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Antifungal Activity of Rice Husk-Derived Liquid Smoke: Growth Suppression of *Rhizoctonia solani* and Bioactive Compound Profiling

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Abstract

Sheath blight poses a major challenge in rice cultivation, with current control methods relying heavily on synthetic fungicides. Environmentally sustainable alternatives, such as bioactive compounds derived from agricultural waste, offer promising potential for integrated disease management. This study investigated the antifungal efficacy of rice husk-derived liquid smoke against *Rhizoctonia solani*, the causal agent of rice sheath blight, and identified its active compounds using gas chromatography-mass spectrometry (GC-MS) analysis. Liquid smoke was produced through the pyrolysis of rice husks and incorporated into potato dextrose agar at concentrations of 1%, 2%, 3%, 4%, and 5% (v/v). *R. solani* was isolated from infected rice plants, and its pathogenicity was confirmed on rice seeds and seedlings. The *in vitro* antifungal activity was assessed by measuring colony diameter and calculating the percentage of mycelial growth inhibition over 7 days. The results demonstrated that liquid smoke significantly inhibited the growth of *R. solani* in a concentration-dependent manner ($p < 0.001$; exact $p = 4.36 \times 10^{-24}$), with the 5% concentration achieving 100% inhibition. Qualitative microscopic observations revealed morphological abnormalities in fungal hyphae at higher concentrations. GC-MS analysis identified 40 bioactive compounds in the liquid smoke, including phenolic compounds and organic acids, which are known for their antimicrobial properties. The findings suggest that rice husk-derived liquid smoke possesses potent antifungal activity against *R. solani* due to the presence of these compounds. This study concludes that rice husk liquid smoke can serve as an effective, eco-friendly alternative to synthetic fungicides for controlling sheath blight disease in rice cultivation.

Keywords: GC-MS; inhibitor; natural pesticide; pyrolysis; sheath blight

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally. In Indonesia, rice is not only a fundamental component of the national diet but also a critical aspect of the country's agricultural economy. The growing demand for rice is driven by increasing population density and rising per capita consumption, which places significant pressure on rice production systems (Tumrani et al., 2015; Zahra et al., 2024). Rice yields are frequently hampered by various

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biotic stressors, among which pest and disease outbreaks are major contributors to yield decline in many rice-growing regions. Plant pests and diseases, if not promptly addressed, can lead to substantial reductions in both the quality and quantity of crops (Liu and Wang, 2016; Subedi et al., 2023). Notably, one of the most detrimental diseases affecting rice is sheath blight, caused by the soil-borne fungus *Rhizoctonia solani*. This pathogen poses a severe threat to rice cultivation due to its widespread prevalence and the difficulty in managing the disease effectively (Morio et al., 2024). *R. solani* is notorious for its ability to form sclerotia or resting mycelia. This pathogen can survive under adverse conditions and complicating control measures (Li et al., 2021).

The initial symptoms of sheath blight typically appear on the lower leaf sheaths near the waterline, where humidity is high. The disease progresses from small, round, white spots to expanding brown lesions that coalesce into irregularly shaped blights. Advanced symptoms include grayish lesions with brown margins, leading to lodging and even death of rice plants under severe infestations (Senapati et al., 2022). The severity of sheath blight is exacerbated by high nitrogen fertilization, dense planting, and warm, humid climates, which are common in many rice-growing regions of Indonesia and other parts of Southeast Asia (Abbas et al., 2023).

Current management strategies for sheath blight encompass cultural practices such as land sanitation, optimal planting density, balanced fertilization, and the application of fungicides (Li et al., 2021). While synthetic fungicides are widely used and effective, their long-term application poses significant environmental and health risks. These risks include the accumulation of pesticide residues in agricultural products, environmental pollution, and the disruption of non-target microbial communities (Asghar et al., 2016). Additionally, the extensive use of synthetic fungicides can lead to the development of fungicide-resistant strains of *R. solani* (Lucas et al., 2015). Such concerns necessitate the exploration of alternative, eco-friendly fungicidal agents that are both effective and sustainable.

One promising approach involves utilizing natural resources rich in lignin, cellulose, and hemicellulose to produce bioactive compounds with antifungal properties (Souto et al., 2021). Rice husk, an abundant agricultural by-product constituting approximately 20% of the total rice grain weight, contains these constituents and is often underutilized due to its recalcitrant nature. In Indonesia alone, millions of tons of rice husk are generated annually, leading to disposal challenges and environmental concerns due to its slow decomposition and potential for pollution if burned openly (Goodman, 2020). Through pyrolysis, rice husk can be converted into liquid smoke. This condensate is rich in phenolic, acidic, and carbonyl compounds, which are known for their antimicrobial activities (Risfaheri et al., 2018). Compared to other biomass sources, rice husk offers a unique advantage due to its high silica content and distinct composition of lignocellulosic polymers, which can yield a different spectrum of bioactive compounds during pyrolysis. These unique chemical constituents may enhance the antimicrobial profile of its liquid smoke. By transforming rice husk into liquid smoke, not only is agricultural waste effectively managed, but a potential natural fungicide is produced. However, the antifungal efficacy of rice husk-derived liquid smoke specifically against *R. solani* is still poorly documented and lacks experimental validation.

Phenolic compounds, which are particularly abundant in liquid smoke, exhibit potent antibacterial and antifungal properties. These compounds can disrupt microbial cell membranes, denature proteins, and interfere with essential metabolic pathways (Ecevit et al., 2022). Higher phenol concentrations correlate with increased antimicrobial efficacy. Acetic acid and carbonyl compounds present in liquid smoke also contribute to its inhibitory effects on microbial growth by lowering pH levels and reacting with microbial proteins. Previous studies have demonstrated the effectiveness of liquid smoke derived from various biomass sources in inhibiting plant pathogens such as *Corynespora cassiicola*, the causal agent of leaf fall disease in rubber plants (Winarni and Komarayati, 2021; Gao et al., 2024). While the antimicrobial activity of liquid smoke from various biomass sources has been reported, the specific effects of rice husk-derived liquid smoke on fungal pathogens remain understudied, with limited efforts to correlate antifungal efficacy with chemical composition. Most existing research treats liquid smoke as a broad-spectrum antimicrobial without systematically linking its bioactive constituents to pathogen-specific outcomes. To date, no studies have specifically evaluated the antifungal activity of rice husk-derived liquid smoke against *R. solani*.

Understanding the chemical composition of rice husk-derived liquid smoke is essential for

elucidating its antifungal mechanisms and enhancing its efficacy as a biofungicide. Identifying these compounds is crucial for understanding the mechanisms of action and optimizing the formulation of biofungicides. This information can facilitate the development of formulations with optimized concentrations of active compounds.

Despite the recognized antifungal properties of liquid smoke, its efficacy against *R. solani* remains largely untested, especially when sourced from rice husk. Previous studies have seldom evaluated rice husk-derived liquid smoke in the context of plant-pathogenic fungi, and none have assessed its specific activity against *R. solani*. This unexplored area limits our understanding of its potential as a targeted biofungicide. Addressing this gap is essential for advancing sustainable disease management strategies in rice cultivation and reducing dependency on synthetic fungicides. Utilizing agricultural waste such as rice husk to develop bio-based plant protection aligns with the principles of sustainable agriculture by promoting waste valorization, reducing chemical inputs, and supporting low-cost alternatives for farmers in developing countries.

MATERIALS AND METHOD

Study site and duration

This research was conducted over 4 months, from January to May 2024. The research took place at the Plant Pest and Disease Laboratory and the Genetics, Microbiology, and Biotechnology (Gembio) Laboratory of the Universitas Jember, Indonesia.

Production of rice husk-derived liquid smoke

A total of 12 kg of rice husks were air-dried to reduce their moisture content. After drying, the moisture content of the rice husks was approximately 10%. The dried husks were placed into a pyrolysis reactor, which is a sealed combustion drum designed for controlled thermal decomposition in an oxygen-limited environment. The reactor was a batch-type reactor with dimensions of 80 cm in diameter and 150 cm in height. The reactor was tightly sealed to maintain the necessary conditions for pyrolysis. The pyrolysis process was initiated by igniting a combustion source using diesel fuel. The temperature within the reactor was carefully monitored and maintained between 200 and 250 °C by regulating the intensity of the flame. This temperature range is optimal for the thermal degradation of lignocellulosic materials into liquid smoke constituents. The process was sustained for 5 hours to ensure complete pyrolysis of the rice husks. Combustion duration was strictly maintained throughout this period. Vapors produced during pyrolysis were directed through a condenser system filled with water, facilitating the condensation of volatile compounds into liquid smoke. The condenser utilized water as the cooling medium with a constant flow rate of 5 l $minute^{-1}$, maintaining a cooling temperature of approximately 25 °C. The efficiency of liquid smoke production was calculated based on the yield, defined as the ratio of the mass of liquid smoke obtained to the initial mass of rice husks used (Hasibuan et al., 2018).

Isolation and identification of *R. solani*

Isolation of *R. solani* was performed following the method described by Al-Fadhal et al. (2019). Rice plants exhibiting characteristic sheath blight symptoms were collected from infected fields. Symptomatic tissues were cut into small sections approximately 1 cm^2 in size and surface-sterilized by immersing them in a 1% sodium hypochlorite (NaOCl) solution for 1 minute. This was followed by rinsing 3 times with sterile distilled water to remove any residual disinfectant.

The sterilized tissue sections were aseptically placed onto potato dextrose agar (PDA) plates and incubated at 25±2 °C for 7 days. Fungal growth emerging from the tissues was subcultured to obtain pure isolates. Hyphal tips of the suspected *R. solani* colonies were transferred to fresh PDA plates using a sterile needle and incubated under the same conditions to establish pure cultures.

Identification of the isolated fungus was conducted through both macroscopic and microscopic examinations. Macroscopically, the colony morphology was observed, noting characteristics such as mycelial color, texture, and the presence of sclerotia. Microscopically, a small amount of mycelium was mounted on a glass slide with a drop of lactophenol cotton blue stain and covered with a coverslip. The slide was examined under a light microscope at 400× magnification. Diagnostic features such as hyphal branching patterns, septation, and the presence of clamp connections were recorded (Ajayi-Oyetunde and Bradley, 2017).

Pathogenicity tests

Two assays were conducted to confirm the pathogenicity of the isolated *R. solani*: one on rice seeds and the other on rice seedlings. For the seed assay, healthy rice seeds of the variety Inpari 32

were surface-sterilized by soaking in a 1% NaOCl solution for 15 minutes, followed by rinsing with sterile distilled water. Ten sterilized seeds were placed on PDA plates fully colonized by a five-day-old culture of *R. solani*. This assay included three replicates and a control consisting of seeds on PDA without the pathogen. The plates were incubated at room temperature (25±2 °C) for 5 days. Seed germination and symptom development were observed daily, focusing on the appearance of brown lesions on emerging radicles and coleoptiles (Khangura et al., 1999).

For the seedling assay, rice seeds were germinated and grown in sterile soil within plastic trays for 14 days to produce uniform seedlings. The seedlings were then transplanted into pots containing 2.5 kg of sterilized soil and maintained under greenhouse conditions for an additional 24 days. Inoculation was performed by wounding the leaf sheaths with a sterile needle to facilitate infection, with each seedling receiving one wound per plant, followed by placing a mycelial plug (7 mm diameter) from an actively growing *R. solani* culture onto the wound site. The inoculated area was wrapped with moist sterile cotton to maintain humidity and covered with aluminum foil to prevent desiccation. Control plants were treated similarly but without the fungal inoculum. Plants were observed daily for symptom development (Adhipathi et al., 2013).

In vitro efficacy test of liquid smoke against *R. solani*

The antifungal activity of rice husk-derived liquid smoke against *R. solani* was evaluated using the poisoned medium technique (Faisa et al., 2018). PDA medium was prepared and allowed to cool to approximately 50 to 55 °C to prevent thermal degradation of the liquid smoke constituents. Sterile liquid smoke was added to the molten PDA at concentrations of 1%, 2%, 3%, 4%, and 5% (v/v) based on the experimental design. The medium was thoroughly mixed to ensure homogeneity and poured into sterile 9 cm diameter petri dishes under aseptic conditions, then allowed to solidify. The final pH of the PDA medium after the addition of liquid smoke was measured and ranged from 5 to 5.5, depending on the concentration used.

A 7 mm diameter mycelial disc was cut from the periphery of a five-day-old *R. solani* culture using a sterile cork borer and placed at the center of each petri dish with the mycelium side down. The inoculated plates were incubated at 25±2 °C for 7 days. A control treatment without liquid smoke was included for comparison. The experiment was arranged in a completely randomized design (CRD) with six treatments (0%, 1%, 2%, 3%, 4%, and 5% liquid smoke concentrations) and five replicates, totaling 30 experimental units.

The radial growth of *R. solani* was measured daily, recording the diameter of the colony along two perpendicular axes and calculating the average. Observations continued until the control plates were fully colonized by the fungus. The antifungal activity was expressed as the percentage of mycelial growth inhibition (PGI) using Equation 1.

$$PGI = \left(\frac{Dc\text{-}Dt}{Dc}\right) \times 100\% \quad (1)$$

where Dc is the average colony diameter in control plates and Dt is the average colony diameter in treated plates.

Analysis of liquid smoke compounds

Chemical characterization of the rice husk-derived liquid smoke was performed using gas chromatography-mass spectrometry (GC-MS). Liquid smoke samples were filtered through a 0.22 μm syringe filter membrane to remove particulate matter. The analysis was conducted using a GC-MS equipped with a split/splitless injector. Chromatographic separation was achieved using a Restek Rtx®-50 capillary column (30 m length × 0.25 mm inner diameter × 0.5 μm film thickness).

Operating parameters included an injection volume of 1 μl of the filtered liquid smoke sample in split mode with a split ratio of 1:50. Helium was used as the carrier gas at a constant flow rate of 1.0 ml minute^{-1}. The injector temperature was set at 250 °C. The oven temperature program started at 80 °C, held for 2 minutes, ramped at 10 °C minute^{-1} to 280 °C, and then held at 280 °C for 10 minutes. The mass spectrometer detector temperature was 280 °C, and the mass scan range was m/z 40 to 500. No internal standards were used for quantification purposes in this analysis. Blank and solvent control injections were included to validate the compound identification and confirm the absence of contamination or artifacts. The mass spectra obtained were analyzed using the Wiley9.LIB mass spectral library for compound identification. Compounds were identified based on match quality and comparison of retention times and mass spectra to known standards (Budaraga et al., 2016).

Data analysis

Experimental data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) to determine the significance of treatment effects on fungal growth inhibition. When significant differences were detected at the 5% probability level, Tukey's Honestly Significant Difference (HSD) test was performed for multiple comparisons among treatment means at a 95% confidence level. Statistical analyses were conducted using DSAASTAT version 1.101 software.

RESULTS AND DISCUSSION

***R. solani* isolate**

The fungus *R. solani* was successfully isolated from rice plants exhibiting sheath blight symptoms collected from paddy fields in Jember Regency, Indonesia. The macroscopic examination of the fungal colonies on PDA revealed brownish-white mycelia that formed concentric ring patterns. Notably, sclerotia were observed; they initially appeared white upon formation and gradually developed into dark brown to black over time (Figure 1a). These sclerotia resembled sand particles with varying shapes (round, oval, and irregular) and possessed wet surfaces with speckled, pore-like appearances, lacking glossiness (Figure 1b). They typically emerged on the 10^{th} day after incubation on PDA medium. Microscopic observations demonstrated that the hyphae were septate with right-angled branching (Figure 1c and 1d). Additionally, nuclei were visible within the hyphae (Figure 1e). The fungal hyphae adhered closely to the media surface, appearing thin and delicate.

These morphological and microscopic characteristics are consistent with previous descriptions of *R. solani*. Nasimi et al. (2024) reported that *R. solani* forms sclerotia as small, dense, irregular aggregates with speckled surfaces and exudates. Similarly, Ajayi-Oyetunde and Bradley (2018) noted that sclerotia of *R. solani* are initially white when young, turning brown to black as they mature, with wet surfaces due to exudate secretion. The right-angled branching of septate hyphae without clamp connections aligns with findings by Hamzah et al. (2021). The presence of nuclei within the hyphae corroborates observations by Nasimi et al. (2024), confirming the identity of the isolated fungus as *R. solani*.

In the seed assay, germination was significantly inhibited in seeds exposed to *R. solani*. Infected seeds exhibited browning of roots and coleoptiles, yellowing of emerging leaves, and overall wilting compared to healthy controls (Figure 1f and 1g). There was a noticeable difference in seedling size between healthy and infected plants. Additionally, sclerotia were observed adhering to the seeds and coleoptiles (Figure 1h, 1i, and 1j). These findings indicate the pathogenic nature of the isolate, corroborating reports by Bigirimana et al. (2015) that *R. solani* causes browning of roots and stem bases in rice, leading to inhibited germination. The presence of sclerotia on seeds and emerging leaves aligns with observations by Kashyap et al. (2019), who found that *R. solani* infection results in brown lesions at the stem base and sclerotial formation on seed surfaces.

In the assay on rice plants, symptoms manifested as brown spots that were initially circular and expanded irregularly in all directions (Figure 1k and 1l). The spots were light brown or cream-colored with dark brown edges. Infected plants exhibited dry blight and shrinkage, particularly at the junction between the leaf sheath and leaf blade, rendering the plant parts brittle and prone to breakage. These symptoms are consistent with descriptions by Neha et al. (2016) that infected rice plants develop brown spots on leaf sheaths starting from the stem base. As the infection progresses, increased severity is observed on the leaf sheaths, potentially leading to overall plant desiccation and browning. The pathogenicity test has confirmed that the isolated fungus is capable of causing sheath blight symptoms, validating its identity as *R. solani* and its use in subsequent experiments.

Efficiency of liquid smoke production and chemical composition

From 12 kg of rice husk, 1.03 l of liquid smoke, and 4 kg of rice husk charcoal were obtained, yielding 8.58%. The liquid smoke had a pH of 4, indicating acidic properties. It exhibited a strong and lasting smoky aroma, a water-like texture with a dark brown color, and sediment formation. The yield obtained was lower than that reported by Nugrahaini et al. (2017), who achieved a yield of 16.15%. Several factors can influence liquid smoke yield, including the type of raw material, moisture content, and combustion method (Xin et al., 2021). Dry rice husk tends to produce less liquid smoke compared to wet materials. Nugrahaini et al. (2017) noted that wet raw materials prolong combustion time, potentially increasing yield. Imperfect

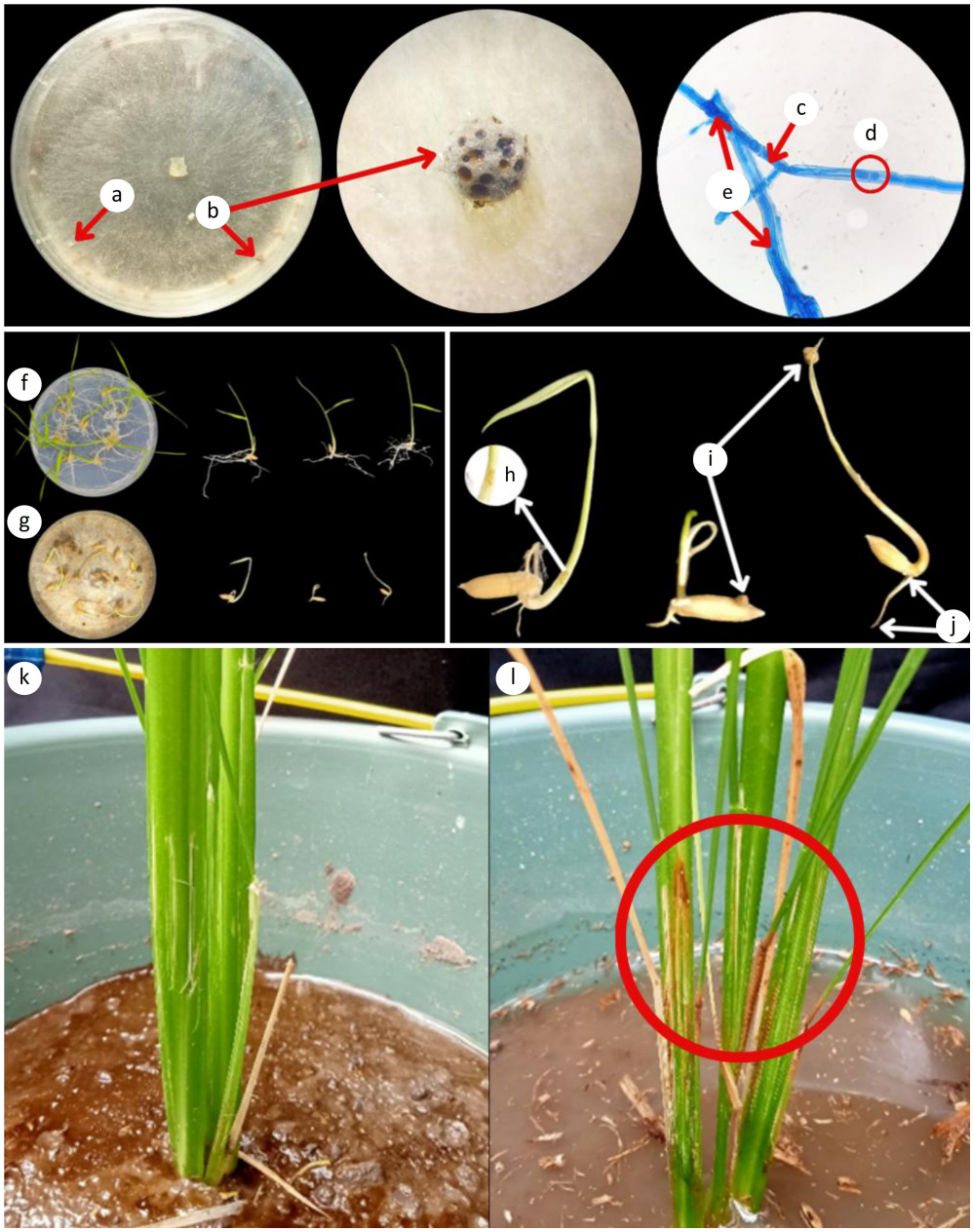

Figure 1. Morphological features *R. solani*: (a) Initial development of sclerotia; (b) Fully formed sclerotia; (c) Right-angle branching of hyphae, a typical characteristic of *R. solani*; (d) Septate hyphae; (e) Hyphal cells with visible nuclei observed at 1000× magnification. Pathogenicity assessment on rice seeds: (f) Normal germination of healthy seeds; (g) Inhibited germination of infected seeds. Disease symptoms on seedlings: (h) Brown lesions on coleoptiles; (i) Sclerotia formation on infected tissue; (j) Discoloration and browning of roots. Pathogenicity assessment on rice plants: (k) Healthy rice plant; (l) Rice plant showing typical sheath blight symptoms following *R. solani* inoculation

combustion conditions, such as leaks or low flame intensity, can also affect the quantity and quality of liquid smoke produced.

The acidity of the liquid smoke is significant for its antimicrobial properties. A pH of 4 can inhibit microbial growth, as low pH environments are unfavorable for many pathogens (Peñalva et al., 2008). The acidic nature is attributed to organic acids formed during pyrolysis, such as acetic acid, which result from the decomposition of cellulose and hemicellulose components of the rice husk.

GC-MS analysis revealed that the liquid smoke contained 40 identifiable compounds, including phenols, acids, and carbonyls (Figure 2, Table 1). Based on the analysis results, the

primary compounds identified in rice husk liquid smoke showed the highest concentration of acetic acid at 13.23%, while the compound with the lowest concentration was Z-7-Hexadecenal at 0.15%. After accumulating the total amounts of compounds from various classes, it was found that the concentrations of phenols, acids, and carbonyls were 26.74%, 21.44%, and 17.52%, respectively. The remaining concentration of 34.3% consisted of various other classes of compounds.

The main components were acetic acid, derived from cellulose decomposition, and phenolic compounds originating from lignin breakdown (Catherine et al., 2016). Phenols are known for their antimicrobial properties and contribute significantly to the antifungal activity of liquid smoke (Teodoro et al., 2015). The phenol content is influenced by the extent of lignin decomposition; more extensive breakdown leads to higher phenol concentrations (Wang et al., 2019).

Effect of liquid smoke on *R. solani* growth

Observations were conducted for 7 days to assess the effects of rice husk-derived liquid smoke on the growth of *R. solani*. Daily measurements of colony diameter were recorded, and the results are depicted in Figure 3a. In the control treatment without liquid smoke, the fungus exhibited rapid growth, completely colonizing the petri dish by the second day. In contrast, the addition of liquid smoke at varying concentrations delayed the growth of *R. solani*. At a 1% concentration, the fungus filled the petri dish by the 3rd day, 2% by the 5th day, and 3% by the 6th day. Notably, *R. solani* did not fully colonize the petri dishes at 4% and 5% liquid smoke concentrations, indicating a significant inhibitory effect at higher concentrations (Figure 3b).

The colony diameter measurements revealed that *R. solani* grew rapidly in the control, with the mycelium spreading across the medium within 2 days. In media supplemented with 1% liquid smoke, the average colony diameter on the second day was 6.94 cm, indicating a slower growth rate compared to the control, although the difference was not statistically significant. At a 2% liquid smoke concentration, the average colony diameter was reduced to 4.14 cm, and at 3%, it further decreased to 2.6 cm. Interestingly, at 2% and 3% concentrations, the fungal colonies exhibited abnormal growth patterns, with the mycelium appearing thicker and tending to grow upwards rather than spreading laterally across the medium (Figure 3c). This contrasts with the normal growth morphology of *R. solani*, where the mycelium is thin, expands laterally, and adheres closely to the medium surface. At 4% and 5% liquid smoke concentrations, there was no observable growth of *R. solani*, suggesting that these concentrations are fungicidal rather than merely fungistatic.

By the 4th day, colonies in the control and 1% treatments had completely filled the petri dishes. In the 2% and 3% treatments, the average colony diameters were 8.38 cm and 6.34 cm, respectively. The colonies at these concentrations displayed septation and appeared thicker compared to the control (Figure 3d). By the 6th day, colonies in the 1%, 2%, 3%, and 4% treatments had filled the petri dishes. However, in the 4% treatment, fungal growth was minimal, with an average colony diameter of 0.24 cm, indicating substantial inhibition. In the 5% treatment, *R. solani* did not exhibit any growth throughout the observation period.

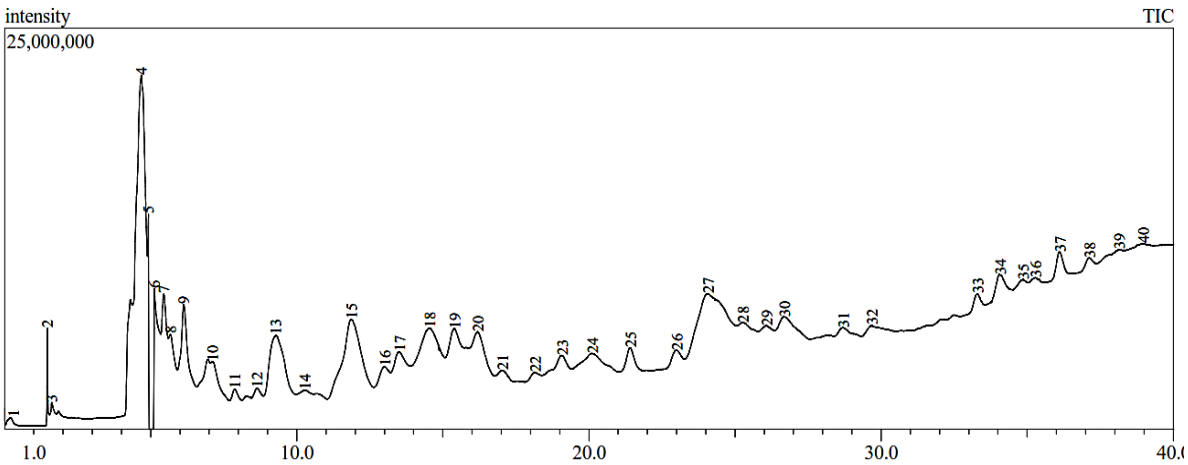

Figure 2. GC-MS chromatogram of rice husk-derived liquid smoke

Table 1. GC-MS analysis of rice husk liquid smoke

Peak	Retention time (minute)	Area (%)	Compound name
1	0.202	0.15	Z-7-Hexadecenal
2	1.464	0.29	Cyclopropane, 1,1-Dibromo-2-Chloro-2-Fluoro
3	1.614	0.30	2-Propanone
4	4.680	13.23	Acetic Acid
5	4.918	0.82	Acetic Acid, Hydrazide
6	5.124	2.69	1,2-Epoxy-3-Propyl Acetate
7	5.444	3.01	Pyridine
8	5.679	2.51	1-Hydroxy-2-Butanone
9	6.132	4.73	Formamide, N,N-Dimethyl
10	7.121	2.59	2-Furanmethanol
11	7.878	1.08	4(3H)-Pyrimidinone
12	8.644	1.08	2-Butanone, 1-(Acetyloxy)
13	9.284	5.62	Cyclopentanone, 2-Methyl
14	10.282	1.16	2-Furanone, 2,5-Dihydro-3,5-Dimethyl
15	11.873	7.47	Phenol
16	13.024	1.58	Phenol, 4-Methoxy
17	13.525	2.82	Acetic Acid, Chloro-, Butyl Ester
18	14.561	6.27	Phenol, 4-Methyl
19	15.412	4.47	Anhydro-Sugar
20	16.206	3.42	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One
21	17.054	0.86	Phenol, 3-Ethyl
22	18.171	0.52	Phenol, 4-Ethyl-2-Methoxy
23	19.091	1.59	2-Propenoic Acid, 2-Methyl-, Ethyl Ester
24	20.133	2.30	2-Furancarboxaldehyde, 5-(Hydroxymethyl)
25	21.431	0.83	Phenol, 2,6-Dimethoxy
26	23.017	0.68	2,8,9-Trioxa-5-Aza-1-Silabicyclo[3.3.3]Undecane, 1-Methyl
27	24.089	8.44	2,8,9-Trioxa-5-Aza-1-Silabicyclo[3.3.3]Undecane, 1-Methyl
28	25.284	2.28	Benzene, 1,2,3-Trimethoxy-5-Methyl
29	26.095	1.48	Ethanone, 1-(2-Hydroxy-6-Methoxyphenyl)
30	26.705	2.64	Hexane-3,3-D2, 1-Bromo
31	28.717	0.68	Benzeneacetonitrile, 4-Hydroxy
32	29.699	0.61	1,6-Anhydro-Beta-D-Glucopyranose
33	33.315	0.60	Hexadecane, 2,6,10,14-Tetramethyl
34	34.087	1.70	Tridecanoic Acid
35	34.871	0.95	9-Benzyl-6-Ethenyl-9H-Purine
36	35.297	0.87	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]
37	36.135	1.85	Octadecanoic Acid, Ethyl Ester
38	37.150	1.45	Hexadecane, 2,6,10,14-Tetramethyl
39	38.167	2.20	Tricyclo[4.3.1.0(2,5)]Decane
40	38.982	2.17	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)
Total		100.00	

On the final day of observation, colonies in the 1%, 2%, and 3% treatments had eventually filled the petri dishes, albeit at a slower rate compared to the control. In the 4% treatment, growth remained very slow, with an average colony diameter of 0.44 cm on the 7th day. The absence of growth in the 5% treatment reinforces the inhibitory potency of higher liquid smoke concentrations. The colonies in the 2% and 3% treatments showed septation and thicker mycelium, suggesting stress responses or morphological adaptations due to the presence of inhibitory compounds in the liquid smoke. These observations are consistent with the findings of Imaningsih et al. (2020) and Rahmat et al. (2020), who reported that increasing concentrations of liquid smoke significantly inhibit fungal colony diameter due to

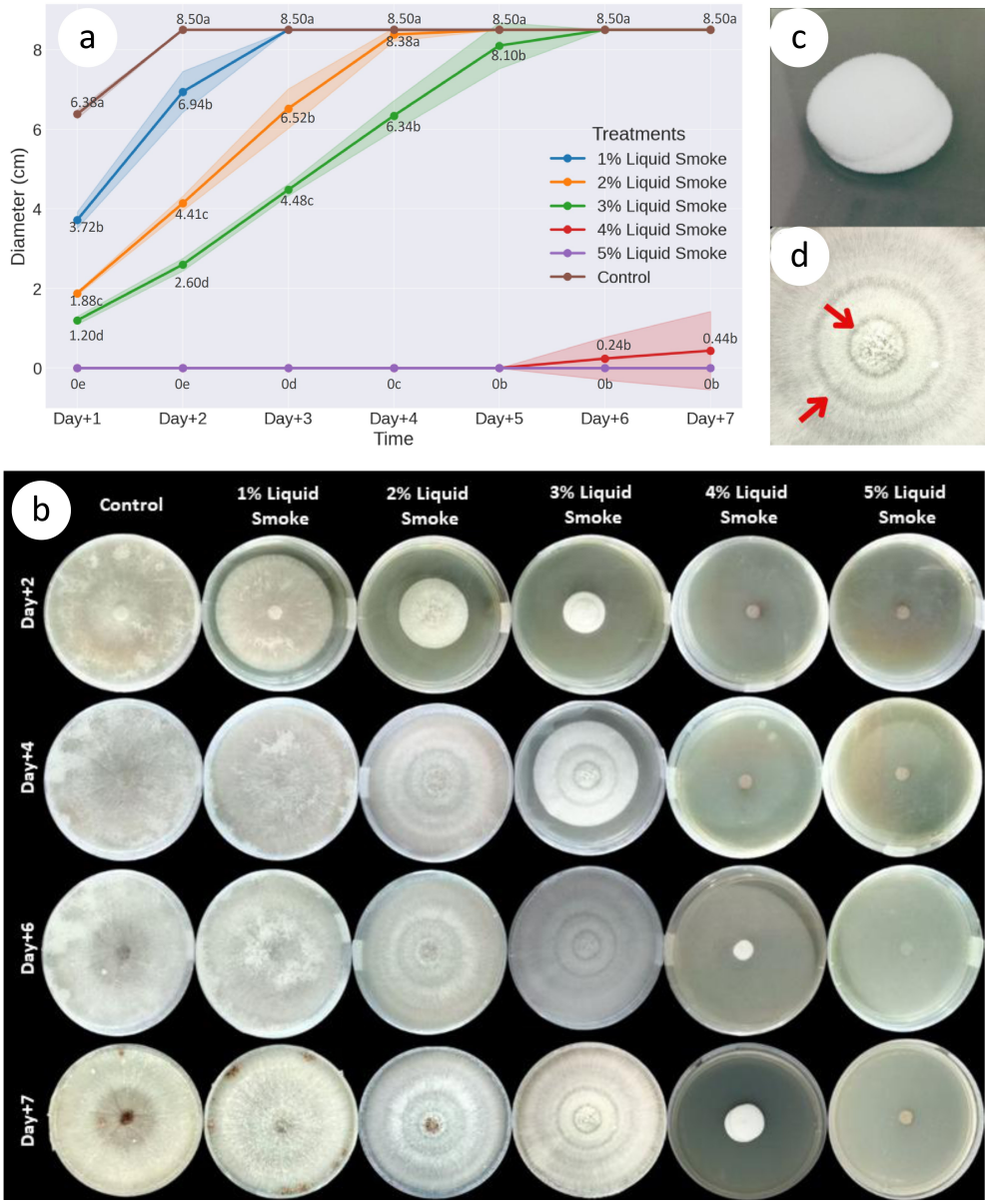

Figure 3. Impact of liquid smoke on the growth of *R. solani*. (a) Colony diameter of *R. solani* under different treatments. (b) Growth patterns of *R. solani* on PDA medium with increasing concentrations of liquid smoke (0%, 1%, 2%, 3%, 4%, and 5%) were monitored over 7 days. Higher concentrations (≥ 3%) significantly suppressed fungal growth, with nearly complete inhibition observed at 4% and 5%. (c) Abnormal upward growth of *R. solani* mycelium. (d) Irregular mycelial growth with pronounced septation

Note: Lines marked with the same letter on the same day indicate no significant difference (Tukey's test, $p < 0.001$; exact p = 4.36×10^{-24}, 95% confidence level)

the presence of phenolic compounds and organic acids.

The growth rates decreased progressively with increasing concentrations of liquid smoke (Figure 4a). In the control and 1% treatments, the growth rate declined over time. However, in the 2% and 3% treatments, there was an initial increase in growth rate on the 2nd day, reaching peaks of 2.17 cm on day 3 for the 2% treatment and 1.62 cm on day 5 for the 3% treatment, followed by a decline. This transient increase may reflect an adaptive response of the fungus to the inhibitory environment before succumbing to the effects of the liquid smoke.

The percentage of PGI demonstrated that the inhibitory effect was concentration-dependent (Figure 4b). For the 1% concentration, the inhibition rates of 41.70% and 18.35% were observed during the first two days, while the 2% concentration sustained the inhibitory effects for

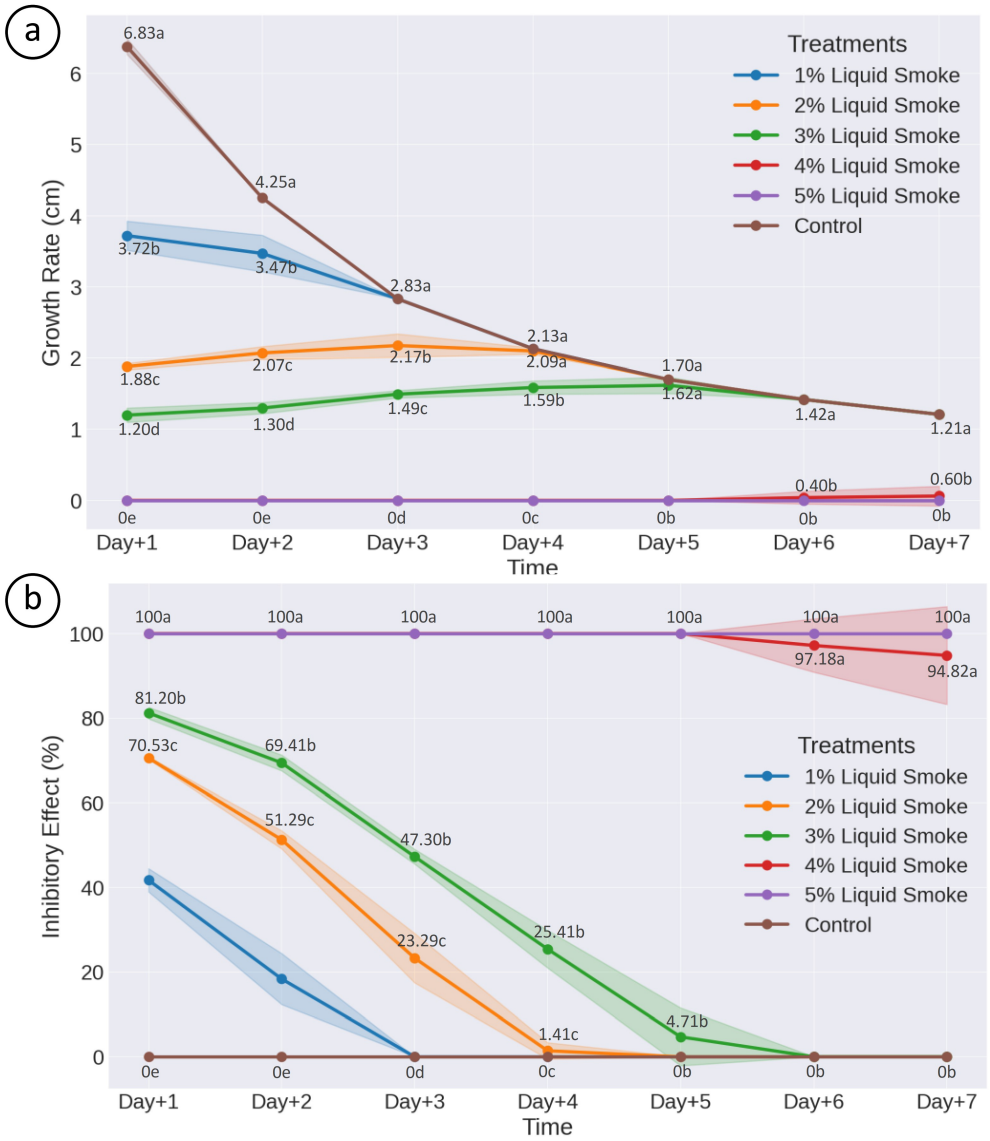

Figure 4. (a) Growth rate of *R. solani* under various treatments, (b) Inhibitory effect of liquid smoke on *R. solani* across different treatment groups

Note: Lines marked with the same letter on the same day indicate no significant difference (Tukey's test, 95% confidence level)

the first four days. The 3% concentration showed significant inhibition from day 1 to 5. The 4% and 5% concentrations exhibited the highest inhibition percentages, with the 5% concentration completely inhibiting fungal growth throughout the experiment. Similar trends have been reported by Imaningsih et al. (2020), emphasizing that increased concentrations of liquid smoke lead to higher inhibition rates due to the cumulative effects of antifungal compounds.

Microscopic examination of the fungal hyphae provided insights into the morphological effects of liquid smoke. In the control, the hyphae appeared robust, with right-angled branching and clear septation. In contrast, hyphae grown in the presence of liquid smoke exhibited structural abnormalities, including curling, shrinkage, thinner cell walls, and fragility (Figure 5). At a 1% concentration, internal hyphal structures appeared abnormal compared to the control. In the 4% concentration, hyphal septa were not visible, suggesting significant disruption of cellular integrity.

These morphological changes suggest that the antifungal mechanisms of liquid smoke may involve disruption of cell wall synthesis, membrane integrity, and metabolic processes (Lucas et al., 2015). The acidic nature of liquid smoke, primarily due to acetic acid and its derivatives, can cause denaturation of enzymes and destabilization of microbial cell membrane permeability (Guimarães et al., 2018; Lourenço

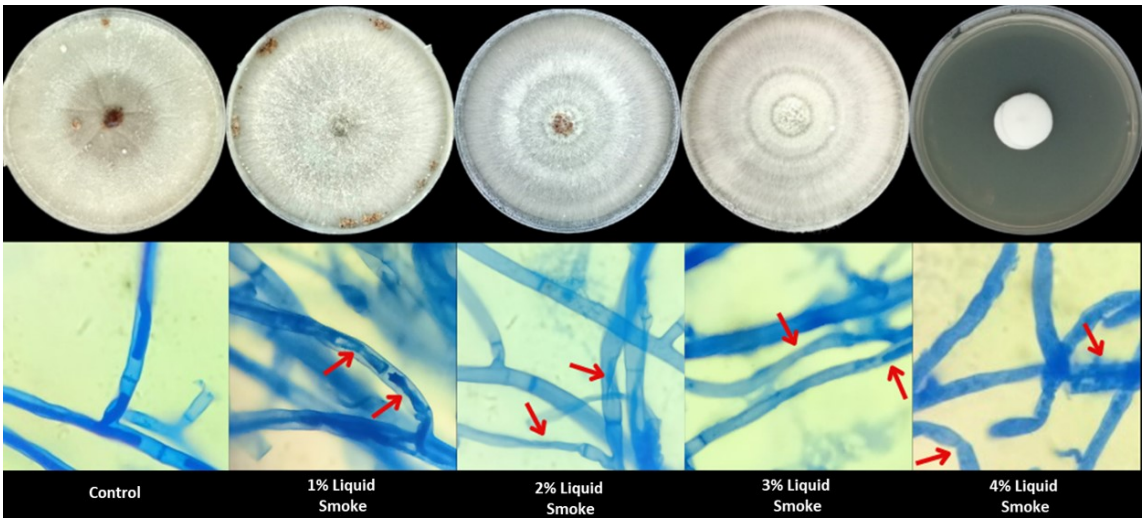

Figure 5. Structural abnormalities of *R. solani* treated with liquid smoke

et al., 2019). Phenolic compounds disrupt pathogen membranes, interfere with metabolic processes and enzymes, and denature proteins in fungal membranes, leading to cell death (Wang et al., 2017; Carvalho et al., 2018). Notably, the combination of organic acids such as acetic acid and phenolic compounds, including phenol, 4-methoxyphenol, 4-methylphenol, and 2,6-dimethoxyphenol, may exhibit synergistic antifungal effects. This synergistic mechanism enhances their collective antimicrobial efficacy. Organic acids can weaken microbial cell membranes and increase permeability, facilitating deeper penetration of phenolic compounds, which subsequently amplify their disruptive effects on cellular proteins and enzymes (Carvalho et al., 2018; Oramahi et al., 2024).

The abnormal growth patterns observed, such as upward growth and thickening of the mycelium in the 2% and 3% treatments, may be attributed to the fungus attempting to avoid contact with inhibitory compounds in the medium. This behavior suggests an adaptive response, where *R. solani* directs its growth away from the unfavorable environment. However, the continued presence of antifungal agents in the medium ultimately impedes its ability to colonize effectively. According to Oramahi et al. (2024), such antifungal compounds in liquid smoke can inhibit hyphal extension and branching, leading to reduced colony biomass.

These findings demonstrate that rice husk-derived liquid smoke effectively inhibits the growth of *R. solani* in a concentration-dependent manner. The significant reduction in colony diameter, growth rate, and observable morphological abnormalities at higher concentrations highlights the potential of liquid smoke as a natural antifungal agent. The presence of phenolic compounds and organic acids, as identified in the chemical analysis, contributes to the antifungal properties of the liquid smoke. The study aligns with previous research by Imaningsih et al. (2020), confirming the efficacy of liquid smoke against phytopathogenic fungi.

CONCLUSIONS

Rice husk-derived liquid smoke demonstrated significant antifungal activity against *R. solani*, with complete inhibition at a 5% concentration. The presence of phenolic compounds and organic acids contributes to its efficacy, causing morphological alterations in the pathogen. These results indicate that rice husk liquid smoke is a promising, environmentally friendly alternative to synthetic fungicides for controlling sheath blight in rice. However, it is important to note that these findings were obtained under *in vitro* conditions, which may not fully represent field-level efficacy due to differences in environmental variables, plant physiology, and microbial interactions. Further research should focus on field trials to validate its effectiveness under natural conditions and explore optimal application methods for practical agricultural use.

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