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RESEARCH PAPER

Identification and sensitivity testing of whip smut pathogen on sugarcane to fungicides and plant extracts

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ABSTRACT

Whip smut symptoms have emerged in sugarcane plantations in Central Lampung Regency, Lampung Province. However, the identity of the pathogen and its sensitivity to various fungicidal active ingredients remain unclear. This study aims to identify the whip smut pathogen in sugarcane plantations in Central Lampung Regency and evaluate its sensitivity to different fungicidal active ingredients.The research involved morphological and molecular identification of the pathogen, along with sensitivity testing against fungicides containing carbendazim, prochloraz, and mancozeb, as well as plant-based fungicides derived from *puyangan* (*Zingiber zerumbet*) and *jamuan* (*Curcuma zedoaria*) extracts. Morphological analysis revealed that the whip smut pathogen has septate hyphae, cylindrical sporidia measuring 7.45–18.31 µm in length and 1.63–3.89 µm in width, and round, yellowish-brown teliospores with an average size of 6.39 × 6.66 µm. Molecular identification confirmed that the LA UKN isolate from Central Lampung Regency belongs to Sporisorium scitamineum, with a bootstrap value of 93%. Sensitivity testing indicated that the pathogen is highly susceptible to carbendazim and prochloraz but less sensitive to mancozeb, *puyangan* extract, and *jamuan* extract.

Key words: *Curcuma zedoaria,* molecular, morphology, *Sporisorium scitamineum,* whip smut, *Zingiber zerumbet*

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a tropical plant belonging to the Poaceae family. It is one of the most economically valuable plantation commodities in the world and has great potential for development in tropical countries like Indonesia. According to the Central Bureau of Statistics (BPS) (2023), national sugar production was only 2.35 million tons, while national sugar consumption reached 5.3 million tons, resulting in a sugar supply deficit of 2.95 million tons. This deficit is covered through imports from countries such as Thailand. One of the main factors limiting sugarcane productivity is disease attacks.

Whip smut is a major sugarcane disease that can cause substantial losses, both quantitatively and qualitatively. Qualitative losses occur when the disease reduces the quality of the sugarcane, while quantitative lossess refer to significant decrease in sugarcane production. Susceptible sugarcane plants can lose more than 60% of their yield (Hidayah, 2020). Malik & Siagian (2022) stated that whip smut can cause up to a 73% reduction in sugarcane weight.

Whip smut is caused by the fungus *Sporisorium scitamineum*, previously known as *Ustilago scitamineum* (Diyasti et al., 2021). This disease leads to the formation of long, black whips that emerge from the top of the sugarcane stalks, which are composed of plant tissue surrounded by black teliospores. Typically, the whips are pencil-sized, unbranched, and rigid. Whip smut is widely recognized for its impact on sugarcane yield and quality, and it is most severe during the active growing stage of the plant (Shuai et al., 2023). To date, control of whip smut has primarily been achieved by planting resistant varieties. Another effective control method is the use of fungicides.

This research aims to identify the pathogen responsible for whip smut symptoms in sugarcane in Central Lampung Regency and to assess the sensitivity of the whip smut pathogen to several fungicide active ingredients, namely carbendazim, mancozeb, and prochloraz, as well as biopesticides derived from rhizome extracts such as *puyangan* (*Zingiber zerumbet*) and *jamuan* (*Curcuma zedoaria*). This study will confirm the cause of the disease in sugarcane plants exhibiting

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whip smut symptoms and evaluate the sensitivity of the pathogen to several synthetic and plant-based fungicides in sugarcane in Central Lampung Regency, which was previously unknown.

MATERIALS AND METHODS

Research Site. This research was conducted from July 2023 to February 2024 at the Laboratory of Plant Disease, Department of Plant Protection, and the Laboratory of Agricultural Biotechnology, Faculty of Agriculture, University of Lampung. Fungal samples were collected from in sugarcane in Central Lampung Regency.

Isolation and Purification of Pathogens in Plants with Whip Smut Symptoms. The pathogen was isolated by collecting spores from the whip and placing them on manually prepared Potato Sucrose Agar (PSA) medium. PSA was prepared by boiling 200 g of potatoes in 1 L of water, straining to obtain the broth, adding 20 g of sucrose and 15–20 g of agar, heating until dissolved, and sterilizing the mixture in autoclave before pouring it into sterile containers.

Once mycelial growth was observed, purification was performed by selecting fungal isolates with distinct macroscopic characteristics, such as colony color and shape. Pathogenicity tests were conducted following Koch's postulates by inoculating each isolate onto healthy plants. Isolates that induced symptoms post-inoculation were further identified using morphological and molecular methods.

Morphological Identification. Morphological identification was based on direct observations of disease signs (spores) in the field, fungal colonies on PSA medium, and microscopic examinations using a compound microscope. Observations of whip smut spores included their shape, color, and size, while colony characteristics on PSA medium were assessed based on shape, surface color, and underside color. These morphological traits were compared with relevant literature (Sundar et al., 2012; Jose et al., 2017; Hidayah, 2020; Shuai et al., 2023).

Molecular Identification. Molecular identification was performed based on the method described by Fitriana et al. (2021) with slight modifications.

DNA extraction began with culturing the isolated fungus in liquid media for seven days, followed by genomic DNA extraction using the CTAB method. The fungal colony was centrifuged at 14,000 rpm for 10 min at 21 ºC, washed with 70% ethanol, and resuspended in a buffer solution. The mixture was homogenized and incubated at 1–2 days.

After incubation, the pellet is ground for 15 minutes, transferred to a 1.5 mL tube, mixed with 2% CTAB, and incubated in a water bath at 65 ºC for 1 hour. The mixture is then extracted using PCI (phenol: chloroform: isoamyl alcohol), centrifuged, and the supernatant is transferred to a new tube. The washing process continues with CI (chloroform: isoamyl alcohol) in a 1:1 ratio, followed by another centrifugation. The obtained supernatant is mixed with cold isopropanol, incubated at -20 ºC for 20 min, and centrifuged to obtain the DNA pellet. The pellet is washed with 70% ethanol, air-dried for 1–2 days, and then dissolved in TE buffer.

The extracted genomic DNA is verified through electrophoresis on a 0.1% agarose gel, run for 60 min at 55 volts, and visualized using the Digi-Doc Imaging System. Subsequently, DNA is amplified using Internal Transcribed Spacer (ITS) primers. The amplification process involves mixing 12.5 µL Red mix, 1 µL forward primer, 1 µL reverse primer, 1 µL DNA, and 9.5 µL sterile water, making a total reaction volume of 25 µL.

The DNA amplification process consists of three main stages: denaturation, annealing, and extension. Amplification is performed using the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990). The reaction begins with pre-denaturation at 95 ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 1 min, annealing at 48 ºC for 1 min, extension at 72 ºC for 1 min, and a final extension at 72 ºC for 5 min.

The resulting PCR product is then analyzed using electrophoresis on a 0.1% agarose gel, run for 60 min at 55 volts, and visualized with the Digi-Doc Imaging System. The amplified product is subsequently sent for sequencing at PT Genetika Science Indonesia.

For phylogenetic analysis, *Paraleptosphaeria orobanches* strain CBS 127945 (JF740230.1) was used as an outgroup. Sequence alignment was performed using CLUSTAL W and BLASTN against the NCBI database. A phylogenetic tree was constructed using MEGA 6 (Neighbor-Joining method) (Tamura et al., 2013). Fungal species were identified based on the closest related species.

Sensitivity Test of Whip Smut Pathogen to Various Fungicide Active Ingredients

Colony Growth of the Whip Smut Fungus. The sensitivity of the whip smut pathogen to various fungicides was evaluated using the poisoned food

technique. The purified pathogen was grown on media containing different fungicide treatments, each applied at the recommended dosage: *puyangan* (*Z. zerumbet*) (10 g/100 mL), *jamuan* (*C. zedoaria*) (10 g/100 mL), carbendazim (2 g/L), prochloraz-manganese-chloride complex (5 g/L), and mancozeb (3 g/L).

To prepare the fungicide test media, PDA was mixed with solutions of each fungicide, homogenized, poured into sterile petri dishes, and allowed to solidify. The whip smut pathogen culture was excised using a 0.5 cm cork borer and placed at the center of each petri dish. The cultures were incubated at room temperature, with each treatment replicated three times.

Fungal colony growth was measured on days 2, 4, and 6 after incubation (DAI) over a 7-day period. The colony diameter was determined using a ruler at four different positions: vertical, horizontal, and two diagonal directions (Figure 1). The final colony diameter for each observation was calculated as the average of these measurements and expressed in centimeters (cm), using the formula from Akin et al. (2023):

$$d = \frac{(A + B + C + D)}{4}$$

d = Diameter of the fungal colony;
A = Measurement of the fungal colony diameter horizontally;
B = Measurement of the fungal colony diameter vertically;
C, D = Measurement of the fungal colony diameter diagonally;

Relative Inhibition Rate (RIR) Calculation. The sensitivity of the whip smut pathogen was determined by calculating the Relative Inhibition Rate (RIR) of each fungicide against fungal colony growth using the method of Joshi et al. (2013):

$$RIR = \frac{d_1 - d_2}{d_1} \times 100\%$$

RIR = Relative Inhibition Rate;
d_1 = Diameter of the fungal colony without treatment (control);
d_2 = Diameter of the fungal colony with treatment.

Whip Smut Spore Germination Test. Spore germination refers to the ability of spores to germinate. To determine germination ability or spore viability, a germination test was conducted using the formula by Akin et al. (2023):

$$S_g = \frac{G_s}{S_o} \times 100\%$$

S_g = Spore germination (%);
G_s = Number of germinated spores;
S_o = Total spores observed.

The germination of whip smut spores was assessed by incubating the spore suspension for 24 h. The suspension was prepared by mixing 0.55 g of spores, collected from plants exhibiting whip smut symptoms in sugarcane plantations, with 350 mL of sterile water. The suspension was then divided into 50 mL aliquots for each treatment, and fungicides were added. Each treatment was shaken for 24 h, after which a drop of the suspension was placed on a glass slide and covered with a cover slip. The number of germinated and ungerminated spores was counted using an Olympus BX53 binocular microscope (Germany) at 40× magnification. Spores were considered germinated if they had formed a germ tube at least half the diameter of the spore.

Data Analysis. The data obtained from the fungicide sensitivity test and spore viability test were tested for homogeneity using Bartlett's test and for additivity using Tukey's test. If these assumptions were met, the data were analyzed using an F-test, followed by the Least Significant Difference (LSD) test at a 5% significance level.

RESULTS AND DISCUSSION

Colony Characteristics of the Whip Smut Pathogen. The isolation of the whip smut pathogen from spore samples revealed distinctive colony characteristics. Macroscopically, the fungal isolate exhibited a white

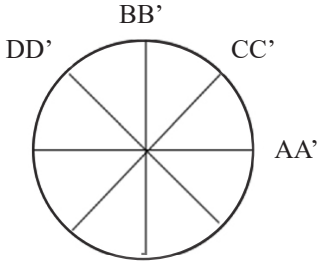

Figure 1. Measurement of average diameter of fungus colonies. AA', BB', CC', DD' = The colony diameter was measured at four different positions: horizontal, vertical, and two diagonal directions.

upper surface, while the underside appeared yellowish-white, gradually turning black. The mycelium was cottony, clustered, and had a rough texture (Figure 2). Microscopically, the fungus displayed septate hyphae and cylindrical sporidia measuring 7.45–18.31 µm in length and 1.63–3.89 µm in width. Additionally, the fungus produced round, yellowish-brown teliospores with an average size of 6.39 × 6.66 µm (Figure 3).

Molecular Identification of the Whip Smut Pathogen. Molecular identification was performed to determine the DNA sequence of the whip smut fungal isolate coded LA UKN. ITS sequence analysis confirmed that the LA UKN isolate clustered within the same group as *Sporisorium scitamineum* (Figure 4).

Fungicide Sensitivity Test on Whip Smut Fungal Colonies. The mean colony diameter test for whip smut fungal growth, analyzed using the LSD test at a 5% significance level, showed that all treatments significantly differed from the control, effectively inhibiting fungal colony growth (Table 1). Synthetic fungicides (carbendazim and prochloraz-manganese-chloride complex) exhibited the highest inhibition rates, both achieving a 100% Relative Inhibition Rate (RIR). In contrast, plant-based fungicides had lower inhibitory effects, with *puyangan* (*Z. zerumbet*) extract demonstrating an RIR of 50.6%, and *jamuan* (*C. zedoaria*) extract showing the lowest RIR at 16.7%. A visual comparison of fungal colony growth in response to different treatments is presented in Figure 5.

Effect of Fungicides on Whip Smut Spore Germination. The spore germination test revealed variations in germination rates among different fungicide treatments. The mean spore germination rate, analyzed using the LSD test at a 5% significance level, indicated that plant extract treatments significantly differed from both the control and synthetic fungicides (Table 2).

Among plant-based fungicides, *jamuan* extract had the highest average spore germination rate at 64.02%, followed by *puyangan* extract at 37.34%. These results suggest that plant-based fungicides were less effective in inhibiting spore germination compared to synthetic fungicides.

Conversely, treatments with carbendazim, prochloraz-manganese-chloride complex, and mancozeb did not significantly differ from the control in terms of spore germination inhibition. The carbendazim treatment had an average spore germination rate of 9.61%, while the control had a rate of 9.91%. The mancozeb treatment resulted in a spore germination rate of 2.84%, while the prochloraz-manganese-chloride complex exhibited the lowest spore germination rate at 0%, making it the most effective treatment. The visual condition of spore germination under each treatment is

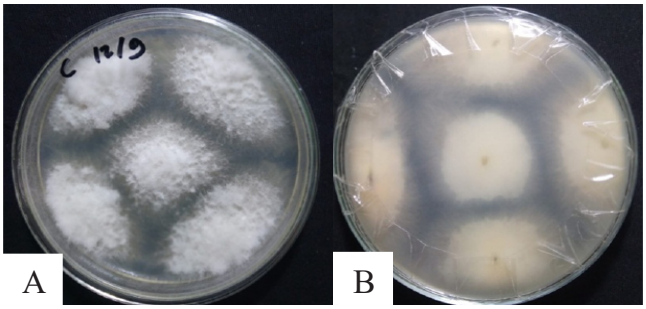

Figure 2. Colonies of the pathogen causing whip smut disease on PDA medium. A. Top surface of the colony; B. Bottom surface of the colony.

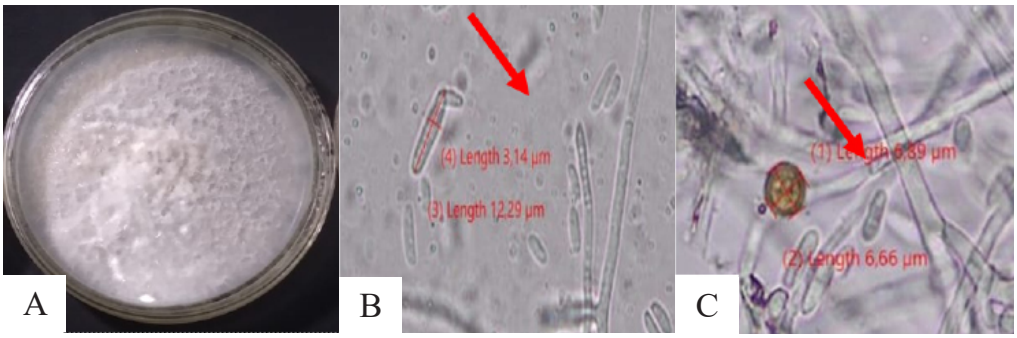

Figure 3. Morphological characteristics of the *S. scitamineum* isolate. A. Top view of the colony; B. Hyphae and sporidia; C. Teliospores.

shown in Figure 6.

Field Observations of Whip Smut in Sugarcane. In Central Lampung Regency, sugarcane plants infected with whip smut initially exhibited symptoms of upright, non-curled leaves. As the disease progressed, long black whips emerged from the apical buds. These whips were densely covered with millions of *S. scitamineum* teliospores, which serve as primary inoculum and can spread via wind dispersal. This aligns with Hidayah (2020), who described whip smut symptoms as highly distinctive due to the formation of black whip-like structures (sori) on infected plants.

Macroscopically, the colony morphology of the whip smut fungal isolate was consistent with previous reports, featuring a white upper surface, a yellowish-white underside that darkened over time, cottony and clustered mycelium, and a rough texture. Microscopically, the septate hyphae and cylindrical sporidia (7.45–18.31 µm × 1.63–3.89 µm) were also in agreement with prior findings. The round, yellowish-brown teliospores (6.39 × 6.66 µm) observed in this study were within the size range reported by Hidayah (2020), who noted teliospore dimensions of 6.87–8.03 µm. Shuai et al. (2023) further described *S. scitamineum* teliospores measuring 5.52–9.69 µm, with germination

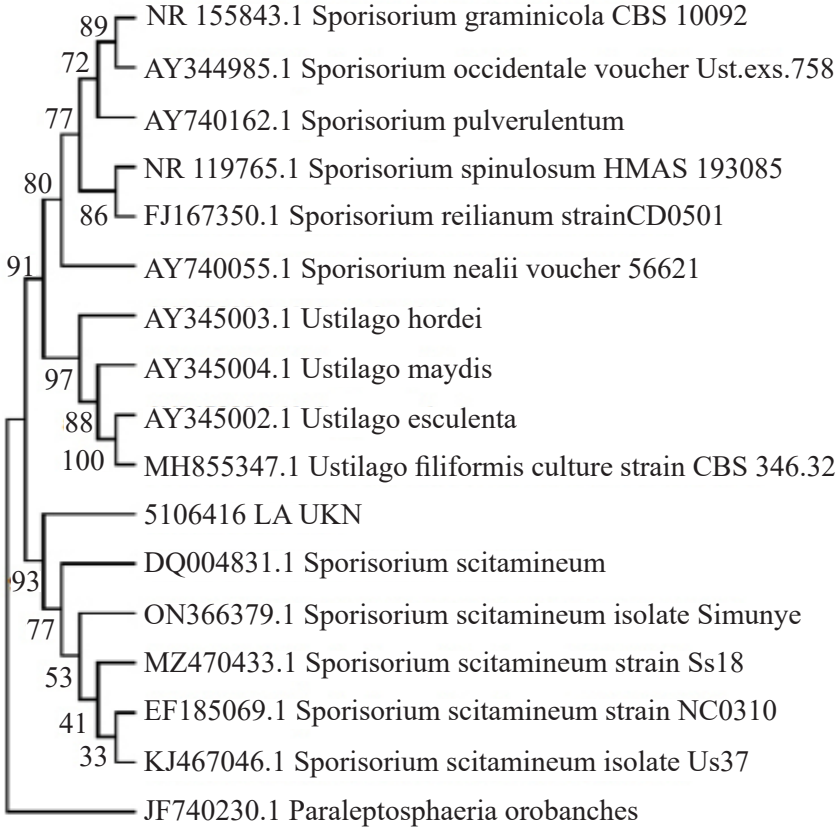

Figure 4. Phylogenetic tree generated using the Neighbor-Joining method for the ITS sequence of the LA UKN isolate.

Table 1. Effect of synthetic fungicides and plant extracts on the diameter of whip smut fungus colonies and Relative Inhibition Rate (RIR)

Treatment	Average diameter of fungal colonies (cm)	RIR (%)
Control	5.50 a	
Puyangan (*Z. zerumbet*)	2.72 c	50.60
Mancozeb	2.56 d	53.00
Jamuan (*C. zedoaria*)	4.58 b	16.70
Carbendazim	0 e	100
Prochloraz-manganese-chloride complex	0 e	100

Note: Values followed by different letters indicate significant differences based on the LSD test at the 5% level.

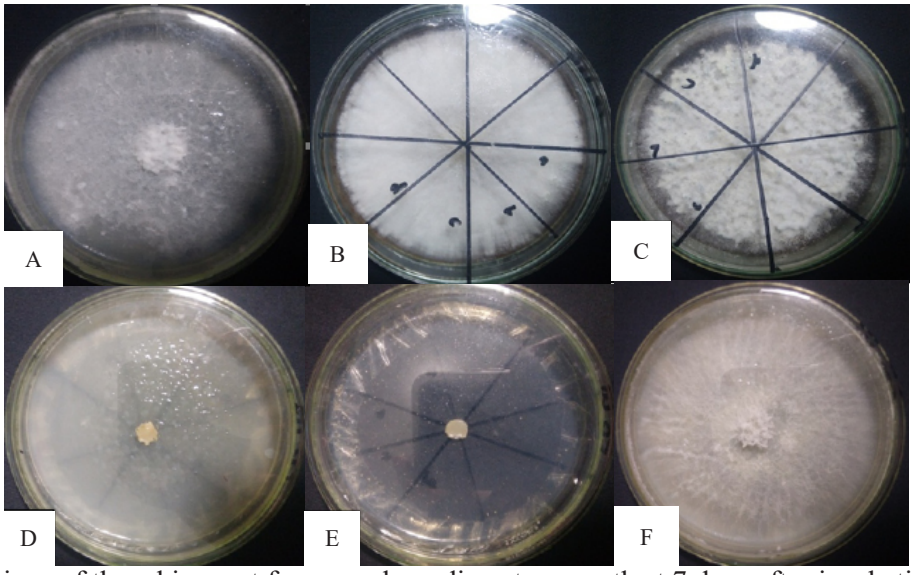

Figure 5. Comparison of the whip smut fungus colony diameter growth at 7 days after incubation. A. control; B. *Puyangan* (*Z. zerumbet*); C. *Jamuan* (*C. zedoaria*); D. Carbendazim; E. Prochloraz-manganese-chloride complex; F. Mancozeb.

Table 2. Spore germination of whip smut against several active ingredients of fungicides

Treatment	Average spore germination (%)
Control	9.91 c
Puyangan (*Z. zerumbet*)	37.34 b
Jamuan (*C. zedoaria*)	64.02 a
Carbendazim	9.61 c
Mancozeb	2.84 c
Prochloraz-manganese-chloride complex	0.0 c

Note: Values followed by different letters indicate significant differences based on the LSD test at the 5% level.

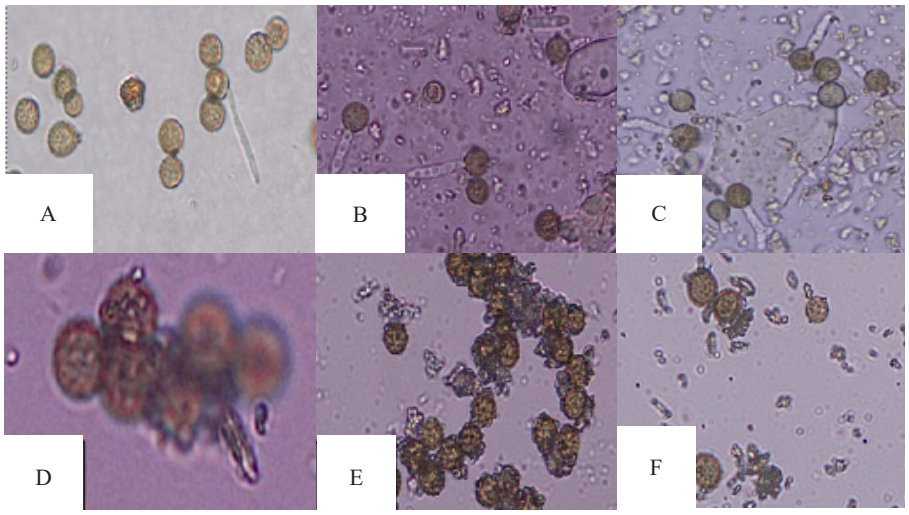

Figure 6. Comparison of whip smut pathogen spore germination at 24 hours after incubation. A. Control; B. *Puyangan* (*Z. zerumbet*); C. *Jamuan* (*C. zedoaria*); D. Carbendazim; E. Prochloraz-manganese-chloride complex; F. Mancozeb.

resulting in a four-celled promycelium that produces sporidia (7.53–20.17 µm × 1.63–3.89 µm), which subsequently reproduce asexually through budding.

Whip Smut Pathogen and Historical Context. Whip smut has been attributed to *S. scitamineum* (formerly *Ustilago scitamineum*) (Diyasti et al., 2021). The disease was first reported in South Africa in 1877 and later

detected in Australia in July 1998, specifically in the Ord River Irrigation Area (ORIA) of Western Australia. In India, whip smut has been documented as caused by *S. scitamineum* (Lal et al., 2009). Phylogenetic analysis using ITS confirmed that the LA UKN isolate from PT GMP clustered within the S. scitamineum group, with a bootstrap value of 93%.

Comparison of Synthetic and Plant-Based Fungicides. In this study, synthetic fungicides were more effective in inhibiting fungal colony growth than plant-based fungicides. This is likely due to their inorganic chemical composition, which enhances pathogen resistance, provides rapid control, and persists longer on plants. However, synthetic fungicides also pose risks, such as increased phytotoxicity and the potential development of pathogen resistance with continuous use. Conversely, plant-based fungicides, derived from natural materials, exhibit lower phytotoxicity and reduced environmental impact (Deresa & Diriba, 2023; Aremu et al., 2024).

Among synthetic fungicides, carbendazim and prochloraz-manganese-chloride complex were the most effective in reducing fungal colony growth. This is attributed to their systemic nature, targeting specific sites within fungal cells. In contrast, mancozeb, a contact fungicide with multi-site action, was comparatively less effective. Carbendazim disrupts mitotic spindles, interfering with fungal protein function, ultimately leading to fungal cell death. Previous studies by Malau et al. (2022) reported that carbendazim achieved an average inhibition rate of 97.57% against *Colletotrichum gloeosporioides*.

Prochloraz-manganese-chloride complex also exhibited strong inhibitory effects, achieving a 100% RIR value. This aligns with findings by Innocenti et al. (2018), who reported that prochloraz inhibited *Trichoderma pleuroticola* growth by 89.5%. As a Demethylation Inhibitor (DMI) fungicide, prochloraz disrupts ergosterol biosynthesis in fungal membranes, thereby inhibiting fungal growth.

The sensitivity test of *S. scitamineum* to mancozeb showed an RIR value of 53%, aligning with Malau et al. (2022), who reported an inhibition rate of 63.84% against *C. gloeosporioides*. Mancozeb functions as a preventive fungicide, inhibiting spore germination through enzyme chelation. Furthermore, it is classified as a contact fungicide with multi-site action, effectively inhibiting the germination of various fungal species by producing ethylene bisisothiocyanate sulfide compounds upon contact with water (Sari & Li'aini, 2022).

The sensitivity test results indicate that *S. scitamineum* remains susceptible to fungicides containing carbendazim and prochloraz-manganese-chloride complex. However, the prochloraz-manganese-chloride complex exhibited the lowest spore germination rate, making it the most effective option. Based on these findings, the use of prochloraz-manganese-chloride complex is recommended for managing whip smut disease in sugarcane plants in Central Lampung Regency.

CONCLUSION

Based on the research results, it can be concluded that the identity of the whip smut pathogen in Central Lampung Regency, based on morphological and molecular identification, is *Sporisorium scitamineum.* Sensitivity tests of the whip smut pathogen to several fungicide active ingredients and plant extracts showed that *S. scitamineum* was sensitive to carbendazim and prochloraz-manganese-chloride complex fungicides. However, *S. scitamineum* was not sensitive to mancozeb fungicide, *puyangan* extract, and *jamuan* extract. Further research is needed to evaluate the effectiveness of fungicides with the active ingredient prochloraz-manganese-chloride complex at various concentrations to develop effective and efficient control techniques.

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AUTHORS' CONTRIBUTIONS

EF and TM considered and planned the experiment. EF coordinated the execution of the experiment and the manuscript writing. TM participated by providing suggestions for some of the experiments and contributing to the manuscript writing. UK contributed to data collection and processing. SP participated by providing suggestions for some of the experiments and partially writing the manuscript. SF and HP contributed by providing advice on sugarcane plants and fieldwork. All authors have read and approved the final manuscript.

COMPETING INTEREST

We declare herewith that there is no competing interest such as financial or non-financial interests, professional or personal relationships that are directly or indirectly connected to the work submitted for publication.

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