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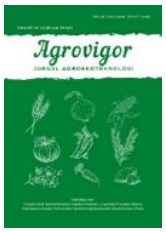

Article

Antimicrobe Activity of Empty Bunches of Palm Tree's Liquid Smoke against *Sclerotium rolfsii* Sacc. and *Puccinia arachidis* Speg. 1884

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is one of the food crops that has high economic value. However, one of the constraints to groundnut production is the stem base rot (*Sclerotium rolfsii*) and rust (*Puccinia arachidis*) diseases. Liquid smoke is known to have antimicrobial activities that need to be further studied for their potential to control various types of pathogens. This study aimed to test the ability of liquid smoke from empty palm bunches in controlling *S. rolfsii* and *P. arachidis* in vitro. The phytotoxicity of liquid smoke to seeds was tested using a rolled paper test established in plastic. Antimicrobial activity was tested by using the poisoning medium method against *S. rolfsii*, while spore germination test against *P. arachidis* was conducted by directly applying liquid smoke drops onto uredospore on a microscope slide. Antimicrobial activity of liquid smoke at concentrations of 1.2%, and 1.4% showed growth inhibition of *S. rolfsii* significantly by relative inhibition level of 78.8%, and 100%, respectively compared to control. The effect of liquid smoke at concentrations of 0.1% and 1.0% inhibited the *P. arachidis* spore germination by 80.2% and 100% at 24 hours and 84.2% and 100% at 48 hours post treatment in compared to control. These indicated that the liquid smoke as an antifungal and a prospective phytochemical to control plant pathogens.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a highly economical food crop as a protein source and plant-based oil (Kurniawan *et al*. 2017). Its productivity in the last 5 years did not increase significantly; in fact, it declined. In 2015, groundnut productivity was 1.72 tonne ha^{-1}, while from 2016 to 2018 it declined to 1.46 tonne $ha^{-1,}$ and in 2019 the productivity was only 0.98 tonne ha^{-1} (BPS 2020). Many factors affect the groundnut production and productivity.

Plant diseases such as stem rot caused by *Sclerotium rolfsii* Sacc., rust caused by *Puccinia arachidis* Speg. 1884, leaf spot by *Cercospora arachidis*/*C. personatum* and mottle virus associated with *Peanut mottle virus* and *Bean common mosaic virus* strain Peanut stripe are one of the groundnut production constraints worldwide, including in Indonesia. The stem rot disease can cause yield loss up to 60% (Kator *et al.* 2015), while rust disease can cause yield loss up to 50-80% (Inayati dan Yusnawan 2016). The yield loss caused by mottle viruses was reported to be about 29.6%-70% in Georgia and 40% in India (Hema *et al.* 2014; Reddy and Thirumala-Devi 2003).

Generally, management efforts of pathogens infecting groundnut by drawing the infected plants when the incidence is still low, crop rotation, utilizing resistant variety, and using chemical synthetic fungicides. However, nowadays, management strategy for plant pathogens approaches by utilizing beneficial microbes such as fungal and bacterial endophytes, rhizobacteria, and rhizosphere fungi as biological control agents, as well as by using plant-based bioactive compounds. Those agents are considered more environmentally friendly methods and cheaper than synthetic pesticides. Previously, *S. rolfsii* able to control by using biological control agents such as *Trichoderma* sp (Kamel *et al*. 2020), *Pseudomonas fluorescens* and rhizobacteria and also by using plant-based volatile oil such as *Piper aduncum* and sereh wangi with inhibition level up to 84.85% and 65.65%, respectively (Idris *et al.* 2023; Nurmansyah 2017). Citronella oil showed the highest antifungal activity against *S. rolfsii* and *Pestalotia* sp. (Nurmansyah *et al*. 2022). The extract of *Hyptis suaveolens* also reported inhibiting the *S. rolfsii* growth (Primayani and Chatri 2018). Recently, plant-derived extracts such as moringa and bougainvillea crude extracts have been reported to be able to decrease the mottle virus titer and increase growth variables on groundnut (Zulfi *et al*. 2024). Furthermore, wood vinegar can reduce the disease incidence of brown spot and dirty panicle on rice, and significantly enhances germination, seedling vigor, shoot height, root length, and fresh weight in comparison to untreated control (Chuaboon *et al*. 2016). Wood vinegar with a concentration less than 1% can increase the growth of banana plantlets, increase defense enzyme activities, and possess antibacterial activities against *Ralstonia syzygii* subsp. *celebesensis* (Nurrahman *et al*. 2021). In vitro study showed that wood vinegar can reduce the growth of *Burkholderia glumae* and increase vigor index and rice germination compared to untreated control (Nurfadillah *et al*. 2022). Based on those facts, utilizing the plant-based phytochemicals is one of the promising agents to control various types of plant pathogens as an alternative, more environmentally friendly control method.

Empty bunches of palm trees are a 23% waste of palm tree fresh bunches. The waste is potentially a source of raw material to produce liquid smoke (Widiastuti and Panji 2007). Empty bunches palm oil tree's liquid smoke (EBPTLS) is synthesized using the pyrolysis process. EBPTLS consist of chemical compounds such as acetate acid, pyridine, benzene sulfonate acid, phenol, and toluene. Acetate acid and phenol are the main compounds of EBPTLS. Generally, phenolic compounds play roles as antibacterials and antifungals (Faisal *et al*. 2020).

EBPTLS reported able to control the growth of *Ganoderma boninense* and *Culvularia* sp in vitro by relative inhibition level 94% and 60% (Mahmud *et al.* 2021). EBPTLS by concentration 3% and 4% can inhibit the growth of rubber leaf fall disease caused by *Pestaliopsis microspora* in vitro up to 60% and 100%, respectively (Sahara *et al*. 2022). EBPTLS can also play a role as an insecticide to control *Plutella xylostella*, *Spodoptera litura*, *Plusia* spp., and *Crocidolomia binotalis* (Sari *et al*. 2018). The potential of EBPTLS in controlling plant pathogens was demonstrated for fungal pathogens infecting palm oil trees, but its potential as a phytochemical needs to be explored against various types of pathogens infecting food and horticulture crops. The research aimed to test the ability of EBPTLS to control the growth of *Sclerotium rolfsii* and the germination of *Puccinia arachidis*. This is the first in vitro

study which demonstrated the EBPTLS as antifungal against essential pathogens on groundnut.

MATERIALS AND METHODS

Preparation of Empty Bunches of Palm Tree's Liquid Smoke (EBPTLS)

The Empty Bunches of Palm Tree's collected from the public palm tree plantation in Pekanbaru. The empty bunches were cut up into small fragments with a size of 2-3 cm, then air-dried for 1 day. The empty bunches of palm tree's liquid smoke was obtained by direct as well as indirect combustion at a temperature of 400°C according to Soldera *et al.* (2008). The condensation fumes (liquid smoke) were collected and stored until use. The liquid smoke synthesis process was conducted at Forestry Laboratory, Riau University.

Isolation of *Sclerotium rolfsii*

The *Sclerotium rolfsii* was isolated from a peanut with stem base rot symptoms. The sclerotia from diseased plants were collected, surface sterilized, then put onto potato dextrose agar (PDA) and incubated at room temperature for 4-7 days. The growing mycelia were transferred to a new PDA to obtain pure *S. rolfsii* isolates.

Effect Empty Bunches of Palm Tree's Liquid Smoke on seed germination and vigor index

Seed treatment was conducted to test the toxicity of EBPTLS on peanut seeds according to Ilyas *et al.*(2007). The peanut seeds were soaked in EBPTLS solution with concentrations of 0.1%, 0.5%, 1.0%, 1.2%, and 1.4%, while untreated control seeds were soaked in sterile water. The soaking duration was done for 1 hr according to Tang *et al.* (2020). After treatments, seeds were air-dried for 48 hr. Each treatment consisted of 20 seeds. The seeds were ordered in 3 pieces of moist paper and rolled using between-paper test method (Sadjad 1993). The experiments were repeated three times as a replication. All of the treated seeds between the paper test were incubated in a germinator at turns temperature of 20-30°C to maintain germination in optimum conditions. The observation of seed germination was conducted twice, namely for the first count at 5 days and the final count at 10 days after seed incubation. The percentage of germination and vigor index was counted, including normal and abnormal seedlings. The percentage of germination (GP) and vigor index (VI) were calculated using the formula provided by ISTA (2010) below:

$$GP\ \% = \frac{\sum normal\ seedling\ at\ first\ count\ + \sum normal\ seedling\ at\ final\ count}{\sum seed\ planted} \times 100\%$$

while formula to determine Vigor Index (VI) is :

$$VI\ \% = \frac{\sum normal\ seedling\ at\ first\ count}{\sum seed\ planted} \times 100\%$$

In vitro test of the empty bunches of palm tree's liquid smoke against *Sclerotium rolfsii*

The effect of EBPTLS on *S. rolfsii* growth was conducted by a media poisoning test. The PDA was added by EBPTLS solution with final concentrations of 0.1%, 0.5%, 1.0%, 1.2%, and 1.4% with a volume of 20 ml. Untreated control media were poured without EBPTLS. The colony of *S. rolfsii* with a 5 mm diameter was put in the middle of the media. Each treatment consisted of 5 Petri dishes as a replication. The diameter of *S. rolfsii* growth was measured when the diameter of the untreated control was fully developed in the Petri dish. The diameter was calculated by measuring the length of the shortest and longest diameters of the colony with the formula adopted from Elfina *et al.* (2015) below, with D the diameter colony of *S. rolfsii*, d1 the shortest diameter, and d2 longest diameter of the colony.

$$D = \frac{d1 + d2}{2}$$

Spore germination test

EBPTLS concentration of 0.1% and 1.0% was dropped on object glass, and *P. arachidis* uredospores from infected leaves were added to the EBPTLS drops using the edge of cover glass. The process was conducted in laminar air flow. Observation of uredospores was carried out at 24 dan 48 hours after treatment, counting the germinated and non-germinated uredospores and documenting with a digital camera. The percentage of spore germination was calculated using the formula adopted from (Gabriel and Riyatno 1989) below. V. spore germination (viability), g. number of germinated spores and u. number of not germinated spores

$$V = \frac{g}{(g + u)} \times 100\%$$

The relative inhibition level (RIL) of *S. rolfsii* growth and *P. arachidis* germination were calculated

using the formula as described below. RIL of *S. rolfsii* calculated based on colony diameter, while RIL of *P. arachidis* based on the number of germinated uredospores.

$$RIL(\%) = \frac{control\ value - treatment\ value}{control\ value} \times 100\%$$

Data analysis

The experiment was arranged in completely randomized design. All obtained data were tabulated in Microsoft Excel and ANOVA analysis was performed using SAS 9.4 and MINITAB software followed by a Tukey test at 95% confidence level.

RESULTS AND DISCUSSION

Effect of the Empty Bunches of Palm Tree's Liquid Smoke (EBPTLS) treatment on seed germination

EBPTLS treatments with a concentration of 0.1%-1.4% did not affect the vigor index of peanut seeds but increased seed germination. The higher the concentration of EBPTLS, the higher the percentage of germinated seeds. Peanut seeds are said to be of good quality if the percentage of seeds that germinate is more than 80% (Sadjad 1993) and have a growth capacity of more than 90% (BPS 2009). Concentration of 1.2% and 1.4% showed the highest germination percentages and were significantly different compared to the untreated control, but did not differ from other concentrations (Table 1).

EBPTLS treatments with a concentration of 1.4% affected root length and were significantly different compared to the untreated control, but all those treatments did not affected plumule length (Table 1). The peanut seeds treated with EBPTLS showed more lateral roots and longer roots compared to the untreated control (Figure 1). The liquid smokes contain Karrikin and cyanohydrin compounds, which can stimulate seed germination through chemical signals involving α/β-fold hydrolase KAI2 receptor (Villaecija *et al.* 2019), and can stimulate various types of plant growth and development (Khatoon *et al.* 2020).

Effect of the Empty Bunches of Palm Tree's Liquid Smoke (EBPTLS) on *Sclerotium rolfsii* growth

The EBPTLS treatments at a concentration of 1.0%, 1.2%, and 1.4% suppressed the colony growth of *S. rolfsii* and were significantly different compared to the untreated control. Increasing the concentration of liquid smoke from 0.1% to 1.4% was proven to be able to suppress the growth of *S. rolfsii* colonies with a relative inhibition level ranging from 2.0% to 100.0%. At the concentration of 1.4%, the *S. rolfsii* was unable to grow (Table 2, Figure 2).

These findings proved the anti-microbe activity of EBPTLS. As previously reported, EBPTLS contain phenol and saponin, both of which have antimicrobial properties (Mahmud *et al.* 2021). Furthermore, saponin content in EBPTLS can function as antifungal, antibacterial, and antiviral, which may help palm oil trees prevent and cope with pest and disease attacks (Ariyanti *et al.* 2024). Increasing the concentration of liquid smoke will increase acid and phenol contents in PDA, thus suppressing the fungal colony growth (Suresh *et al.* 2019; Araujo *et al.* 2018). The acid and phenol content of liquid smokes in fungal media will disrupt fungal membrane cells' function, mainly on cell membrane permeability and ultimately resulting in fungal fatality (Turecka *et al.* 2018).

Table 1. The effect of the empty bunch of palm oil liquid smoke on peanut seeds germination

Concentration (%)	Vigor index (%)	Germination (%)	Plumule length (cm)	Root length (cm)
Control	84.0 ± 5.5 a	82.0 ± 8.5 c	5.8 ± 1.5 a	12.0 ± 3.9 b
0.1	87.0 ± 8.4 a	83.0 ± 8.4 bc	6.0 ± 1.5 a	12.4 ± 2.4 ab
0.5	88.0 ± 7.6 a	85.0 ± 7.9 abc	6.3 ± 2.6 a	14.0 ± 3.4 ab
1.0	89.0 ± 6.5 a	91.0 ± 5.5 abc	6.3 ± 2.1 a	14.4 ± 3.1 ab
1.2	91.0 ± 8.9 a	92.0 ± 4.5 ab	6.8 ± 1.4 a	15.0 ± 1.8 ab
1.4	91.0 ± 6.5 a	93.0 ± 5.7 a	7.2 ± 1.5 a	16.0 ± 3.1 a

*Numbers in the same column followed by the same letters were not significantly different by Tukey test at α=5%.

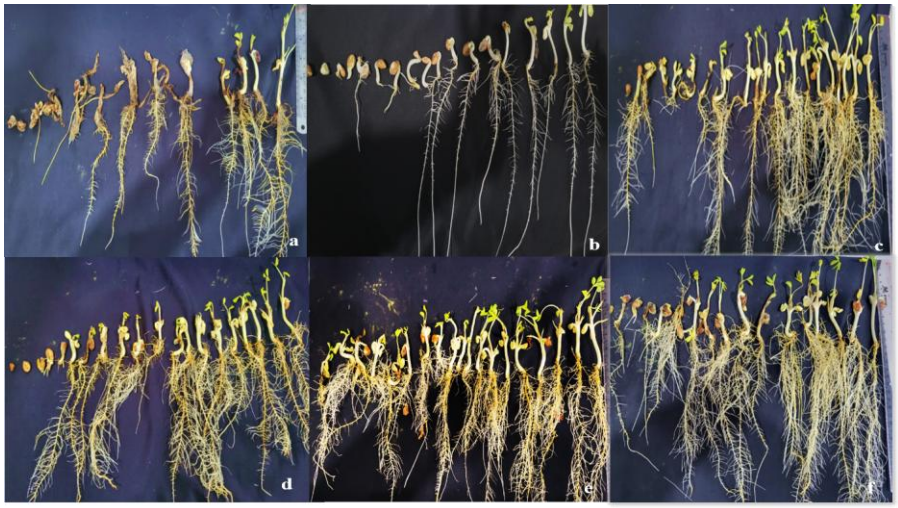

Figure 1. Performance of groundnut seeds germination treated by liquid smoke (LS). A. control, b. LS 0.1%, c. LS 0.5%, d. LS 1%. E. LS 1.2%, and f. LS 1.4%

Table 2. Effect of empty bunch of palm oil liquid smoke on *Sclerotium rolfsii* colony growth.

Liquid smoke concentration (%)	ø Colony (cm)	RIL (%)**
0.0	8.5 ± 0.0 a*	0.0 ± 0.0 d
0.1	8.3 ± 0.2 a	2.0 ± 2.0 d
0.5	8.0 ± 0.1 a	5.4 ± 1.7 d
1.0	5.5 ± 0.4 b	35.0 ± 5.1 c
1.2	1.8 ± 0.8 c	78.8 ± 8.8 b
1.4	0.0 ± 0.0 d	100.0 ± 0.0 a

*Numbers in the same column followed by the same letters were not significantly different byTukey test at α 5%.

** RIL. Relative inhibition level

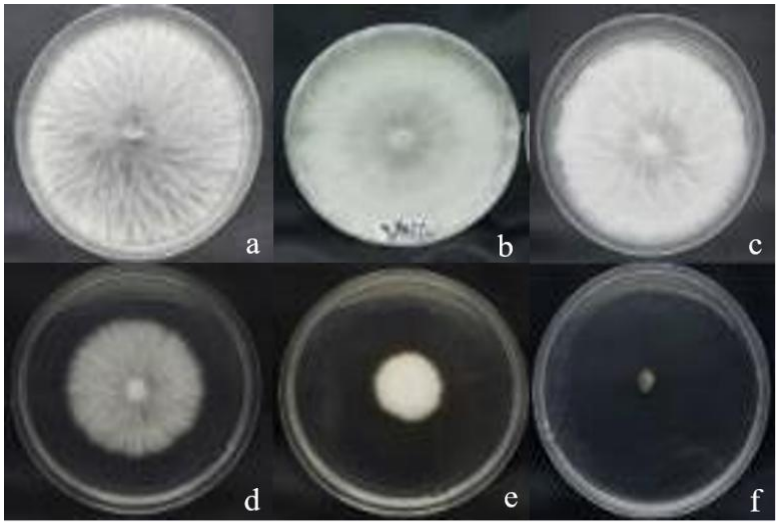

Figure 2. Effect of empty bunches oil palm liquid smoke on the colony growth of *Sclerotium rolfsii*. Liquid smoke concentration a. Untreated Control, b. 0.1%, c. 0.5%, d. 1.0%, e. 1.2%, and f. 1.4%.

Table 3. The effect of thr empty bunches oil palm liquid smoke on germination of *Puccinia arachidis*'s uredospore.

Liquid smoke concentration (%)	Spore viability (%)			
	24 hr	RIL**(%)	48 hr	RIL (%)
Control	8.40 ± 7.06 a*	0.0 a	9.68 ± 7.47 a	0.0 a
0.1	1.66 ± 0.90 ab	80.2 b	1.53 ± 0.92 b	84.2 b
1.0	0.00 ± 0.00 b	100.0 b	0.00 ± 0.00 b	100.0 b

*Numbers in the same column followed by the same letters were not significantly different by Tukey test at α 5%.
** RIL. Relative inhibition level

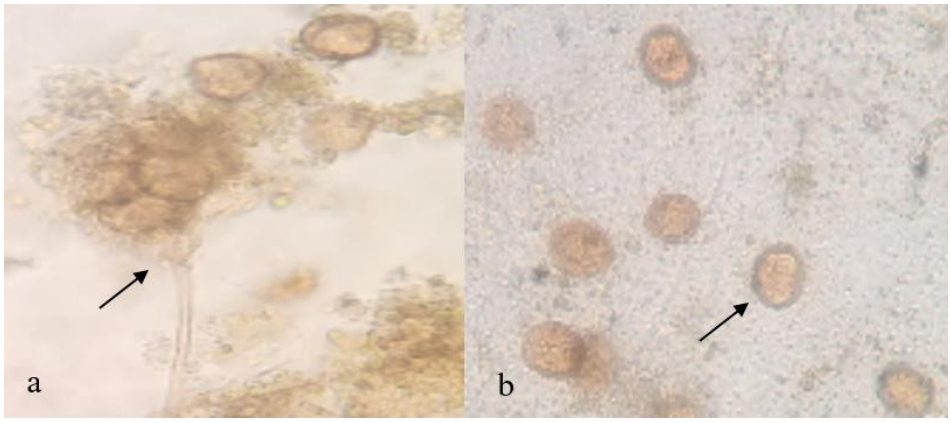

Figure 3. The effect of empty bunches oil palm liquid smokes on uredospore *Puccinia arachidis* at 24 hours after treated by 1.0% liquid smoke. a. Germination of untreated control uredospore, and b. uredospores treated by 1.0% liquid smoke were not germinate. Magnification 400 times.

The mechanism of liquid smokes inhibited the development of fungi by acidifying cytoplasm and by damaging the membrane surface, causing food active transport via membrane to be disrupted; it also caused destabilization of various functions and cell components, whereas alcohol content of liquid smokes can destroy microbes' membrane cells such as lignin and cellulose (Komarayanti and Wibowo 2015). The liquid smokes from coconut shell, pinecone, and oil palm branch have been shown to effectively control blood disease on banana by inducing resistance of banana seedlings by increasing levels of ethylene, auxin, lignin, activity of POD and PAL, and plant growth, significantly (Aisyah *et al*. 2018). Taken together, the roles of liquid smokes vary, including the ability to increase plant growth, inhibit fungal and bacterial growth, and induce plant systemic resistance by enhancing antioxidant activity.

Effect of Empty Bunches of Palm Tree's Liquid Smoke (EBPTLS) treatment on *Puccinia arachidis* germination

The EBPTLS treatment at a concentration of 0.1% and 1.0% can inhibit germination of uredospores of *P. arachidis* either at 24 hours or 48 hours post-treatment in comparison to the untreated control. The uredospore treated by 0.1%, and 1.0% liquid smoke showed significantly lower spore germination than the untreated control uredospore, with the maximum relative inhibition level reaching 100% at a concentration of 1.0% (Table 3, Figure 3). Active compounds found in liquid smoke include acetic acid 41.47%, phenol 23.68%, ethylene glycol 16.29%, ethanol 7.65% and acetone 6.02%. Among them, phenol and acetic acid are well known as anti-microbes because they hamper the metabolism of microbial cell systems and reduce intercellular pH, resulting in inhibition and cell death (Priatni *et al*. 2017). Additionally, the acetic acid compound, phenol, and alcohol in liquid smokes can suppress spore germination and fungal growth (Chuaboon *et al*. 2016). Liquid smokes can change the structure of fungal morphology and inhibit spore germination by altering fungal membrane cell

permeability and protein synthesis, hence hindering fungal metabolism (Zhou *et al*. 2024).

CONCLUSION

Liquid smokes from empty oil palm bunches have been shown to demonstrate antifungal activities, inhibiting the growth of *Sclerotium rolfsii* colonies and uredospore germination of *Puccinia arachidis*. The relative inhibition level in reducing colony growth and uredospore germination increases up to 100% depending on the concentration. At concentrations ranging from 0.1% to 1.4%, the liquid smoke was not phytotoxic to peanut germination. The future direction will be addressed by evaluating the effectiveness of the EBPTLS treatments in controlling the infection of pathogens in planta.

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AUTHORS CONTRIBUTIONS

RNP conducted the experiment, collected data and wrote the original draft, ETT supervised the methodology of in vitro test and data analysis, and TAD contributed to conceptualization, interpreted data and reviewed the manuscript. All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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