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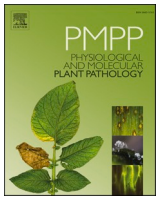

Biocontrol potential of *Trichoderma* culture filtrates against *Schizophyllum commune*: Metabolite profiling and oxidative stress mechanisms

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ABSTRACT

Abnormal germination, brown germ, and seed rot significantly compromise oil palm seed viability, primarily due to fungal contamination. Among 52 fungal isolates from defective seeds, SOPRC-07, exhibiting high pathogenicity, was identified as *Schizophyllum commune* via morphological and molecular (ITS and LSU rDNA) analyses. Two *Trichoderma* strains, *T. asperelloides* SKRU-01 and *T. asperellum* NST-009, demonstrated potent antifungal activity, inhibiting mycelial growth by up to 85% on PDA and more than 94% in PDB. LC–QTOF–MS profiling of culture filtrates revealed several putatively identified metabolites. Cyclo-(Ala–Pro) and sapidolide A were detected in both *T. asperelloides* SKRU-01 and *T. asperellum* NST-009, whereas anisomycin was detected only in SKRU-01. These metabolites may contribute to oxidative stress in *S. commune*, as indicated by elevated reactive oxygen species (ROS) and activation of antioxidant defenses (SOD, CAT, and glutathione). Comparatively, selected chemical fungicides—prochloraz, azoxystrobin, metalaxyl, propiconazole, and thiram—also inhibited fungal growth, with propiconazole achieving complete inhibition at ≥0.4% (v/v). On germinated seeds, SKRU-01 and NST-009 reduced disease incidence from approximately 73% to 40% and 43%, corresponding to 45% and 41% control efficacy, whereas fungicides further lowered incidence to 27–37%, with propiconazole providing 64% control. These results highlight the potential of *Trichoderma*-derived metabolites as natural antifungal agents with a biochemical mode of action, offering a sustainable alternative to chemical pesticides for managing seedborne *S. commune* in oil palm.

1. Introduction

The production of high-quality oil palm (*Elaeis guineensis* Jacq.) seedlings relies on successful seed germination, which directly influences nursery efficiency and plantation productivity [1]. Despite advances in tissue culture technology [2], most oil palm plantations still depend on seed-based propagation [1,3]. Therefore, the use of certified seeds with high germination capacity and good seed health remains essential for sustainable production. Seed germination failure may result from poor seed quality, improper storage, or fungal infection. Among these factors, seedborne fungi represent a major and preventable cause of germination loss [4]. Several fungal genera, including *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Thielaviopsis* spp., and *Schizophyllum* spp., have been associated with oil palm seeds, causing seed decay and reduced seedling vigor [4,5]. Notably, *Schizophyllum commune* Fr. was reported as a seedborne pathogen responsible for brown germ and seed rot in oil palm [5]. Our recent investigation also detected *S. commune* in ungerminated seeds, indicating its potential role in germination failure.

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Pathogenicity studies have shown that *S. commune* can reduce oil palm seed germination by up to 65% [5]. Although this fungus is recognized as an edible mushroom in some regions, it has also been associated with plant diseases, including heart rot in apples and wood decay in forestry in the USA [6], as well as wood rot in almond trees in Uzbekistan [7]. Under high humidity and inadequate seed processing, the fungus colonizes the seed surface and may obstruct germ pores, thereby interfering with embryo respiration and germination [5].

Chemical fungicides are widely used to manage seedborne fungal infections [8–12]. In Thailand, commonly applied fungicides such as propiconazole, prochloraz, metalaxyl, azoxystrobin, and thiram play important roles in fungal disease management [13,14]. However, increasing concerns regarding chemical residues and environmental impact have encouraged the exploration of biological alternatives. Biological control using *Trichoderma* species has emerged as a promising strategy. These fungi suppress pathogens through multiple mechanisms, including the production of antifungal metabolites, cell wall-degrading enzymes, and fungitoxic antibiotics [15,16], while also promoting plant growth [17]. Among locally studied strains, *T. asperelloides* SKRU-01 has demonstrated effectiveness against food spoilage fungi such as *A. parasiticus* and *A. flavus* [18–20], whereas *T. asperellum* NST-009 has shown efficacy against several plant pathogens, including *Phytophthora* leaf fall, *Cercospora* leaf spot [21,22], and damping-off caused by *Pythium aphanidermatum* [23]. These strains therefore represent promising candidates for controlling *S. commune* in oil palm seeds.

The present study aims to (i) confirm *S. commune* as the causal agent of defective oil palm seeds through morphological and molecular identification, (ii) evaluate the efficacy of selected *Trichoderma* strains (*T. asperelloides* SKRU-01 and *T. asperellum* NST-009) and commonly used fungicides against the pathogen both *in vitro* and on germinated seeds, and (iii) characterize the bioactive metabolites of effective strains using LC–QTOF–MS and investigate their potential oxidative stress–mediated antifungal mechanisms.

2. Materials and methods

2.1. Source of materials

2.1.1. Microorganisms

Trichoderma asperelloides SKRU-01, previously isolated from loam soil [18], and *T. asperellum* NST-009, obtained from the culture collection of the Agricultural Microbial Production and Service Center, Walailak University, Thailand, were used in this study. The cultures were grown on potato dextrose agar (PDA) and incubated at 28 ± 2 °C for one week.

2.1.2. Chemical fungicides

A total of five commercial fungicides were used in this study, including thiram (80% WG), propiconazole (25% w/v), azoxystrobin (25% w/v), prochloraz (45% w/v), and metalaxyl (25% WP). All fungicides were registered and approved for agricultural use and were commercially available in Thailand. Each fungicide was prepared to obtain final concentrations ranging from 0.1% to 0.8% (v/v) based on the active ingredient (a.i.), and the required amounts of each commercial formulation were calculated according to their respective active ingredient contents.

2.1.3. Defective and germinated oil palm seed samples

Samples of defective seeds, rotten seeds, and abnormally germinated seeds obtained during the oil palm seed germination process were kindly provided by the Suratthani Oil Palm Research Center, Kanchanadit, Thailand, and the Krabi Oil Palm Research Center, Khlong Thom, Thailand. The defective oil palm seed samples were placed in 1 L zip-lock bags (30 × 45 cm) and transported to the Plant Pathology Laboratory, Faculty of Agricultural Technology, Songkhla Rajabhat University, Mueang District, Songkhla, Thailand, for further experiments. Surface sterilization was carried out by immersing the seeds in 10% (v/v) sodium hypochlorite solution for 10 min, followed by three rinses with sterile distilled water. The seeds were then gently dried under sterile conditions in a laminar airflow chamber for 30 min.

Germinating oil palm seeds (Tenera hybrid, cv. Golden Tenera Cativa) with radicle lengths of approximately 2 cm were kindly provided by Golden Tenera Public Company Limited, located in Mueang District, Krabi Province, Thailand. The seeds were surface-sterilized by immersion in 2% (v/v) sodium hypochlorite solution for 3 min, followed by three rinses with sterile distilled water. They were then gently dried in a laminar airflow cabinet for 30 min under aseptic conditions.

2.2. Fungal isolation

Defective oil palm seed samples were placed on a metal rack inside a sealed container maintained at 90% relative humidity and incubated at 28 ± 2 °C for 10 days. Hyphal tips emerging from the micropyle region were aseptically excised and transferred to PDA plates. After obtaining pure cultures, all isolates were used for morphological observation.

2.3. Morphology study

Fungal isolates obtained from defective oil palm seeds were cultured on PDA and incubated at 28 ± 2 °C for 7 days. Macroscopic colony characteristics, including colony diameter, texture, pigmentation, and the presence of fruiting bodies (basidiocarps), were recorded. Microscopic features were examined using a compound light microscope (Nikon Eclipse E100, Nikon Instruments Inc., Japan). Fungal mycelia were stained with lactophenol cotton blue, mounted on glass slides, and covered with cover slips for observation.

2.4. Molecular identification

The fungal pathogen isolate SOPRC-07 was identified by phylogenetic analysis based on the internal transcribed spacer (ITS) region and large subunit (LSU) rRNA gene sequences. Both gene regions were amplified and sequenced by Macrogen Co., Ltd. (Seoul, South Korea) using the primer pairs ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) for the ITS region [24], and LR0R (5′-ACC CGC TGA ACT TAA GC-3′) and LR7 (5′-TAC TAC CAC CAA GAT CT-3′) for the LSU rRNA gene [25]. The obtained sequences were compared with reference sequences in the GenBank database using the Basic Local Alignment Search Tool program (BLAST: http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) to assess sequence homology. Phylogenetic trees based on ITS and LSU sequences were constructed using MEGA 12, including reference sequences of related species, to confirm the identification of isolate SOPRC-07 as *Schizophyllum commune*.

2.5. Pathogenicity test

Due to the technical difficulty of directly inoculating fungal pathogens into dormant oil palm seeds, pathogenicity tests were carried out using germinated oil palm seeds. Germinated seeds were selected because they allow easier inoculation of the pathogen while still representing an early stage of seedling development, which is critical for evaluating the pathogenic potential of the isolated fungi. A 5 mm-diameter mycelial plug of the fungal isolate was placed adjacent to the radicle of each germinated seed, while seeds in the control group were left uninoculated. All seeds were placed on a metal rack inside a sealed container maintained at 90% relative humidity and incubated at 28 ± 2 °C for 20 days. During this period, the growth of fungal mycelia, colonization of seed tissues, and visible symptoms such as rotting, tissue necrosis, and inhibition of radicle growth were monitored and compared with those of the control group. The experiment was performed in three replicates, with each replicate consisting of five germinated seeds.

2.6. In vitro antagonistic activity of Trichoderma strains against S. commune

2.6.1. Dual culture effect

In vitro confrontation assays were performed following the method described by Amira et al. [26], with slight modifications. In the first setup, a mycelial plug (5 mm in diameter) of each *Trichoderma* strain (SKRU-01 or NST-009) and *S. commune* was placed 1 cm apart on a PDA plate to evaluate direct antagonistic interaction. In the second setup, two plugs of the same *Trichoderma* strain were placed on opposite sides of a PDA plate, 1 cm from the edge, while a plug of *S. commune* was positioned at the center. This setup was used to assess the inhibitory ability of *Trichoderma* under increased antagonist density. Plates inoculated with *S. commune* alone served as the control. All treatments were incubated at 28 ± 2 °C for 5 days and performed in two independent trials, each with three replications. The radial growth of *S. commune* was measured, and the percentage of mycelial growth inhibition was calculated using the formula: Inhibition (%) = [(R1 – R2)/R1] × 100, where R1 is the radial mycelial growth in the control plate (without *Trichoderma*), and R2 is the radial growth in the presence of the *Trichoderma* strain (SKRU-01 or NST-009).

2.6.2. Culture filtrates effect

The antifungal activity of culture filtrates obtained from *Trichoderma* strains SKRU-01 and NST-009 was evaluated against *S. commune* using both solid (PDA) and liquid (PDB) media assays. Each strain was cultured in PDB at 25 ± 2 °C for 10 days. Mycelial mats were removed by filtration through Whatman No. 1 filter paper, and the resulting filtrates were sterilized by passage through a 0.45 μm Millipore membrane filter (Sartorius®) to obtain culture filtrates (CFs).

For PDA assays, double-strength PDA was prepared, autoclaved at 121 °C for 15 min, maintained at 60 °C, and then mixed with appropriate volumes of CFs to yield final concentrations of 1%, 15%, 30%, 45%, and 60% (v/v), with a total volume of 10 mL per Petri dish. Plates containing PDA without filtrates served as controls. A 5-mm mycelial plug from a 3-day-old *S. commune* culture was placed at the center of each plate, and plates were incubated at 28 ± 2 °C for 5 days. Colony diameters were recorded, and the percentage of mycelial growth inhibition was calculated as previously described. Each experiment was conducted twice, with three replications per treatment.

For PDB assays, PDB was prepared and autoclaved at 121 °C for 15 min. The CFs were added in appropriate volumes to obtain final concentrations of 1%, 15%, 30%, 45%, and 60% (v/v), with the total volume adjusted to 10 mL in 125 mL Erlenmeyer flasks. Sterile distilled water was added in equivalent volumes to PDB and served as the control. A 5-mm mycelial plug from a 3-day-old *S. commune* colony was inoculated into each flask, and cultures were incubated at 28 ± 2 °C on a rotary shaker for 5 days. After incubation, mycelial mats were collected by filtration through pre-weighed filter papers, dried at 60 °C for 3 days, and weighed. The percentage inhibition of mycelial growth based on dry weight (ponderal growth inhibition) was calculated using the formula: Inhibition (%) = [(W1 – W2)/W1] × 100, where W1 is the dry weight of mycelia in the control treatment, and W2 is the dry weight of mycelia in the presence of the *Trichoderma* strain (SKRU-01 or NST-009).

2.7. Metabolite profiling of putative antifungal compounds in culture filtrates from T. asperelloides SKRU-01 and T. asperellum NST-009 by LC–QTOF–MS

The CFs obtained from *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 were analyzed to profile secondary metabolites and tentatively identify compounds with reported antifungal activity. Metabolomic analysis was performed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC–QTOF–MS) on an Agilent 1290 Infinity II LC system interfaced with a 6545 Q-TOF mass spectrometer (Agilent Technologies, USA). Sample preparation of CFs was carried out according to previously established procedures.

Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 Rapid Resolution HD column (150 × 2.1 mm, 1.8 μm; Agilent) maintained at 25 °C. The mobile phase comprised solvent A (0.1% acetic acid in ultrapure water) and solvent B (LC–MS–grade methanol), delivered at a flow rate of 0.2 mL/min. The elution program began with 95% solvent A for 2 min, followed by a linear decrease to 0% A over 40 min. This composition was held for 5 min before returning to the initial conditions for column re-equilibration. Each sample was injected at a volume of 10 μL.

Mass spectrometric detection was conducted in both positive and negative electrospray ionization modes (+ESI and −ESI) using an AutoMS/MS acquisition strategy. Full-scan spectra were collected over an *m/z* range of 100–1200, while MS/MS fragmentation data were acquired across *m/z* 50–1200 at collision energies of 10, 20, and 40 V. Accurate mass calibration was continuously maintained using reference ions at *m/z* 121.0509 and 922.0098 for positive ionization, and *m/z* 112.9856 and 1033.9881 for negative ionization. Putative metabolite identification was performed by comparing exact mass measurements and MS/MS fragmentation patterns with entries in the METLIN database using MassHunter PCD/PCDL software (version 8.0).

2.8. Mechanism of action of T. asperelloides SKRU-01 and T. asperellum NST-009 against S. commune

The antifungal mechanisms exerted by CFs of *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 against *S. commune* were elucidated through physiological and biochemical analyses. Actively growing mycelia of *S. commune* were obtained by excising 5-mm agar plugs from the colony margin of 5-day-old cultures and transferring them into 100 mL of PDB. The cultures were incubated at 28 ± 2 °C with continuous shaking at 150 rpm for three days to allow sufficient biomass development.

Following incubation, the fungal mycelia were collected, thoroughly washed with sterile distilled water to remove residual medium components, and adjusted to 1 g (wet weight). The mycelial samples were subsequently resuspended in 10 mL of PDB supplemented with 60% (v/v) CFs of either SKRU-01 or NST-009. Control treatments consisted of mycelia incubated in PDB without CFs. All treatments were maintained under identical incubation conditions for an additional three days. After treatment, fungal samples were prepared for downstream biochemical analyses.

For enzyme and antioxidant assays, harvested mycelia from both treated and control groups were homogenized in 3 mL of phosphate-buffered saline (PBS; 100 mM, pH 7.4) and centrifuged at 8000 rpm for 10 min at 4 °C, following the method described by Das et al. [27]. The clarified supernatants were used as crude extracts for biochemical determinations.

Oxidative stress induction and antioxidant defense responses in *S. commune* were evaluated by quantifying intracellular reactive oxygen species (ROS) levels and key antioxidant-related parameters, including catalase (CAT), superoxide dismutase (SOD), and glutathione redox status (GSH and GSSG). These indicators collectively reflect cellular redox imbalance and defensive responses to antifungal stress [28,29].

Intracellular ROS accumulation was assessed using the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) according to Keston and Brandt [30]. Fluorescence intensity was measured at excitation and emission wavelengths of 485 and 530 nm, respectively, and ROS levels were quantified based on a DCF standard curve. Catalase activity was determined by monitoring the decomposition rate of hydrogen peroxide following Beers and Sizer [31] and expressed as units per minute per milligram of protein, using a molar extinction coefficient of 43.6 M^{-1} cm^{-1} [27]. Superoxide dismutase activity was quantified using the quercetin auto-oxidation inhibition assay described by Kostyuk and Potapovich [32] and expressed as enzyme activity per milligram of protein.

For glutathione analysis, fungal tissues were homogenized in phosphate buffer containing EDTA and metaphosphoric acid to preserve redox integrity. After centrifugation, reduced glutathione (GSH) levels were determined fluorometrically using o-phthalaldehyde (OPA) at an excitation wavelength of 350 nm and emission at 420 nm, while oxidized glutathione (GSSG) was quantified following derivatization with N-ethylmaleimide (NEM) prior to OPA reaction [32]. Total protein concentrations were measured using the Lowry method to normalize enzymatic activities [33]. All experiments were conducted in triplicate and independently repeated twice.

2.9. In vitro antagonistic activity of five chemical fungicides against S. commune

The antifungal activity of five chemical fungicides—prochloraz, metalaxyl, azoxystrobin, propiconazole, and thiram—against *S. commune* was evaluated using both solid (PDA) and liquid (PDB) culture media. Each fungicide was tested at concentrations ranging from 0.1% to 0.8% (v/v).

For PDA medium, an appropriate volume of each fungicide was mixed with melted sterile PDA to a final volume of 10 mL and poured into 9-cm Petri dishes. Sterile distilled water mixed with melted sterile PDA at the same volume served as the control. A 5-mm mycelial plug from a 3-day-old *S. commune* colony was placed at the center of each plate. Cultures were incubated at 28 ± 2 °C for 5 days. Radial growth was measured, and the percentage inhibition of mycelial growth was calculated as described previously. Each experiment was conducted twice with three replications per trial.

For the PDB medium, the appropriate volume of each fungicide was added to sterile PDB to achieve a final volume of 10 mL in 150-mL flasks, while sterile distilled water was added to PDB in equivalent volumes for the control. A 5-mm mycelial plug from a 3-day-old *S. commune* colony was inoculated into each flask, which was then incubated at 28 ± 2 °C on a rotary shaker for 5 days. After incubation, mycelial mats were collected by filtration through pre-weighed filter papers, dried at 60 °C for 3 days, and weighed. The percentage inhibition of mycelial growth was calculated as described previously. Each experiment was conducted twice with three replications per trial.

2.10. Efficacy of biological and chemical fungicide treatments for controlling S. commune using an oil palm seed model

The concentration of 60% (v/v) of CFs from two *Trichoderma* strains (SKRU-01 and NST-009) was selected because it showed the highest antifungal activity *in vitro* in both PDA and PDB assays. This concentration was used to compare their efficacy with three chemical fungicides—prochloraz, azoxystrobin, and propiconazole—against *S. commune* using a germinated oil palm seed model. This model was employed because the hard seed coat of oil palm prevents direct inoculation into the seed, and the radicle region provides a suitable infection site for *S. commune*. Sterile germinated oil palm seeds (Section 2.1.3) were soaked in either the CFs of SKRU-01 or NST-009, or in each chemical fungicide at 0.8% (v/v), for 10 min. After air-drying, a 5 mm-diameter mycelial plug of the fungal isolate was placed adjacent to the radicle of each seed. In the negative control group, seeds were neither inoculated nor treated, whereas in the positive control group, seeds were inoculated with the pathogen only. All seeds were placed on a sterile metal rack inside a sealed plastic container maintained at 90% relative humidity and incubated at 28 ± 2 °C for 20 days. After incubation, each germinated oil palm seed was examined for the presence of brown germ or seed rot symptoms, characterized by brown discoloration and fungal mycelial growth covering the germ or radicle. The percentage of infected seeds was calculated as: Disease incidence (%) = (Number of infected seeds/Total number of seeds) × 100. The experiment was conducted in triplicate, with each replicate consisting of ten germinated seeds.

2.11. Statistical analysis

Significant differences among the control and treatments were assessed using one-way analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test was applied to compare means at $p < 0.05$. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) software (version 26).

3. Results

3.1. Sample collection and disease symptoms

A total of 52 fungal isolates were obtained from defective, rotten, and abnormally germinated oil palm seeds collected from two research centers in southern Thailand. Of these, 32 isolates originated from the Suratthani Oil Palm Research Center (SOPRC-01 to SOPRC-32), and 20 from the Krabi Oil Palm Research Center (KOPRC-33 to KOPRC-52). All isolates produced white, coarse mycelial colonies that completely covered the surface of PDA within five days of incubation. Due to the uniform colony morphology observed among isolates, one representative isolate (SOPRC-07) was selected for further pathogenicity and morphological characterization.

3.2. Evaluation of the pathogenicity potential on oil palm seed germination

The pathogenic potential of the fungal isolate SOPRC-07 was evaluated using germinated oil palm seeds with a root length of approximately 2 cm. Seeds were inoculated with the isolate and compared with uninoculated controls (Fig. 1). In the control group, seeds exhibited normal growth with continuous root elongation and cotyledon development (Fig. 1A). In contrast, seeds inoculated with SOPRC-07 displayed fungal growth covering the seed surface, resulting in stunted development of both stem and root tissues, which is consistent with symptoms of brown germ and seed rot (Fig. 1B). Although re-inoculation of non-germinated seeds to fully fulfill Koch's postulates could not be conducted due to limitations in biological material and environmental conditions, the ability of SOPRC-07 to colonize and damage germinated seeds provides preliminary evidence of its pathogenic potential. These findings suggest that SOPRC-07 may contribute to brown germ and seed rot in oil palm seeds, indicating its relevance as a seedborne pathogen.

3.3. Morphology study

The fungal strain SOPRC-07 was characterized based on its morphological features (Fig. 2). After 7 days of incubation on PDA, colonies appeared cottony and white, with irregular margins and a yellow-brown coloration on the reverse side (Fig. 2A–a). By 25 days of incubation, fan-shaped fruiting bodies (basidiocarps) were observed (Fig. 2A, b–c). Microscopic examination revealed hyaline, septate, and branched hyphae with clamp connections and the presence of chlamydospores (Fig. 2B–a–c).

3.4. Molecular identification

The genomic DNA of strain SOPRC-07 was amplified using primers targeting the ITS and LSU regions. Molecular identification of the pathogen is shown in Fig. 3. The obtained sequences were compared with reference sequences in the GenBank database using the BLAST tool. Both the ITS (Fig. 3A) and LSU (Fig. 3B) sequences showed a 100% match with *Schizophyllum commune* (AB369910.1 and FJ372704.1). Therefore, based on the combined morphological characteristics and molecular analyses, strain SOPRC-07 was conclusively identified as *S. commune*.

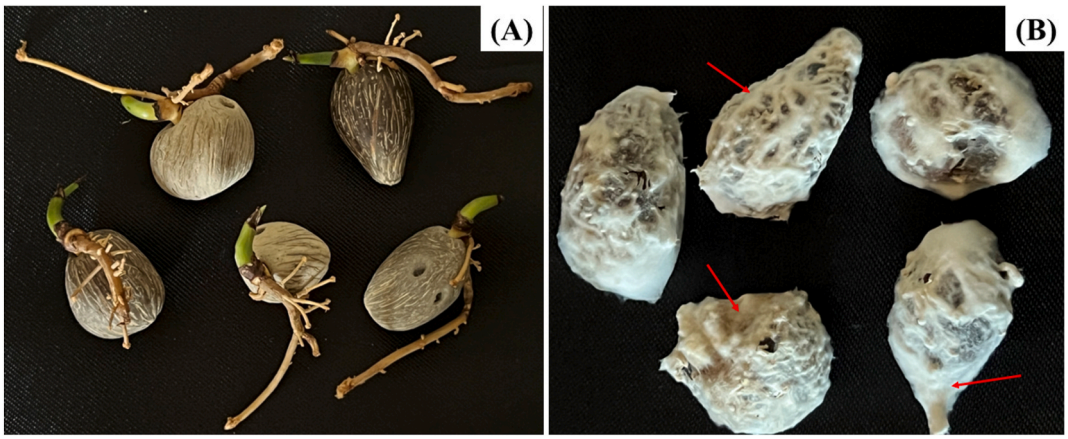


Fig. 1. Pathogenicity assessment of *S. commune* on germinating oil palm seeds after incubation at 28 ± 2 °C in a plastic box under 90% relative humidity for 20 days. **(A)** Shoot emergence and root development in control seeds. **(B)** Growth of the *S. commune* covering the surface of germinating seeds, resulting in the inhibition of shoot and root tissue development. Red arrows indicate germinating seeds showing extensive mycelial colonization by *S. commune*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. *In vitro antagonistic activity of Trichoderma strains against S. commune*

The antagonistic activity of *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 against *S. commune* was evaluated using dual-culture assays (Fig. 4). *T. asperelloides* SKRU-01 inhibited the pathogen by 57.62%, whereas *T. asperellum* NST-009 achieved 53.30% inhibition (Fig. 4A). Upon establishing greater colonization by the antagonists, inhibition substantially increased, reaching 86% for SKRU-01 and 80% for NST-009 (Fig. 4B). These results suggest that rapid mycelial growth and competitive colonization are key factors determining the biocontrol efficacy of *Trichoderma* strains.

The antifungal effects of CFs from *T. asperelloides* SKRU-01 (Table 1A) and *T. asperellum* NST-009 (Table 1B) against the mycelial growth of *S. commune* were evaluated on PDA medium at concentrations ranging from 1% to 60% (v/v) (Table 1). Both SKRU-01 and NST-009 significantly inhibited ($p < 0.05$) fungal growth in a concentration-dependent manner. In the untreated control, the colony growth of *S. commune* reached 8.50 cm in diameter, whereas treatment with CFs reduced growth to 1.23 cm for SKRU-01 and 1.73 cm for NST-009. The inhibition rates of SKRU-01 ranged from 2.94% to 85.49% (Table 1A), while NST-009 ranged from 1.96% to 79.61% (Table 1B). Notably, at 15% (v/v), both CFs achieved more than 50% growth inhibition. At 60% (v/v), SKRU-01 and NST-009 showed inhibition values of 85.49% and 79.61%, respectively.

Similar trends were observed when the antifungal activity of SKRU-01 (Table 2A) and NST-009 (Table 2B) was evaluated in PDB medium, showing significant inhibition ($p < 0.05$) of fungal growth (Table 2). At 60% (v/v), CFs from both SKRU-01 and NST-009 almost completely suppressed *S. commune* growth, with inhibition rates of 94.11% and 94.48%, respectively, indicating strong antifungal activity in liquid culture.

3.6. *Metabolite profiling of putative antifungal compounds in culture filtrates from T. asperelloides SKRU-01 and T. asperellum NST-009 by LC–QTOF–MS*

The metabolite profiles of CFs from *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 were analyzed using LC–QTOF–MS in both negative (−ESI) and positive (+ESI) ionization modes (Supplementary Table S1–S4). In the −ESI mode, 59 metabolites were putatively identified in SKRU-01 (Table S1) and 94 in NST-009 (Table S3), while the +ESI mode revealed 147 metabolites in SKRU-01 (Table S2) and 124 in NST-009 (Table S4). These metabolites belonged to diverse chemical classes, including diketopiperazines (DKPs), alkaloids, flavonoids, terpenoids, peptides, organic acids, ketones/aldehydes, and furan derivatives.

Among the detected metabolites, several compounds previously reported to exhibit antifungal activity were putatively identified. In SKRU-01 (−ESI, Table S1), these included cyclo-(Ala–Pro) diketopiperazine, levoamine (chloramphenicol D base), and bisacurone epoxide, while the +ESI mode (Table S2) revealed additional compounds such as anisomycin and sapidolide A. Similarly, NST-009 (−ESI, Table S3) contained cyclo-(Ala–Pro) diketopiperazine, levoamine, and formylfusarochromanone, with anisomycin also detected in the +ESI mode (Table S4).

Notably, several metabolites were detected in both strains, including cyclo-(Ala–Pro) diketopiperazine and levoamine in the negative ESI mode, and anisomycin in the positive ESI mode. The presence of these metabolites indicates similarities in the metabolite profiles of the two strains. Overall, LC–QTOF–MS analysis revealed that both *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 produce diverse secondary metabolites, some of which have been previously associated with antifungal activity.

3.7. *Mechanism of action of T. asperelloides SKRU-01 and T. asperellum NST-009 against S. commune*

Exposure of *S. commune* to CFs from *T. asperellum* NST-009 and *T. asperelloides* SKRU-01 induced statistically significant ($p < 0.05$) alterations in oxidative stress–related parameters compared with the control (Fig. 5A–F). As shown in Fig. 5A, intracellular ROS levels increased markedly from 15.77 μM/mg protein in the control to 194.17 μM/mg protein following NST-009 treatment and further to 426.26 μM/mg protein in the SKRU-01 treatment. Correspondingly, SOD activity (Fig. 5B) increased from 7.63 Unit/min/mg protein in the control to 37.22 Unit/min/mg protein in NST-009 and 99.79 Unit/min/mg protein in SKRU-01 treatments. CAT activity (Fig. 5C) showed a slight increase in the NST-009 treatment (12.22 Unit/min/mg protein) compared with the control (10.89 Unit/min/mg protein), whereas a pronounced elevation was observed in the SKRU-01 treatment (32.56 Unit/min/mg protein). In parallel, GSH content (Fig. 5D) increased from 0.33 μM/mg protein in the control to 2.25 μM/mg protein in NST-009 and 5.67 μM/mg protein in SKRU-01 treatments, accompanied by increases in GSSG levels (Fig. 5E) from 0.14 μM/mg protein to 0.93 and 2.23 μM/mg protein, respectively. Despite these substantial changes in glutathione pools, the GSH/GSSG ratio (Fig. 5F) remained relatively stable across treatments, with values of 2.40 in the control, 2.42 in NST-009, and 2.55

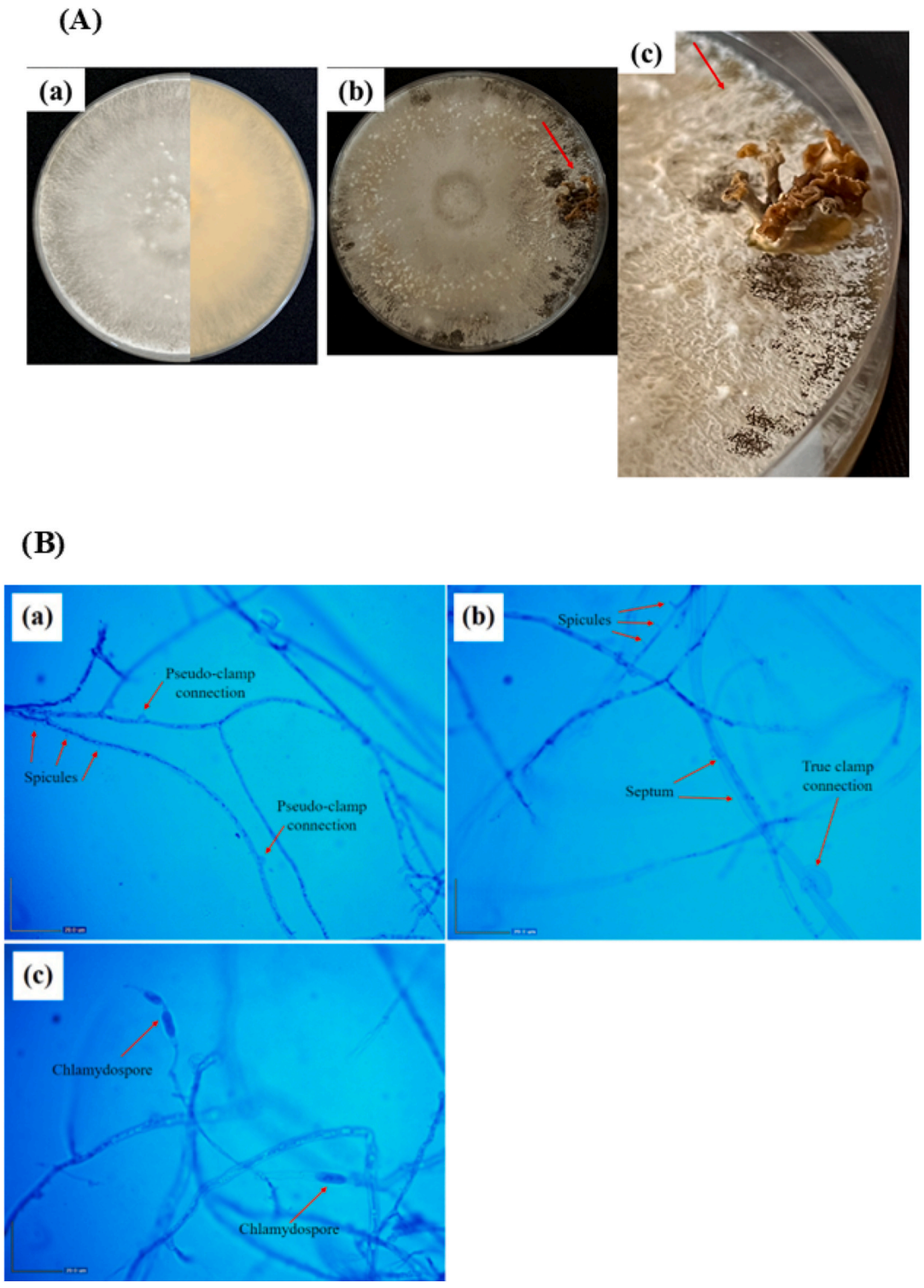


Fig. 2. Morphological characteristics of *S. commune*. **(A, a)** Colony growth on PDA after 7 days of incubation at 28 ± 2 °C. **(A, b, c)** Fan-shaped fruiting bodies (basidiocarps) on PDA after 25 days of incubation at 28 ± 2 °C. **(B, a-c)** Microscopic features showing hyaline, septate, and branched hyphae with clamp connections and chlamydospores.

in SKRU-01. Overall, all oxidative stress–related parameters exhibited higher levels in *S. commune* exposed to SKRU-01 CFs than to NST-009, indicating a stronger oxidative stress response induced by SKRU-01.

3.8. *In vitro antagonistic activity of five chemical fungicides against S. commune*

The antifungal activity of five chemical fungicides—prochloraz, metalaxyl, azoxystrobin, propiconazole, and thiram—against *S. commune* mycelial growth on toxic PDA and PDB media is shown in Table 3. All fungicides significantly inhibited ($p < 0.05$) mycelial growth

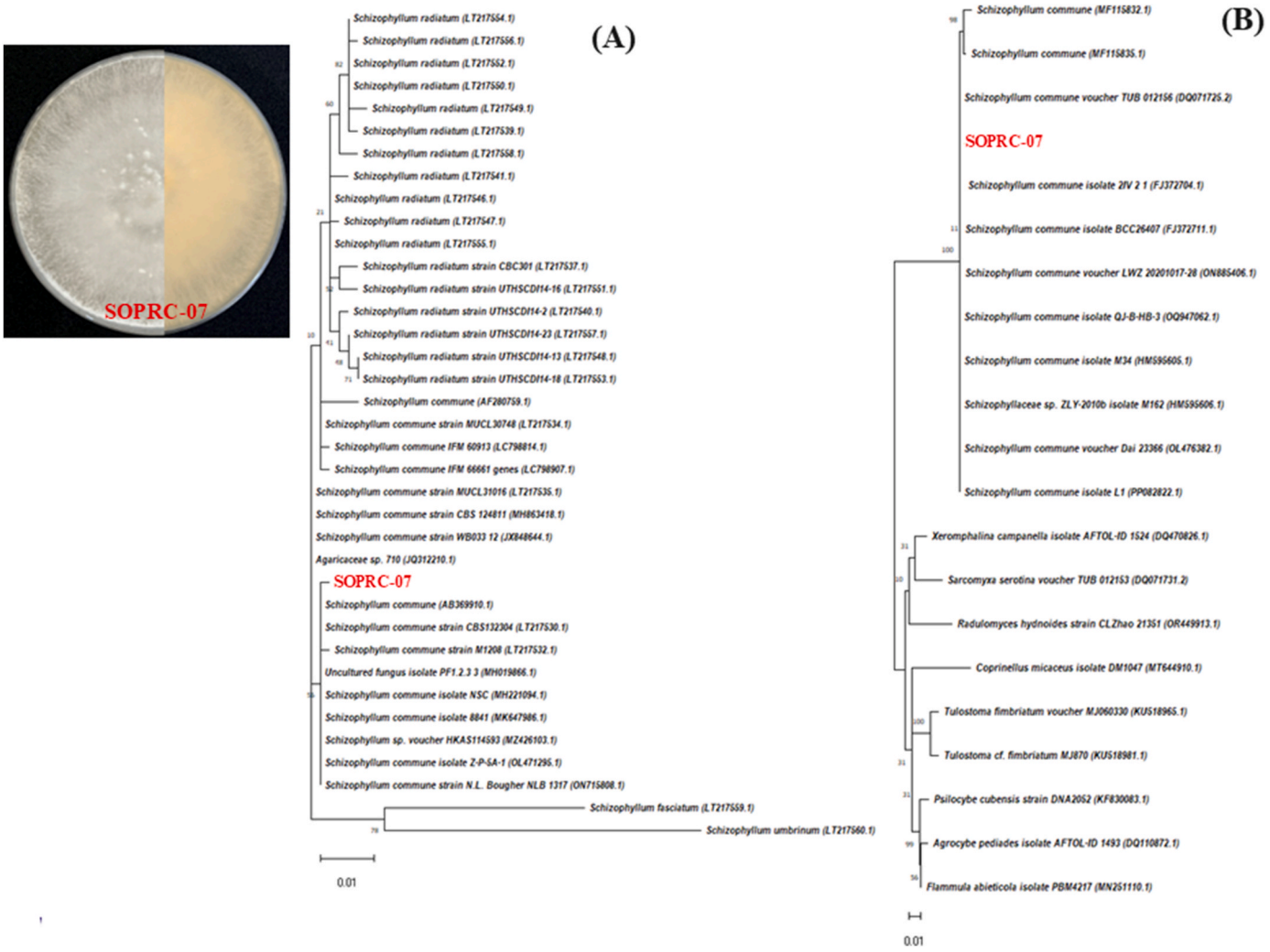


Fig. 3. Identification of the fungal pathogen SOPRC-07. Phylogenetic trees were constructed using the maximum-likelihood method based on sequences of the **(A)** internal transcribed spacer (ITS) region and **(B)** large subunit (LSU; 26S rRNA) gene. These sequences were compared with those of related *Schizophyllum* spp. type strains. The scale bars represent 0.01. Bootstrap support values were calculated from 1000 replicates. The isolate SOPRC-07, identified as *Schizophyllum commune*, is highlighted in bold red font. The phylogenetic trees were midpoint-rooted. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and percent in a dose-dependent manner, although efficacy varied among compounds.

On PDA, prochloraz strongly reduced the diameter of colonies from 9.00 cm in the control to 1.88 cm at 0.8% (v/v), corresponding to 79.07% inhibition. Azoxystrobin reached 76.67% inhibition at 0.8% (v/v). Propiconazole completely inhibited growth at ≥0.4% (v/v), achieving 100% inhibition. Metalaxyl showed moderate activity, increasing from 5.74% at 0.1% (v/v) to 100% at 0.8% (v/v). Thiram suppressed growth up to 80.56% at 0.8% (v/v).

In PDB, prochloraz, azoxystrobin, and propiconazole completely inhibited fungal growth at all tested concentrations. Metalaxyl reduced mycelial dry weight from 115.80 mg (control) to 38.43 mg at 0.4% (v/v) and completely inhibited growth at ≥0.6% (v/v). Thiram showed dose-dependent inhibition, reaching complete inhibition at 0.8% (v/v). Overall, propiconazole was the most effective, followed by prochloraz, azoxystrobin, thiram, and metalaxyl, demonstrating clear differences in antifungal potency in both solid and liquid media.

3.9. Efficacy of biological and chemical fungicide treatments for controlling S. commune using an oil palm seed model

The efficacy of two *Trichoderma* culture filtrates (SKRU-01 and NST-009; 60% v/v) and three chemical fungicides (0.8% v/v) against *S. commune* was evaluated using germinated oil palm seeds. Significant differences ($p < 0.05$) among treatments were observed in disease incidence and disease control efficacy (Fig. 6). The untreated control exhibited the highest disease incidence (73.3%), whereas all treatments markedly reduced infection levels (Fig. 6A). CFs from SKRU-01 and NST-009 reduced disease incidence to 40.0% and 43.3%, corresponding to control efficacies of 45.3% and 41.1%, respectively.

Among the chemical fungicides, prochloraz, azoxystrobin, and propiconazole reduced disease incidence to 36.7%, 33.3%, and 26.7%, respectively, corresponding to control efficacies of 50.0%, 54.5%, and 63.7% (Fig. 6B). Although chemical fungicides showed higher control efficacy, CF from SKRU-01 at 60% (v/v) demonstrated comparable suppression to prochloraz and showed potential as a biological alternative for controlling brown germ and seed rot in oil palm seeds.

4. Discussion

Research on *S. commune* as a pathogen of oil palm seeds remains extremely limited. To date, only a few studies, mainly by Dikin et al. [5, 34], have reported its association with brown germ and seed rot, focusing primarily on pathogen isolation and preliminary biological

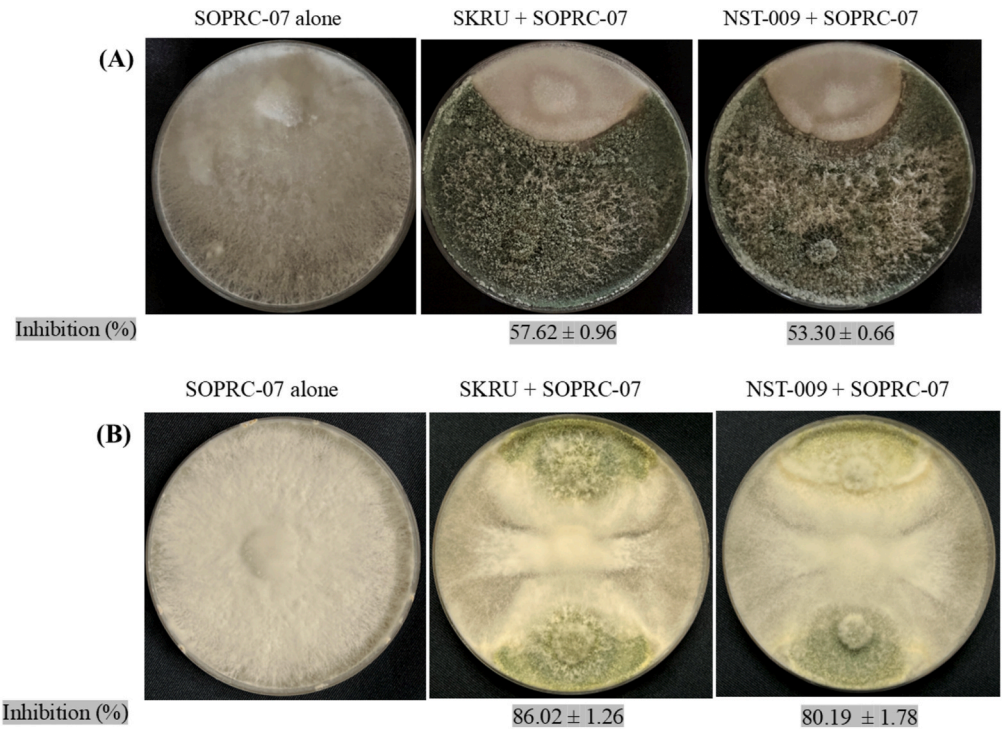
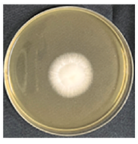
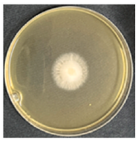
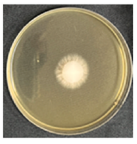
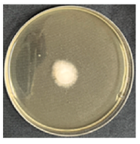
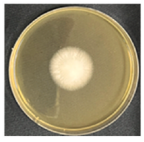
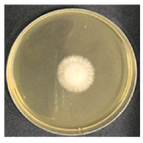
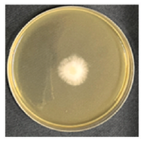
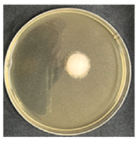


Fig. 4. *In vitro* antagonistic activity of *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 against *S. commune* using a dual culture assay on PDA after 5 days of incubation at 28 ± 2 °C. **(A)** One mycelial plug of each *Trichoderma* strain. **(B)** Two mycelial plugs of each *Trichoderma* strain.

Table 1
Antifungal activity of culture filtrates (CFs) concentrations (1–60% v/v) of *T. asperelloides* SKRU-01 **(A)** and *T. asperellum* NST-009 **(B)** on the colony growth of *S. commune* in PDA medium after 5 days of incubation at 28 ± 2 °C.

(A)						
Parameters	SKRU-01 CFs (% v/v)					
	Control	1	15	30	45	60
Colony diameters (cm)	$8.50^{a} \pm 0.00$	$8.25^{b} \pm 0.27$	$3.05^{c} \pm 0.08$	$2.42^{d} \pm 0.20$	$1.78^{e} \pm 0.21$	$1.23^{f} \pm 0.10$
Colony growth inhibition (%)	-	$2.94^{e} \pm 0.13$	$64.12^{d} \pm 1.34$	$71.57^{c} \pm 1.04$	$79.02^{b} \pm 0.02$	$85.49^{a} \pm 0.05$
Colony morphology						
(B)						
Parameters	NST-009 CFs (% v/v)					
	Control	1	15	30	45	60
Colony diameters (cm)	$8.50^{a} \pm 0.00$	$8.33^{a} \pm 0.26$	$3.23^{b} \pm 0.48$	$2.55^{c} \pm 0.08$	$1.82^{d} \pm 0.36$	$1.73^{d} \pm 0.15$
Colony growth inhibition (%)	-	$1.96^{e} \pm 0.26$	$61.95^{d} \pm 0.48$	$70.00^{c} \pm 0.08$	$78.63^{b} \pm 0.35$	$79.61^{a} \pm 0.15$
Colony morphology						

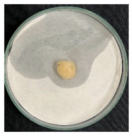
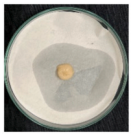
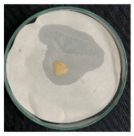
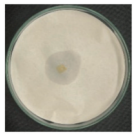
Note: Data are presented as the mean ± standard deviation (SD) of three replicates. Values within the same row that share the same letter do not differ significantly, as determined by one-way ANOVA followed by Tukey's HSD test at $p > 0.05$.

control using antagonistic bacteria. Despite the economic importance of oil palm, knowledge regarding the epidemiology, pathogenicity, and management of *S. commune* in germinated seeds has progressed little over the past two decades.

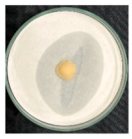
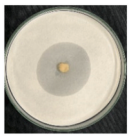
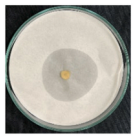
In this study, we investigated potential causes of non-germination in oil palm seeds during the germination process, hypothesizing that fungal infection may contribute to seed failure. *S. commune* was consistently isolated from defective and germinated seeds, particularly from the micropyle region, and identified using morphological characteristics and ITS–LSU sequence analysis. Because direct inoculation of intact oil palm seeds posed technical constraints due to their hard endocarp and low permeability, which hindered reliable pathogen penetration and uniform infection, germinated seeds with radicles approximately 2 cm in length were used as a practical infection model. In addition, the limited

Table 2
Antifungal activity of culture filtrates (CFs) concentrations (1–60% v/v) of *T. asperelloides* SKRU-01 **(A)** and *T. asperellum* NST-009 **(B)** on the mycelial growth of *S. commune* in PDB medium after 5 days of incubation at 28 ± 2 °C.

(A)

Parameters	SKRU-01 CFs (% v/v)					
	Control	1	15	30	45	60
Mycelial dry weight (mg)	$133.50^{a} \pm 3.25$	$123.40^{a} \pm 13.07$	$42.60^{b} \pm 2.20$	$16.63^{b} \pm 3.20$	$7.63^{b} \pm 1.50$	$7.87^{b} \pm 0.56$
Inhibition (%)	-	$8.38^{c} \pm 0.61$	$68.22^{b} \pm 1.60$	$87.54^{a} \pm 2.40$	$94.28^{a} \pm 2.63$	$94.11^{a} \pm 3.88$
Colony morphology						

(B)

Parameters	NST-009 CFs (% v/v)					
	Control	1	15	30	45	60
Mycelial dry weight (mg)	$133.50^{a} \pm 3.25$	$105.63^{b} \pm 3.22$	$25.80^{c} \pm 2.61$	$16.20^{c} \pm 2.78$	$5.43^{c} \pm 0.55$	$7.37^{c} \pm 0.54$
Inhibition (%)	-	$21.61^{b} \pm 1.17$	$80.68^{a} \pm 1.88$	$87.87^{a} \pm 2.08$	$95.93^{a} \pm 4.74$	$94.48^{a} \pm 1.86$
Colony morphology						

Note: Data are presented as the mean ± standard deviation (SD) of three replicates. Values within the same row that share the same letter do not differ significantly, as determined by one-way ANOVA followed by Tukey's HSD test at $p > 0.05$.

availability of uniformly viable seeds and the prolonged germination period under controlled environmental conditions restricted the ability to perform reinoculation experiments on ungerminated seeds. Under these conditions, inoculated germinated seeds exhibited extensive fungal colonization, accompanied by reduced root and shoot development compared with uninoculated controls. These observations provide experimental support for the pathogenic potential of *S. commune* and offer a feasible model for studying seedborne infection in structurally complex oil palm seeds, although full confirmation of Koch's postulates using intact seeds warrants further investigation.

The antagonistic interaction between *Trichoderma* and *S. commune* was evaluated using a confrontation assay (dual culture), a method widely used for the *in vitro* screening of potential biocontrol agents against plant pathogens. In this study, two experimental configurations were employed to examine the inhibitory interaction between the antagonist and the pathogen. The classical dual culture setup allowed direct observation of the interaction between *Trichoderma* and *S. commune*. In addition, a modified configuration with two plugs of *Trichoderma* were included to increase antagonist density and to assess whether a higher antagonist presence could enhance pathogen suppression. This approach provided additional information on the inhibitory capacity of the antagonist under increased antagonist density, which may better represent situations where the antagonist colonizes the substrate more extensively.

Consistent with these observations, CFs of *Trichoderma* strains SKRU-01 and NST-009 significantly inhibited mycelial growth in co-cultivation and CF-based assays, suggesting a dominant role of diffusible antifungal metabolites. At 60% (v/v), CFs of SKRU-01 and NST-009 inhibited mycelial growth by 85.49% and 79.61% on PDA and by 94.11% and 94.48% in PDB, respectively, with consistently higher inhibition in liquid medium. These results are consistent with previous reports demonstrating strong antifungal activity of *Trichoderma* metabolites. For example, Naglot et al. [35] reported 73.3% inhibition of *Fusarium solani* using 50% CFs of *T. viride*, while Zhu et al. [36] showed that fermentation broth of *T. longibrachiatum* T6 reduced *Valsa mali* growth and conidial germination by 75.7% and 98.1%, respectively. Abdelmoteleb et al. [37] further demonstrated dose-dependent inhibition of *F. solani* biomass, reaching 87.23% at 60% CFs. Our findings also align with previous work on SKRU-01 and NST-009. CFs of *T. asperelloides* SKRU-01 at only 5% (v/v) suppressed *Aspergillus flavus* and *A. parasiticus* by over 70% [20], while CFs of SKRU-01 and NST-009 completely inhibited *Colletotrichum gloeosporioides* PSU-03 [38]. Together with reports on other *Trichoderma* species [39–42], these results confirm the robustness and broad-spectrum efficacy of *Trichoderma* CFs and support their suitability as liquid biocontrol formulations.

LC–QTOF–MS profiling of culture filtrates from *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 revealed multiple secondary metabolites with potential antifungal properties. Compounds tentatively identified in SKRU-01 included cyclo-(Ala–Pro) diketopiperazine, levoamine, bisacurone epoxide, anisomycin, and sapidolide A, whereas NST-009 produced cyclo-(Ala–Pro) diketopiperazine, levoamine, formylfusarochromanone, anisomycin, and sapidolide A. Several of these compounds have previously been reported to exhibit antifungal activity [42–46]. For example, diketopiperazines (DKPs) such as cyclo-(Ala–Pro) diketopiperazine have demonstrated broad-spectrum antifungal effects, while anisomycin and sapidolide A have been associated with inhibition of fungal growth [42–46].

Notably, SKRU-01 exhibited greater antifungal efficacy than NST-009 *in vitro*. This enhanced activity may be related to differences in metabolite composition, particularly the presence of bisacurone epoxide and the overall diversity of detected compounds in SKRU-01. Such compositional variation suggests that metabolite diversity may influence the overall biocontrol performance of the two strains. However, metabolite identification in this study was based on LC–QTOF–MS profiling and database matching, and the specific contribution of individual compounds to the inhibition of *S. commune* was not experimentally verified. Therefore, confirmation through isolation, purification, and functional characterization of the identified metabolites will be necessary to clarify their respective roles and relative contributions to pathogen suppression. Although culture filtrates exhibited antifungal activity comparable to or greater than some tested chemical fungicides, direct comparisons between purified metabolites and commercial fungicides were beyond the scope of this study.

ROS regulate fungal growth and development by functioning as signaling molecules essential for differentiation processes [47]. However, excessive ROS accumulation can cause oxidative damage to lipids,

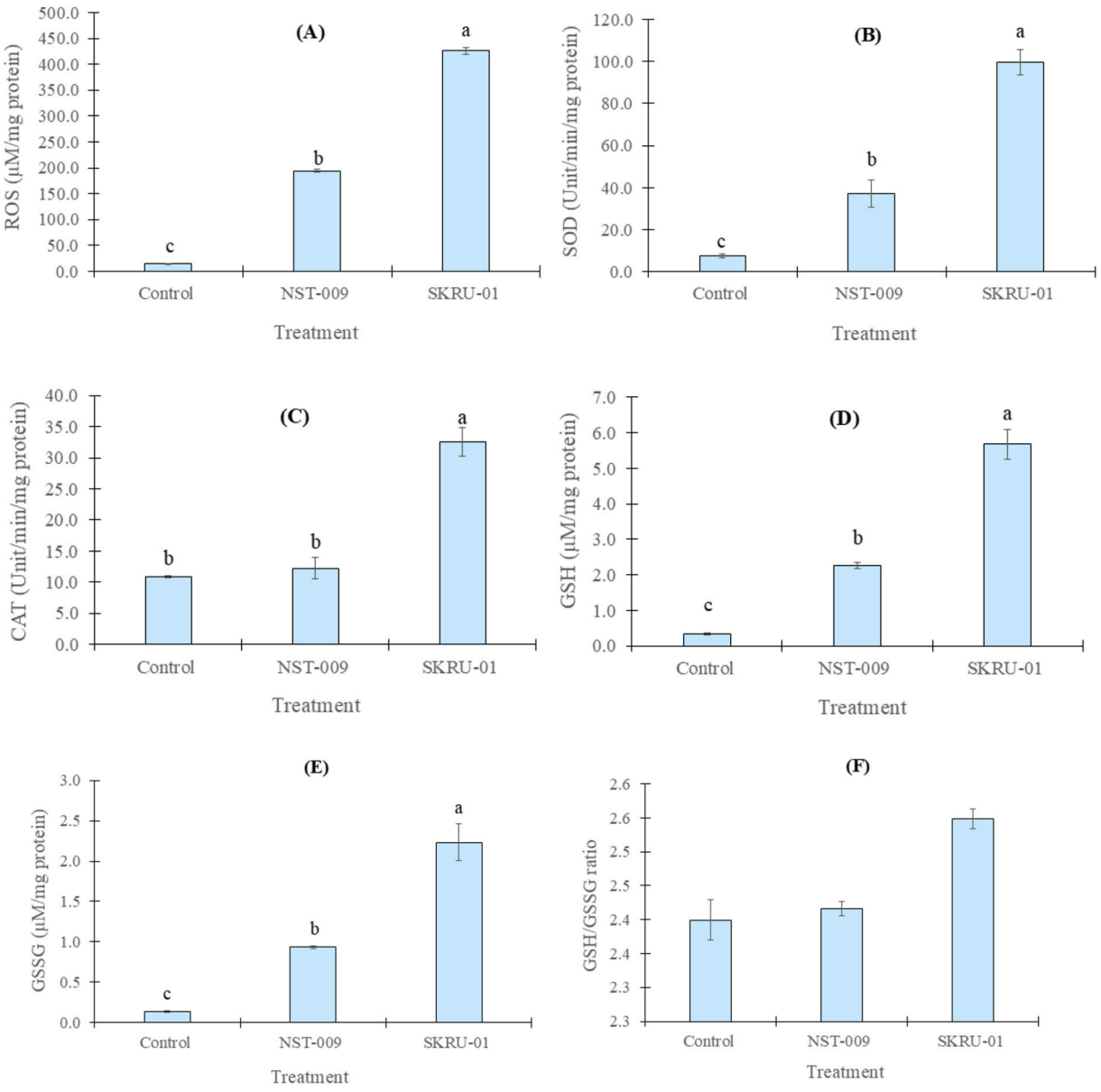


Fig. 5. Effect of culture filtrates from *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 on oxidative stress and antioxidant system of *S. commune*. **(A)** Reactive oxygen species (ROS) levels; **(B)** superoxide dismutase (SOD) activity; **(C)** catalase (CAT) activity; **(D)** reduced glutathione (GSH) content; **(E)** oxidized glutathione (GSSG) content; and **(F)** GSH/GSSG ratio.

proteins, and DNA, resulting in impaired cellular function [29]. Fungi employ antioxidant defense systems, including SOD, CAT, and GSH, to maintain redox balance and protect against oxidative stress [48]. In the present study, exposure of *S. commune* to culture filtrates led to increased ROS accumulation and elevated antioxidant enzyme activities, indicating induction of oxidative stress. SKRU-01 triggered a stronger oxidative stress response than NST-009, which is consistent with its greater antifungal activity. The distinct metabolite profile of SKRU-01 may underlie this enhanced stress induction, although the precise compounds responsible and their mechanistic interactions with fungal cells remain to be elucidated. It should also be noted that oxidative stress parameters were measured at a single time point to enable direct comparison between treatments. Given that ROS production and antioxidant responses are dynamic processes, future time-course analyses would provide deeper insight into the temporal progression of oxidative stress and its contribution to the antifungal mechanism of *Trichoderma*-derived metabolites. Therefore, the present findings provide preliminary mechanistic insight rather than a complete temporal characterization of oxidative stress responses.

Chemical fungicides also effectively suppressed *S. commune*, although efficacy varied with compound type and concentration. Propiconazole exhibited the strongest activity, completely inhibiting mycelial growth at 0.4% (v/v), followed by prochloraz and azoxystrobin. Higher concentrations were required for thiram and metalaxyl, reflecting dose-dependent sensitivity [49,50]. In general, complete inhibition was achieved at lower concentrations in liquid medium than on solid medium, consistent with improved diffusion and bioavailability. These effects correspond well with the known modes of action of each fungicide, including disruption of ergosterol biosynthesis, mitochondrial respiration, enzyme activity, and RNA synthesis [51–53].

The higher efficacy of chemical fungicides compared with *Trichoderma* culture filtrates is likely attributable to their well-defined target-site specificity and optimized formulation, which enable rapid and concentrated inhibition of essential fungal pathways. In contrast, culture filtrates contain complex mixtures of secondary metabolites that may act synergistically but at lower effective concentrations, resulting in

Table 3
Antifungal activity of five chemical fungicides—prochloraz, metalaxyl, azoxystrobin, propiconazole, and thiram —at concentrations of 0.1–0.8% (v/v) against *S. commune* in solid (PDA) and liquid (PDB) cultures after 5 days of incubation at 28 ± 2 °C.

Treatment	Concentration (% v/v)	PDA medium		PDB medium	
		Diameter of colony (cm)	Inhibition (%)	Mycelial dry weight (mg)	Inhibition (%)
Control	-	$9.00^{a} \pm 0.00$	-	$115.80^{a} \pm 0.01$	-
Prochloraz					
	0.1	$3.67^{h} \pm 0.26$	$59.26^{n} \pm 0.80$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.2	$3.22^{i} \pm 0.28$	$64.26^{m} \pm 1.03$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.4	$2.50^{l} \pm 0.00$	$72.22^{j} \pm 2.80$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.6	$2.05^{op} \pm 0.08$	$77.22^{gh} \pm 1.45$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.8	$1.88^{q} \pm 0.08$	$79.07^{f} \pm 1.38$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
Azoxystrobin					
	0.1	$7.42^{d} \pm 0.20$	$17.59^{r} \pm 0.58$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.2	$5.92^{e} \pm 0.49$	$34.26^{q} \pm 0.17$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.4	$4.75^{g} \pm 0.76$	$47.22^{o} \pm 2.65$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.6	$2.87^{j} \pm 0.29$	$68.15^{l} \pm 0.21$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.8	$2.10^{o} \pm 0.47$	$76.67^{h} \pm 2.08$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
Propiconazole					
	0.1	$1.00^{t} \pm 0.00$	$88.89^{c} \pm 0.16$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.2	$0.50^{u} \pm 0.00$	$94.44^{b} \pm 0.23$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.4	$0.00^{v} \pm 0.00$	$100^{a} \pm 0.00$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.6	$0.00^{v} \pm 0.00$	$100^{a} \pm 0.00$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.8	$0.00^{v} \pm 0.00$	$100^{a} \pm 0.00$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
Metalaxyl					
	0.1	$8.48^{b} \pm 0.04$	$5.74^{t} \pm 0.12$	$113.47^{b} \pm 0.08$	$2.02^{g} \pm 1.13$
	0.2	$8.35^{c} \pm 0.14$	$7.22^{s} \pm 0.05$	$72.93^{e} \pm 0.03$	$37.79^{d} \pm 0.73$
	0.4	$5.17^{f} \pm 0.61$	$42.59^{p} \pm 0.13$	$38.43^{f} \pm 0.12$	$66.81^{c} \pm 0.38$
	0.6	$1.58^{s} \pm 0.30$	$82.41^{d} \pm 0.17$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
	0.8	$0.00^{v} \pm 0.00$	$100^{a} \pm 0.00$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$
Thiram					
	0.1	$2.73^{k} \pm 0.19$	$69.63^{k} \pm 2.17$	$113.67^{b} \pm 2.05$	$1.84^{g} \pm 0.08$
	0.2	$2.40^{m} \pm 0.31$	$73.33^{i} \pm 1.42$	$102.97^{c} \pm 0.08$	$11.08^{f} \pm 0.11$
	0.4	$2.33^{n} \pm 0.21$	$74.07^{i} \pm 0.28$	$93.60^{d} \pm 1.84$	$19.17^{e} \pm 1.92$
	0.6	$2.00^{p} \pm 0.46$	$77.78^{g} \pm 0.61$	$13.33^{g} \pm 1.15$	$88.49^{b} \pm 2.85$
	0.8	$1.75^{r} \pm 0.54$	$80.56^{e} \pm 0.28$	$0.00^{h} \pm 0.00$	$100^{a} \pm 0.00$

Note: Data are presented as the mean ± standard deviation (SD) of three replicates. Values within the same row that share the same letter do not differ significantly, as determined by one-way ANOVA followed by Tukey's HSD test at $p > 0.05$.

comparatively moderate suppression. This distinction highlights fundamental differences between synthetic fungicides and biologically derived metabolites in terms of mode of action and potency.

While chemical fungicides provided rapid and complete suppression, biological treatments also significantly reduced disease incidence (>40%), reinforcing the value of *Trichoderma*-based strategies for sustainable disease management. Although the control efficacy of SKRU-01 was slightly lower than that of propiconazole and azoxystrobin, its performance was comparable to prochloraz in the oil palm seed model, suggesting that metabolite-based treatments may offer practical potential as complementary or alternative control strategies. Importantly, biological treatments may reduce the risk of resistance development and environmental persistence associated with repeated fungicide applications. These findings are consistent with earlier studies demonstrating effective control of *S. commune* using antagonistic bacteria or their metabolites in oil palm seeds [5,40].

5. Conclusion

Defective germination and seed rot caused by *S. commune* pose a significant threat to oil palm seed quality. Among 52 fungal isolates, SOPRC-07 was identified as *S. commune* through morphological and molecular analyses. Two *Trichoderma* strains, *T. asperelloides* SKRU-01 and *T. asperellum* NST-009, significantly inhibited fungal growth *in vitro* (up to 85% on PDA and over 94% in PDB). Chemical analysis revealed diverse secondary metabolites, including diketopiperazines (DKPs), anisomycin, and sapidolide A, which may contribute to ROS-mediated inhibition of *S. commune*. *Trichoderma* culture filtrates induced ROS accumulation and activated fungal antioxidant defenses. On germinated seeds, *Trichoderma* treatments reduced disease incidence from 73.3% (control) to 40.0–43.3%, while chemical fungicides further decreased incidence to 26.7–36.7%, with propiconazole achieving the highest control (63.5%). These findings confirm the potential of *T. asperelloides* SKRU-01 and *T. asperellum* NST-009 as biocontrol agents and highlight the link between metabolite diversity, oxidative stress–mediated mechanisms, and practical disease management strategies for seedborne *S. commune* in oil palm propagation.

CRediT authorship contribution statement

Wanida Petlamul: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Jirayu Buatong:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Karistsapol Nooprom:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Benjamas Cheirsilp:** Writing – review & editing, Funding acquisition. **Sirasit Srinuanpan:** Writing – review & editing. **Krittin Chumkaew:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Siriporn Yossan:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Sunipa Chankaew:** Investigation, Formal analysis, Data curation. **Amornrat Chumthong:** Writing – review & editing. **Sawai Boukaew:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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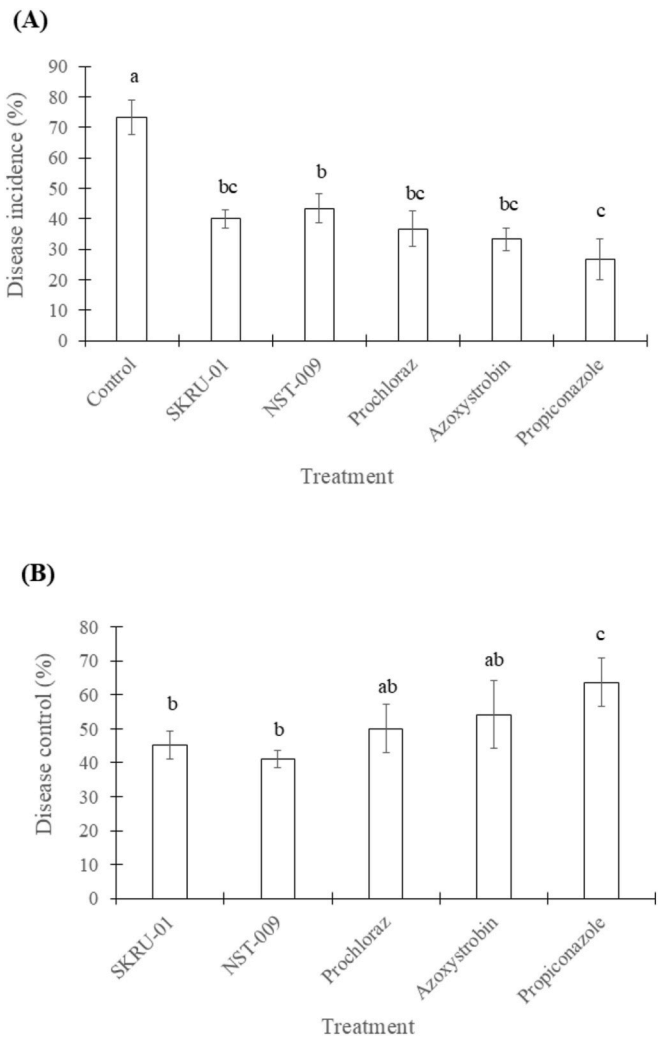

Fig. 6. Efficacy of CFs from two *Trichoderma* strains (SKRU-01 and NST-009) and three chemical fungicides (prochloraz, azoxystrobin, and propiconazole) in controlling *S. commune* infection in germinated oil palm seeds after 20 days of incubation at 30 °C under humid conditions. **(A)** Disease incidence (%) and **(B)** Disease control (%). ***Note:*** Disease incidence was evaluated based on the presence of brown germ or seed rot symptoms, characterized by brown discoloration and fungal mycelial growth covering the germ or radicle. Data represent the mean ± standard deviation (SD) of three replicates (n = 30 seeds per treatment). Bars with the same letter are not significantly different according to Tukey's HSD test ($p > 0.05$) after one-way ANOVA.

Declaration of competing interest

The authors declare that they have no conflicts of interest, financial or personal, that could have inappropriately influenced the work reported in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2026.103195.

Data availability

The authors do not have permission to share data.

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