no effect on *P. digitatum*. Treatment of the concentration of liquid smoke had no effect on the lesion diameter of the green rot infection on citrus fruit. Whereas in blue rot disease, the concentration of 50, 75, and 100% liquid smoke treatment gave different lesion diameter compared to the control, but among the treatments there was no difference in the lesion diameter. This proved that the treatment of liquid smoke concentration of 50% was established to have fungicidal action against blue rot disease on citrus fruit.

Keywords: Citrus, Fruit Rot, Fungicidal, Liquid Smoke, *Penicillium*

1. Introduction

Food and Agriculture Organization reported that global average loss due to the food postharvest losses was about 38% in industrialized Asia, Africa, Latin America and South East Asia. Among all the factors for reducing the losses on food supply, postharvest diseases of fruits present a major factor that causes the postharvest losses and limits the duration of storage [1].

Citrus are vulnerable to the postharvest decay caused by *Penicillium digitatum*, *Penicillium italicum*, and *Geotrichum citri-aurantii*, which are responsible for the green mold, blue mold, and sour rot post-harvest disease,

respectively [2].

Diseases that often infect citrus fruits are green and blue rot, each caused by the pathogenic fungus *Penicillium digitatum* and *Penicillium italicum*. The occurrence of blue rot higher than green rot. Two symptoms of this disease can be distinguished based on the color of the spores or conidia of the fungus that forms on the surface of citrus fruit, which green on the green rot and blue on the blue rot [3].

The imazalil fungicide used to control *Penicillium* rot diseases was proven to give good results. The use of synthetic pesticides was considered an effective procedure to prevent infection. However, the use of these pesticides always reaps polemics about their negative adverse effects on human and the

environment health [4]. In the further study, proved of the nine *P. digitatum* strains tested, six of them had imazalil resistance with different levels. While the results of testing on five strains of *P. italicum* had not shown resistance to imazalil [5].

Alternative solutions to synthetic pesticide problems can be solved by using botanical pesticides. One kind of safe botanical pesticides are liquid smoke or wood vinegar as a product of the thermochemical conversion of woody biomass into organic acid compounds, phenols, alcohols, carbonyls, ketones, pyridine, etc. which act as antimicrobials and antioxidants [6]. Phenol and furfuraldehyde compounds present in liquid smoke are thought to be the main components, both of which work to inhibit the growth of fungi, so that liquid smoke has the potential for the substitution of synthetic fungicides [7].

Liquid smoke also produced from agricultural waste such as corncobs, fruit bunches, and various fruit shells which also contain lignin, cellulose and hemicellulose compounds or also called lignocellulosic wastes. One of the fruit shells produced by young coconut ice traders in the City of Tasikmalaya was not utilized properly, so the waste accumulated to the landfill site. The waste takes a long time to decompose by microbiological and weather decomposition. Thus, the pyrolysis process of this waste will reduce its flow to the landfill [8].

The aim of this research was to produce liquid smoke from young coconut waste [24] and to test its fungicidal action on fungus-pathogens of citrus fruit, i.e. *Penicillium digitatum* (green rot) and *Penicillium italicum* (blue rot).

2. Material and Methods

2.1. Material Preparation

Materials used include Siamese citrus that were ripe picked from the farmer's garden in Mekarjaya Village, Bayongbong District, Garut Regency, marked by a yellow tinge on the fruit. Young coconut shell waste was collected from four young coconut ice traders in Tasikmalaya City, potato dextrose agar (PDA), aquades, tween 20 (polisorbat 20) and pure pathogenic cultures of *Penicillium digitatum* and *Penicillium italicum*.

While the tools used were hemocytometer, light microscope, disposable petridish, petridish, laminar air flow, tray, glassware, measuring pipette, micro pipette, digital balance, hot plate magnetic stirrer, drying place, thermometer, hygrometer, mini photo studio light box, wood moisture meter and a set of pyrolysis tools.

The young coconut liquid smoke was made by pyrolysis following the procedure of Rahmat *et al.* [8]. Then the resulting coarse liquid smoke was redistilled using a glass distillator in the temperature range of 100 to 110°C. Redistillation was done to remove tar and purify liquid smoke so as not to stain during application. Furthermore, liquid smoke was characterized based on Japanese liquid smoke quality standards, which include: specific gravity, color, transparency, pH, phenol compound content and acid content.

2.2. Experimental Procedures

The *in vivo* experiment consisted of 30 treatments and was repeated three times, so that 30 experimental units were needed. If each unit was filled with three citrus fruits, then the total fruit needed were 90. The fruits were cleaned using a 5% soap solution, then rinsed with water and dried. The uniformity of maturity was treated with the degreening method. [9].

The pathogen was inoculated by dripping 20 µL of conidia suspension onto the surface of the injured fruit. The conidia density was regulated at 10^6 ml^{-1} [4]. Determination of density was calculated using a hemocytometer. The wound was done using a needle that has been sterilized first. The purpose of injury was for infection to occur considering that *Penicillium* is a weak pathogen that attacks through wounds or weak tissue [3, 10]. Furthermore, the fruits were incubated in a plastic box with more than 90% humidity to trigger an infection.

2.3. Data Observation

Data observation included: (i) Increase of lesion diameter. Increased diameter of the lesion was measured every 24 hours after inoculation. The diameter of the infection was determined from the average diameter data transversely, longitudinally, and diagonally minus the diameter of the initial lesion. Data were analyzed using analysis of variance (ANOVA) and followed by Duncan's multiple test; and (ii) Retardation of infection. Retardation of infection was calculated by comparing the infections that occur in the treatment and control. The effectiveness of treatment can be assessed by the extent of infection by smaller pathogens [11]. Retardation of infection was calculated by the formula:

$$Ri (\%) = \frac{dC - dT}{dC} \times 100\%$$

Note: Ri=Retardation of infection (%); dT=diameter in treatment (cm); dC=diameter in control (cm).

Liquid smoke solutions were made in five levels of concentration, namely: C₀, C₁, C₂, C₃, and C₄, each of which were 0, 25, 50, 75, and 100%. The treatment of liquid smoke were applied to the citrus fruit by surface wetting by using different brushes at each concentration level, and they stored in labeled plastic boxes respectively.

3. Result

3.1. Liquid Smoke Properties

Table 1. Quality of coconut-waste liquid smoke.

Parameter	Sample	International Standards
Yield (mL/kg)	300	-
Color	Brownish yellow	Pale yellow-brown
pH	2	1.5 – 3.7
Transparency	Without dispersion	Without dispersion
Density (g/mL)	1.02	≤1.0005
Phenol presence	exist	-
Acid value (%)	10.7	1-18

The overall characteristics of liquid smoke from coconut waste (Table 1) meet international liquid smoke standards, which include specific gravity, color, acidity, and pH in the required range and the absence of dispersed substances.

3.2. Pathogenic Characteristics

The results of macroscopic and microscopic observations of both pathogenic fungi *P. digitatum* and *P. italicum* culture in PDA media with characteristics as in Table 2 and Figure 1. *P.*

digitatum isolate was characterized by mycelium with an olive-green to brown-green top surface due to the presence of a colored conidia mass, smooth hyphae, the lower part of the mycelium which was white until cream, no exudate, and the smooth surface texture of the mycelium. Whereas *P. italicum*, this gray-blue mold when viewed from the top surface was orange to brown from the bottom surface, had a smooth and septated hyphae and an exudate was produced at the top of the mycelium. The surface of the mycelium of *P. italicum* is more bumpy than that of *P. digitatum* [12, 13].

Table 2. Isolate identification on *Penicillium digitatum* and *Penicillium italicum*.

Parameter	<i>P. digitatum</i>	<i>P. italicum</i>
Mycelium surface	Smooth as velvet	Rough bumpy
Color of colony mass	Olive green, then after 14 days it turned brownish green	Blue gray, then after 14 days turned gray
Hyphae shape	Have septa	Have septa
Conidiophore shape	Diverse <i>terverticillate</i>	Nearly uniform <i>terverticillate</i> ,
Conidia shape	Oval or ellipsoidal, diverse and larger sizes	Ellipsoidal and subglobose, smaller and relatively uniform size

3.3. Fungicidal Activity in Vivo Test

a) Increase of lesion diameter

Table 3. Increase of lesion diameter in 24, 48, 72, and 96 hour after incubation.

Pathogen	LC (%)	28 hour	48 hour	72 hour	96 hour
<i>P. digitatum</i>	0	2.07 b	5.36 a	8.62 a	10.19 a
		B	B	B	B
	25	1.45 ab	4.64 a	7.83 a	10.85 a
		B	B	B	B
	50	1.49 ab	4.74 a	8.03 a	11.14 a
		B	B	B	B
	75	1.21 a	4.32 a	7.67 a	11.41 a
		B	B	B	B
	100	1.38 ab	4.76 a	7.85 a	10.62 a
		B	B	B	B
<i>P. italicum</i>	0	1.30 b	2.03 b	2.54 b	3.38 b
		A	A	A	A
	25	0.24 a	0.72 a	1.31 ab	1.90 ab
		A	A	A	A
	50	0.20 a	0.48 a	0.77 a	1.09 a
		A	A	A	A
	75	0.30 a	0.38 a	0.49 a	0.78 a
		A	A	A	A
	100	0.23 a	0.43 a	0.66 a	0.97 a
		A	A	A	A

* Note: LC 25 liquid smoke concentration; Numbers followed by the same letter in uppercase indicate no significant differences for variations in pathogens, and lowercase indicate no significant differences for variations in concentration according to the Duncan's multiple range test at a confidence level of 5%.

b) Retardation of Infection

Table 4. Retardation of infection in 24, 48, 72, and 96 hour after incubation.

Pathogen	LC* (%)	24 hour	48 hour	72 hour	96 hour
<i>P. digitatum</i>	0	0.0	0.0	0.0	0.0
	25	23.1	13.7	7.7	4.2
	50	19.5	11.9	6.4	3.9
	75	42.7	18.6	8.8	5.3
	100	22.5	10.7	8.3	4.9
<i>P. italicum</i>	0	0.0	0.0	0.0	0.0
	25	81.6	63.7	47.9	43.0
	50	84.0	76.1	69.6	67.9
	75	76.3	80.5	80.2	76.1
	100	83.2	79.5	74.1	71.3

*Note: LC=liquid smoke concentration

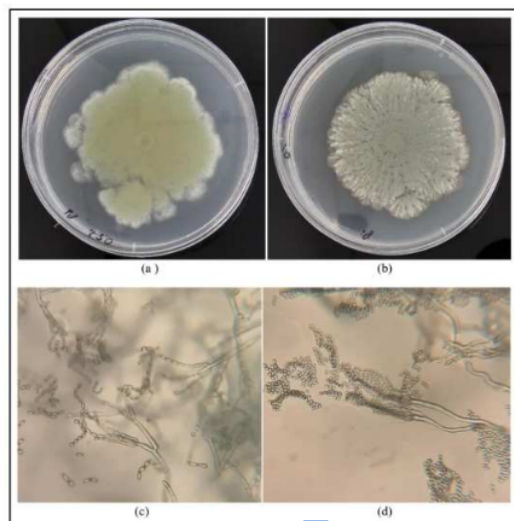


Figure 1. (a) and (b) are morphologies of *P. digitatum* and *P. italicum* mycelium that grow in PDA medium. (c) and (d) are microscopic morphology of conidia and conidiophores of *P. digitatum* and *P. italicum*.

4. Discussion

The reason for testing the content of phenols and acids, because phenol compounds as antioxidants that can retard the growth of microbes. While the content of acetic acid and other organic acids that work as retarder for microbial growth [14]. Phenol and acid levels in liquid smoke can be used as a reference for its quality as a natural pesticide. The high phenol content was positively correlated in retarding the growth of microorganisms [6]. Phenol and terpenoid compounds in liquid smoke from pine wood turned out to damage the cell membrane and interfere with the process of respiration *Colletotrichum sp* [15]. Some organic acids are produced from the breakdown of C=C and C-O-C bonds found in cellulose and hemicellulose. Meanwhile, phenols and other aromatic compounds are produced from decomposition of lignin [16].

Microscopically *P. digitatum* and *P. italicum* have the general characteristics of the genus *Penicillium* including: insulated hyphae, conidiophores formed on hypha that branched near the tip, conidia consisting of globulos-shaped cell units that elongate like chains [17]. *P. digitatum* conidiophore was characterized by its verticillate shape, diverse branching and consisting of a few short-stemmed matulae and branches divided into three to six phialides, conidia in a cylindrical or elliptical shape, thin-walled and sized around 3.5 to 8.0 x 3.0 to 4.0 µm. Whereas conidiophores in *P. italicum* were verticillate, hyaline, sometimes there were branches with stems measuring 100 to 250 x 3.5 to 5.0 µm and the matulae approaches cylindrical, with three to six phialides [12, 13].

Observations in the *in vivo* experiment were the diameter of

the lesion and the percentage of infections in citrus fruits. It turns out that there was a difference in the initial formation of mycelium in *in vivo* and *in vitro* experiments. In the *in vivo* experiments, mycelium began to form at 72 hours after inoculation (Table 3). Meanwhile the *in vitro* testing was formed at 24 hours after inoculation. Environmental factors and host species appeared to be influential in retarding infection. Citrus fruit has the ability to protect itself from pathogens by forming peroxide compounds that were toxic to pathogens. But pathogens also adapt by forming peroxidase enzymes that catalyze the breakdown of peroxide compounds into water and oxygen. The success of a pathogen to attack was determined by the speed of the pathogen in breaking of peroxide compounds [18].

In *Penicillium* rot, the infection occurred in environmental conditions with high humidity, ie humidity reached 90% with temperatures between 25 to 30°C. The reason, humidity was related to the water content in the air, if the water content was lacking, it causes dehydration pathogens and unable to carry out physiological activities. Temperature was also an important factor in cell physiology, where temperature affects the respiration of pathogenic cells. Pathogenic cell respiration will increase pathogen growth. Conversely the respiration of host plant cells will decrease their resistance to infection, so that at this temperature will be suitable for pathogens in conducting infections [19].

Table 3 shows that the increase in diameter of the infectious lesions in green rot gave a higher rate than in blue rot at all concentrations of liquid smoke treatment. It was also known that *P. digitatum* attacked citrus fruits faster than *P. italicum*. That fact was accordance with the previous study, that the green rot caused by *P. digitatum* attacks was very massive, while the blue rot occurred more slowly [20].

In green rot disease, the treatment of liquid smoke concentration not gave a difference in the diameter of the lesion of infection. Whereas in the blue rot disease, the treatment of liquid smoke treatment gave a difference in the diameter of the lesion compared to the control, except for the concentration treatment of 25%, but among fellow treatments the concentration not gave a difference in effect. This proved that, 50% liquid smoke concentration treatment gave the same effectiveness as 75 and 100%, which had anti-fungal activity against blue rot but not against green rot.

Based on datas in Table 4, the liquid smoke treatment on *P. digitatum* provided retardation at the onset of infection, but continued to decrease until 96 hours after application. In *P. italicum*, application of liquid smoke gave a higher retardation value of infection increment until the end of the observation period. This shows that *P. italicum* was more sensitive to liquid smoke treatment than *P. digitatum*. The highest retardation of *P. italicum* was treated with 75% smoke treatment concentration, namely 76.1% in the last observation.

The concentration needed to retard the growth of hyphae or mycelium was much higher than the retarding of conidial growth. This phenomenon was caused by *Penicillium* hyphae could survive in conditions of liquid smoke with low pH

acidity. Postharvest diseases in citrus fruits were dominated by fungal pathogens, because their characteristics are tolerant of the environment with a low pH [3]. Citrus fruits naturally had a low pH, which of 4 to 5 [10]. High acid levels in citrus fruits were caused by the presence of free carbonyl compounds (COOH) which were very important in creating sour taste in various fruits and vegetables [20]. This caused the low concentration of liquid smoke could not retard the growth of *Penicillium* hyphae.

The content of phenol in liquid smoke is closely related to its antifungal properties. Simple phenols in botanical pesticides caused cell membrane disruption which could result in cell damage. Phenol compounds could also retard protein synthesis in cells directly so that the cell physiology process was disrupted [21]. Phenol compounds in clove oil and thyme oil had strong antifungal activity against *Penicillium digitatum* fungus, which, by scanning electron microscope (SEM), showed that hyphae exposed to essential oils had abnormal morphology and growth points of hyphae not fully developed, so that the growth of fungi was hampered [22]. Likewise, phenol compounds contained in liquid smoke, it turned out that the retardation of blue rot disease had more intense hyphal damage compared to fungal green rot disease. In addition, hyphae penetration into fruit tissue also affects the occurrence of disease. Green rot develops faster in warm temperatures between 24 and 28°C, while blue rot was slower [13]. The temperature at the time of the experiment was suitable with the development of green rot disease characterized by hyphae growing deeper before application of liquid smoke, so the hyphae were difficult to be inhibited after application. The use of liquid smoke can also increase the resistance of host plant tissues to attack pathogens. Enzymes peroxidase (POD) and polyphenol oxidase (PPO) in blueberries significantly that could produce oxidative compounds in cells so that plant tissue was more resistant to pathogenic attack. The emergence of these enzymes could be increased by combining liquid smoke with other substances such as peach plant resins [23]. In citrus fruits, the use of liquid smoke could also induce disease resistance. This was proven by the presence of lignification in the wound tissue treated with liquid smoke. However, if the concentration is too high it can damage the fruit so it is not marketable.

5. Conclusion

Liquid smoke from pyrolysis of young coconut waste has proven good quality [38] according to international [17] quality standards, and was able to inhibit the growth of green rot pathogens (*Penicillium digitatum*) and blue rot (*Penicillium italicum*) in citrus fruits.

The treatment of [33] id smoke at a concentration of 2.5% could be retarded *Penicillium digitatum* and *Penicillium italicum* in *in vitro* experiments with retardation of 100%.

In vivo testing, liquid smoke treatment had a retarding effect on the development of the diameter of the blue rot infection (*P. italicum*). The highest retardation occurred in the treatment with 75% concentration of liquid smoke. Whereas for green

fruit rot (*P. digitatum*) the treatment had no effect on retardation.

Acknowledgements

The authors are grateful for the support of the Directorate General of Research and Development of the Ministry of Research, Technology and the Higher Education Republic of Indonesia.

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