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Linalool-dominated volatile organic compounds from *Bacillus aryabhattai* control *Lasiodiplodia theobromae* and preserve postharvest quality of nutmeg fruit

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ABSTRACT

Postharvest fungal diseases caused by *Lasiodiplodia theobromae* significantly reduce the quality and marketability of nutmeg fruit. This study investigated the antifungal potential of *Bacillus aryabhattai* strain KT-21 and its volatile organic compounds (VOCs). Among five bacterial isolates, strain KT-21 exhibited the strongest VOC-mediated inhibition (50.18%) and broad-spectrum antifungal activity against 10 plant pathogenic fungi. The strain combined VOC-mediated inhibition, direct antagonism, and mycolytic activity, indicating a multi-mechanistic mode of action. Genome analysis confirmed strain KT-21 as *B. aryabhattai* and revealed multiple biosynthetic gene clusters, including terpene pathways. GC–MS profiling identified 32 VOCs, with linalool (30.73%) as the dominant compound. Linalool exhibited the strongest antifungal activity, showing fungicidal effects at $\geq$ 20 μL and an IC_{50} of 5.27 μL per plate. *In vivo* assays demonstrated that strain KT-21 VOCs significantly reduced disease severity on nutmeg fruit (up to 82.86%), while linalool achieved up to 93.33% efficacy when applied at the early stage of infection and preserved fruit quality by maintaining key physicochemical attributes. Ultrastructural observations confirmed severe cellular damage in fungal hyphae. These findings highlight strain KT-21 as a promising biocontrol agent with complementary VOC and contact-dependent mechanisms, supporting the application of linalool for postharvest disease management and quality preservation.

1. Introduction

Plant pathogenic fungi pose a major threat to agricultural production and postharvest systems, causing significant yield losses and quality deterioration (Khan et al., 2020; Guo et al., 2023). Among these pathogens, *Lasiodiplodia theobromae* is recognized as a destructive fungus affecting a wide range of economically important crops, including mango, avocado, and nutmeg, leading to diseases such as fruit rot, stem canker, and dieback (Huda-Shakirah et al., 2022; Guerrero et al., 2025; Jiang et al., 2025). Its broad host range and aggressive infection behavior make it a major concern in tropical and subtropical agriculture (Guerrero et al., 2025).

Nutmeg (*Myristica fragrans* Houtt.) is an economically important tropical spice crop widely cultivated in Southeast Asia and other tropical regions (Biju et al., 2021; Luo et al., 2026). In Thailand, nutmeg production contributes to local agriculture and the spice industry (Keereekoch et al., 2018). However, the fruit is highly susceptible to fungal infection due to its soft tissue and the warm, humid conditions typical of tropical environments. Infection by *L. theobromae* during postharvest handling often leads to rapid fruit decay, quality

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deterioration, and significant economic losses.

Control of *L. theobromae* has largely relied on synthetic fungicides such as thiabendazole, imazalil, and azoxystrobin. However, the long-term use of these chemicals has resulted in the emergence of resistant pathogen populations and raised concerns regarding food safety and environmental contamination (Chen et al., 2020; Yang et al., 2021). These limitations highlight the urgent need for safer and more sustainable approaches for managing postharvest diseases.

Biological control agents (BCAs) have gained increasing attention as eco-friendly alternatives for suppressing plant pathogens. In particular, microbial VOCs have emerged as promising antifungal agents due to their ability to inhibit pathogens without direct contact, making them suitable for postharvest applications (Kanchiswamy et al., 2015; Weisskopf et al., 2021). VOCs produced by antagonistic microorganisms such as *Bacillus*, *Streptomyces, Pseudomonas, Trichoderma,* and *Scheffersomyces* have been reported to exhibit broad-spectrum antifungal activity through multiple mechanisms, including disruption of cell membranes, induction of oxidative stress, and interference with fungal metabolism (Garbeva and Weisskopf, 2020; Zhao et al., 2022; Ling et al., 2023; Zou et al., 2023; Wang et al., 2025).

Among these compounds, terpene-derived volatiles such as linalool have been widely recognized for their strong antifungal activity. Previous studies have demonstrated that linalool can disrupt membrane integrity, alter permeability, and induce structural damage in fungal cells, leading to growth inhibition or cell death (Yue et al., 2023; Wang et al., 2025).

Despite these advances, several knowledge gaps remain. First, most studies have focused primarily on antifungal activity, while limited information is available on the integration of VOC profiling with genomic analysis to elucidate the biosynthetic origin of these compounds, particularly terpene-associated pathways. Second, although antagonistic effects are frequently reported, comprehensive evaluation of multiple mechanisms, including contact-mediated inhibition, fungal lysis (mycolytic activity), and ultrastructural alterations, remains limited. Third, the effects of microbial VOCs on postharvest fruit quality, especially in nutmeg, have not been thoroughly investigated.

Therefore, this study aimed to evaluate the antifungal potential of VOCs produced by a bacterial isolate against *L. theobromae*, identify key bioactive compounds using GC–MS, and link these compounds to biosynthetic gene clusters through genome analysis. In addition, multiple antagonistic mechanisms were investigated, and the efficacy of VOCs and linalool in controlling disease and preserving postharvest quality of nutmeg fruit was assessed.

2. Material and methods

2.1. Microorganisms and growth conditions

The five antagonistic bacterial isolates (KT−6, KT−21, C-KT−3, R-KT−26, and SB−01) were isolated from the rhizosphere soil of rice (*Oryza sativa* L.). These isolates exhibited strong antifungal activity against *Rhizoctonia solani* in a direct dual culture assay; however, their inhibitory effects via VOCs have not yet been evaluated. All isolates were cultured on nutrient agar (NA) and maintained at 4 °C for subsequent use in this study.

The pathogen *Lasiodiplodia theobromae* was isolated from nutmeg fruits exhibiting rot symptoms collected from cultivation fields in Ron Phibun Subdistrict, Nakhon Si Thammarat Province, Thailand (8.193139° N, 99.845305° E). The isolate was identified based on morphological characteristics and molecular phylogenetic analysis of the internal transcribed spacer (ITS) and large subunit (nrLSU) regions of nuclear ribosomal DNA (rDNA) (Chuprom et al., 2026). The confirmed strain was cultured and maintained on potato dextrose agar (PDA) slants at 4 °C before use.

A range of plant pathogenic fungi, including *Schizophyllum commune* Fr. (SOPRC−07), *Sclerotium rolfsii* Sacc. (KKU 01), *Peniophora salaccae* Boukaew et al. (2024a) (SKRU002), *Curvularia oryzae* Bugnicourt (SOPRC−9), *Aspergillus parasiticus* Speare (TISTR 3276), *Aspergillus flavus* Link (PSRDC−4), *Rhizoctonia solani* J.G. Kühn (PTRRC−9), and *Colletotrichum musae* (Berk. & M.A. Curtis) Arx (NYB−03), were obtained from the Plant Pathology Laboratory, Faculty of Agricultural Technology, Songkhla Rajabhat University, Songkhla, Thailand. In addition, *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei (PSU−01) and *Fusarium incarnatum* (Desm.) Sacc. (PSU−01) were kindly provided by Prof. Dr. Anurag Sunpapao, Agricultural Innovation and Management Division (Pest Management), Faculty of Natural Resources, Prince of Songkla University, Thailand. All fungal isolates were routinely maintained on PDA slants at 4 °C prior to use in subsequent experiments.

2.2. Screening of inhibitory effect of bacterial VOCs against *L. theobromae*

Five bacterial isolates were initially screened for their ability to produce VOCs with antifungal activity against *L. theobromae*. For VOC production, each isolate was cultured on NA plates (90 mm diameter) by uniformly spreading 100 µL of a bacterial suspension adjusted to 1×10^7 CFU/mL. The plates were incubated at 36 °C for 24 h to allow VOC accumulation (Ling et al., 2022). The pre-incubated cultures were subsequently used as VOC sources in antifungal assays.

Antifungal activity was evaluated using a sealed dual-plate technique. A 5-mm-diameter mycelial plug, taken from the actively growing margin of *L. theobromae*, was placed at the center of a PDA plate (90 mm diameter). The lid of the PDA plate was replaced with the base of an NA plate containing the bacterial culture, while control plates were paired with sterile NA medium. The paired plates were sealed with parafilm to minimize VOC loss and incubated at 28 ± 2 °C for 3 days (Calvo et al., 2020). Fungal growth was assessed by measuring colony diameter, and the inhibitory effect was expressed as a percentage relative to the control. Growth inhibition was calculated as: Percentage inhibition (%) = [(C − T) / C] × 100, where C and T represent colony diameters in the control and treatment, respectively. Each treatment was performed in triplicate and repeated twice.

2.3. Broad-spectrum antifungal activity of VOC produced by strain KT−21

The antifungal activity of VOC emitted by strain KT−21 was evaluated against ten plant pathogenic fungi. VOC production by strain KT−21 and antifungal activity assessment were conducted using the sealed dual-plate assay described previously. Briefly, strain KT−21 was cultured on NA plates (90 mm diameter) and incubated at 36 °C for 24 h. A 5-mm-diameter mycelial plug obtained from the actively growing margin of each fungal pathogen was placed at the center of a PDA plate. The PDA plate was then paired with the NA plate containing the strain KT−21 culture, while control treatments included PDA plates paired with sterile NA medium. All paired plates were sealed with parafilm and incubated at 28 ± 2 °C for 3–7 days, depending on the growth rate of each fungal species. After incubation, fungal colony diameters were measured, and the percentage inhibition of mycelial growth was calculated relative to the control as described previously. Each treatment was performed in triplicate and repeated twice.

2.4. In vitro contact-mediated antagonistic mechanisms of strain KT−21 against *L. theobromae*

To differentiate between volatile- and contact-mediated antifungal mechanisms, the antagonistic activity of strain KT−21 was further evaluated using dual culture, co-cultivation, and mycolytic assays.

Dual culture assays were conducted on PDA to evaluate the direct antagonistic activity of strain KT−21 against *L. theobromae*. In one setup, strain KT−21 was streaked along one side of a 90-mm Petri dish, with a

5-mm mycelial plug of *L. theobromae* placed on the opposite side. In the second setup, strain KT−21 was streaked on two opposite sides of the plate, and the fungal plug was placed at the center. Plates were incubated at 28 ± 2 °C for 3 days. Fungal colony diameters were measured, and percentage inhibition was calculated relative to the control using the formula described previously.

Co-cultivation in liquid medium was performed to quantify the effect of strain KT−21 on fungal biomass. Bacterial cells were harvested from overnight nutrient broth (NB) cultures, washed twice with 10 mM $MgSO_4$, and adjusted to an OD_{600} of 0.6 (~1 × 10^8 CFU/mL). *L. theobromae* conidial suspension was prepared at 1 × 10^6 conidia/mL. Equal volumes (100 μL each) of bacterial and fungal suspensions were added to 9.8 mL of potato dextrose broth (PDB) in 50-mL tubes. Tubes were incubated at 28 ± 2 °C with shaking at 150 rpm for 3 days. Fungal mycelia were then collected on Whatman #1 filter paper, dried at 60 °C for 2 days, and dry weight was measured.

The potential ability of strain KT−21 to utilize fungal biomass as a nutrient source was evaluated following modifications of Mannaa et al. (2023). *L. theobromae* mycelia were grown in PDB at 28 ± 2 °C, shaking 150 rpm, for 2 days. Mycelia were harvested, washed with sterile distilled water, and 6 mg of biomass was added to 3 mL of NB diluted 1:1000 (v/v) to create nutrient-limited conditions and promote potential utilization of fungal biomass. Bacterial suspension (100 μL, OD600 = 0.6) was added to tubes containing fungal biomass (+fungi), while control tubes contained bacteria alone (−fungi). Tubes were incubated at 28 ± 2 °C, shaking at 150 rpm, for 2 days. After incubation, bacterial populations were determined by plating 100 μL of serially diluted cultures onto NA and counting colony-forming units (CFUs) after 2 days at 36 °C

All experiments were conducted in triplicate and independently repeated twice.

2.5. Identification of strain KT−21 by genome analysis and biosynthetic gene clusters

Whole-genome sequencing of strain KT−21 was performed using the Illumina platform. Raw reads were quality-filtered and de novo assembled using Unicycler (Wick et al., 2017). Genome annotation was conducted with PROKKA to predict coding sequences and structural RNA genes (Seemann, 2014). Phylogenomic analysis was performed using the AutoMLST2 pipeline with publicly available genomes of closely related *Bacillus* species. Pairwise genome similarity was estimated based on average nucleotide identity (ANI), and conserved single-copy marker genes were concatenated for alignment. A maximum-likelihood phylogenomic tree was inferred using IQ-TREE with 1000 bootstrap replicates. Based on phylogenomic analysis and ANI, strain KT−21 was identified as *Bacillus aryabhattai*. Biosynthetic gene clusters (BGCs) associated with secondary metabolite production were predicted using antiSMASH (v6.0.1) and compared with reference clusters in the MIBiG database for similarity and potential functional annotation.

2.6. Identification and profiling of VOC produced by strain KT−21

The VOCs emitted by bacterial strain KT−21 were characterized using a headspace-based analytical approach with slight modifications from previously described methods (Ling et al., 2022). For VOC production, 10 μL of bacterial suspension of strain KT−21 adjusted to 1 × 10^7 CFU/mL was inoculated into 3 mL of NA medium contained in 5 mL sealed glass vials. The vials were incubated at 36 °C for 24 h to allow VOC accumulation in the headspace. Sterile NA vials prepared under identical conditions served as negative controls. Volatile compounds in the headspace were extracted using headspace solid-phase microextraction (HS-SPME) and subsequently analyzed by gas chromatography–mass spectrometry (GC–MS). Instrumental conditions, chromatographic separation, and mass spectral identification were performed according to previously established procedures (Boukaew et al., 2024b). Each treatment was conducted with two independent replicates.

2.7. Antifungal activity of synthetic VOCs against L. theobromae

The antifungal activity of four major purified VOCs, namely acetophenone, phenylethyl alcohol, 2-mercaptoethanol, and linalool, was evaluated against *L. theobromae* using a sealed I-plate (two-compartment Petri dish) assay. A mycelial plug (5 mm in diameter) excised from the actively growing margin of *L. theobromae* was placed at the center of one compartment containing PDA. In the second compartment, sterile filter paper discs were placed, and defined volumes of each purified VOC were applied to achieve final concentrations of 2.5, 5, 10, 20, 40, and 60 μL per sealed Petri dish. Control treatments received no VOCs. After VOC application, the Petri dishes were immediately sealed with Parafilm to prevent vapor loss and incubated at 28 ± 2 °C for 2 days. At the end of the incubation period, fungal colony growth was determined by measuring colony diameter, and the percentage inhibition of mycelial growth was calculated relative to the control as described previously.

To assess fungal viability, mycelial plugs showing complete growth inhibition were transferred to fresh PDA plates and incubated for an additional 2 days under the same conditions. Fungal viability was determined based on visible regrowth, where regrowth was recorded as alive (A) and the absence of regrowth as dead (D). Each treatment consisted of three replicates, and the experiment was repeated twice. The half-maximal inhibitory concentration (IC_{50}) of the selected VOC exhibiting fungicidal effects was estimated using a linear regression model based on the relationship between compound concentration and percentage inhibition, as described by Sebaugh (2011).

2.8. Control efficacy of strain KT−21 VOCs against nutmeg black rot caused by L. theobromae

Uniform, symptomless nutmeg fruits of similar size were collected from local orchards of the Baan Suan Chan Community Enterprise Group for Nutmeg Product Processing (8.193139° N, 99.845305° E), located in Ron Phibun Subdistrict, Nakhon Si Thammarat Province, southern Thailand. The inhibitory effect of VOCs produced by strain KT−21 on the development of *L. theobromae* was evaluated using nutmeg fruits as the host substrate. Fruits were surface-wounded (3 × 3 mm) and inoculated with 10 μL of a conidial suspension (1 × 10^6 spores/mL). The inoculated fruits were then placed in sterile plastic containers (19.2 × 28 × 10.9 cm^3) and maintained at 85–90% relative humidity (Xu et al., 2026). To generate VOCs, NA plates previously inoculated with strain KT−21 at concentrations of 10^6, 10^7, and 10^8 CFU/mL and incubated at 37 °C for 24 h were placed inside each container. NA plates without bacterial inoculation served as the positive control (pathogen only), whereas fruits that were neither inoculated with *L. theobromae* nor exposed to strain KT−21 VOCs were used as the negative control. All containers were sealed with parafilm, inverted to prevent direct contact between bacterial cultures and fruit surfaces, and incubated at 28 ± 2 °C for 5 days. Disease severity (DS) was assessed by measuring the average lesion diameter on the fruit surface. Biocontrol efficacy was calculated using the following formula: [(DS of control - DS of treated)/DS of control] × 100%. Each treatment was replicated three times, with six fruits per replicate (18 fruits per treatment).

2.9. Comparative efficacy of strain KT−21 VOCs and linalool against nutmeg black rot caused by L. theobromae

Nutmeg fruit preparation, inoculation with *L. theobromae*, and placement in containers were performed as described previously. NA plates inoculated with strain KT−21 (10^8 CFU/mL; 37 °C, 24 h) were placed inside each container as the VOC treatment. For comparison, linalool was tested at concentrations corresponding to IC_{50}, 2 ×IC_{50}, 3 ×IC_{50}, and 4 ×IC_{50}. Each concentration was applied onto sterile filter

paper discs (Whatman No. 1) placed on small Petri dishes and introduced into the containers to allow volatile exposure. Containers without strain KT−21 or linalool served as the control. All containers were sealed with parafilm, inverted, and incubated at 28 ± 2 °C for 5 days. Disease severity and biocontrol efficacy were assessed as described previously. Each treatment was replicated three times, with six fruits per replicate (18 fruits per treatment).

2.10. Effects of linalool on L. theobromae and nutmeg fruit quality

2.10.1. On L. theobromae development

Nutmeg fruit preparation, inoculation with *L. theobromae*, and placement in containers were performed as described previously. Linalool at 4 ×IC_{50} was applied onto sterile filter paper discs (Whatman No. 1), which were placed on small Petri dishes and introduced into the containers at different time points: simultaneously with pathogen inoculation and at 1, 2, and 3 days after inoculation. Plates without linalool served as the positive control, whereas fruits that were neither inoculated with *L. theobromae* nor exposed to linalool served as the negative control. All containers were sealed with parafilm, inverted, and incubated at 28 ± 2 °C for 5 days. Disease severity and biocontrol efficacy were assessed as described previously. Each treatment was replicated three times, with six fruits per replicate (18 fruits per treatment).

2.10.2. On nutmeg fruit quality

Following disease evaluation, nutmeg fruits subjected to different linalool treatments were selected for postharvest quality analysis. Only fruits that had undergone the respective treatments were used, and all samples were prepared according to standard procedures prior to measurement of the following quality parameters:

2.10.2.1. Determination of pH and total soluble solid content. The pH of nutmeg fruit samples was measured using a pH meter. Total soluble solids (TSS) were determined using a refractometer and expressed as °Brix, following standard methods (AOAC International, 2019). The instrument was calibrated with deionized water prior to analysis. Samples were homogenized, and 1–2 drops were applied to the prism surface, with readings recorded after stabilization.

2.10.2.2. Determination of total titratable acidity. Total titratable acidity (TTA) was determined using an acid–base titration method with phenolphthalein as an indicator (AOAC International, 2019; Li et al., 2024a). A 2.0 mL sample was diluted with 20 mL deionized water, followed by the addition of two drops of 1% (w/v) phenolphthalein. The mixture was titrated with 0.1 N NaOH until a faint pink color persisted for 30 s. Titratable acidity was expressed as % citric acid using the following equation: $TTA\ (\%) = [(V \times N \times 0.064) / S] \times 100$, Where V is the volume of NaOH used (corrected by blank titration), N is the normality of NaOH (0.1 N), and S is the sample volume (mL).

2.10.2.3. Determination of total phenolic content. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method (Limcharoen et al., 2022). Absorbance was measured at 765 nm, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

2.10.2.4. Determination of free radical scavenging activity. Free radical scavenging activity was determined using the DPPH assay (Baliyan et al., 2022). Absorbance was measured at 517 nm after 30 min incubation in the dark. Radical scavenging activity (%RSA) was calculated as: [(Absorbance of control - Absorbance of treated)/ Absorbance of control] × 100%.

All experiments were conducted in triplicate and repeated twice.

2.11. Ultrastructural analysis of L. theobromae by SEM and TEM

Ultrastructural alterations in fungal mycelia following linalool treatment were examined using electron microscopy. Mycelia of *L. theobromae* were exposed to linalool at 2 ×IC_{50} and incubated at 28 ± 2 °C for 48 h, while untreated mycelia served as the control. After treatment, mycelial samples were fixed in 2.5% glutaraldehyde and rinsed three times with 0.1 M phosphate buffer (10 min each). The samples were then dehydrated through a graded ethanol series.

For SEM analysis, dehydrated samples were sputter-coated with gold and observed using a scanning electron microscope (SEM; FEI Apreo, Netherlands). For TEM analysis, samples were embedded in Epon 812 resin, sectioned using an ultramicrotome, and examined under a transmission electron microscope (TEM; Thermo Scientific Talos F200i, Czech Republic).

2.12. Statistical analysis

All data were subjected to statistical analysis using SPSS software (IBM SPSS Statistics, version 26.0; IBM Corp., Armonk, NY, USA). Differences among treatment means were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons. A significance level of $p < 0.05$ was applied throughout the analysis. For pairwise comparisons between each treatment and the untreated control, independent samples *t*-tests were performed ($p < 0.05$). Asterisks indicate significant differences between treated samples and the untreated control within each isolate.

3. Results

3.1. Screening of inhibitory effect of bacterial VOCs against L. theobromae

The VOCs produced by five bacterial isolates were initially evaluated for their inhibitory effect on *L. theobromae* (Fig. 1). All isolates significantly ($p < 0.05$) suppressed fungal growth compared with the control (Fig. 1A–C). Among them, strain KT−21 exhibited the strongest inhibitory activity, reducing colony diameter to 4.48 cm^2 and achieving 50.18% inhibition. Moderate inhibition was observed for strain SB−01 (32.96%) and strain C-KT−3 (30.37%), whereas strain R-KT−26 (22.59%) and strain KT−6 (18.33%) displayed lower levels of inhibition. Based on these results, strain KT−21 was selected for further investigation due to its highest VOC-mediated antifungal potential.

3.2. Broad-spectrum antifungal activity of VOCs produced by strain KT−21

To evaluate the broad-spectrum antifungal activity of VOCs produced by strain KT−21, its effects were further tested against ten plant pathogenic fungi (Fig. 2). VOCs from strain KT−21 significantly ($p < 0.05$) inhibited the mycelial growth of all tested fungi (Fig. 2A–C). Complete inhibition was observed against *S. rolfsii* (100%), followed by *R. solani* (83.26%). Strong suppression was recorded for *A. flavus* (71.71%) and *C. cassiicola* (55.26%), while moderate inhibition occurred in *C. oryzae* (48.85%), *F. incarnatum* (45.71%), *P. salaccae* (43.20%), and *C. musae* (41.13%). The lowest inhibition was observed in *A. parasiticus* (39.28%) and *S. commune* (37.25%). These results confirm that strain KT−21 VOCs exhibit potent broad-spectrum antifungal activity, with varying sensitivity among different pathogens.

3.3. In vitro contact-mediated antagonistic mechanisms of strain KT−21 against L. theobromae

Strain KT−21 exhibited strong inhibitory activity against *L. theobromae in vitro*. In dual-culture assays on PDA, clear inhibition zones formed around the bacterial streaks, indicating the production of

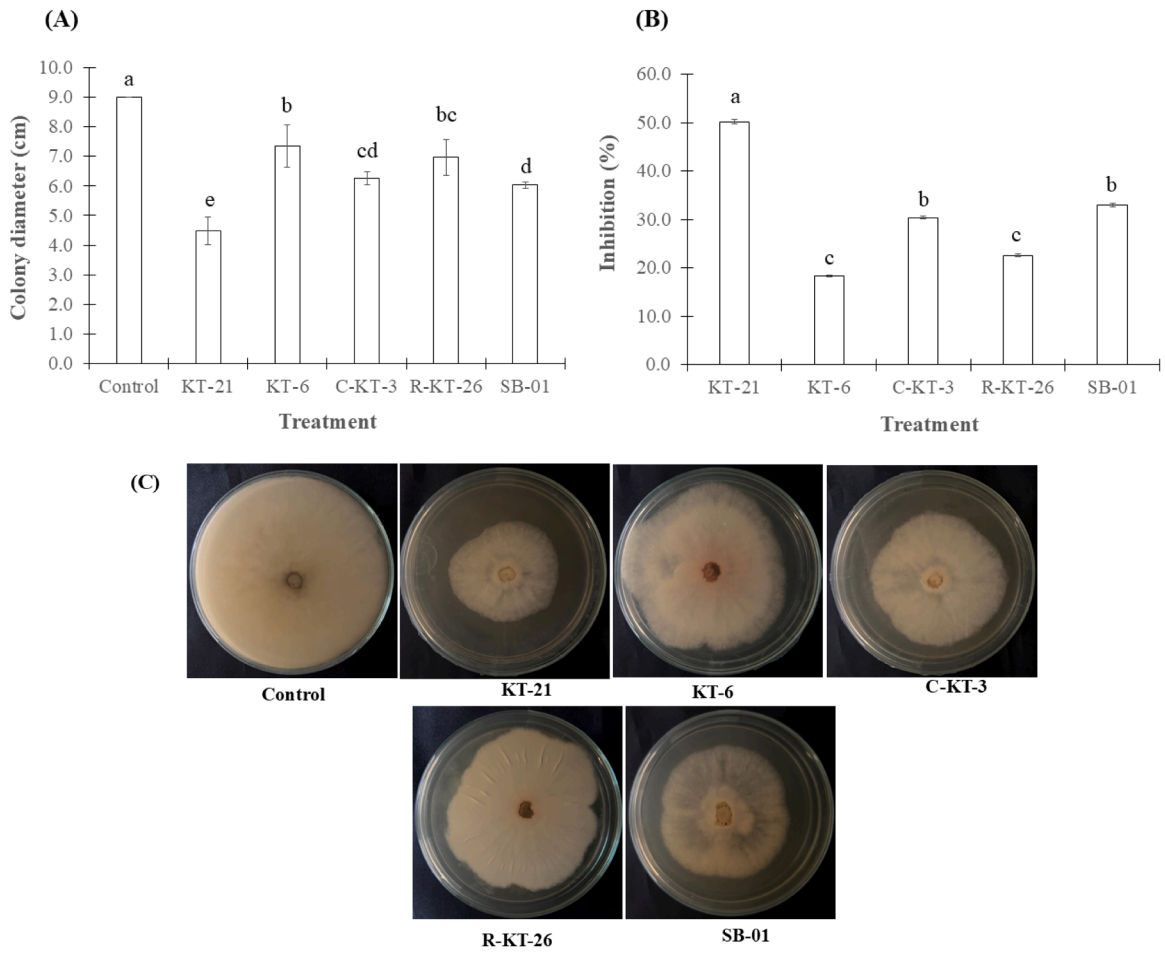

Fig. 1. Antifungal activity of VOCs produced by five bacterial isolates against the mycelial growth of *L. theobromae* on PDA after incubation at 28 ± 2 °C for 3 days. **(A)** Representative colony growth, **(B)** percentage of growth inhibition, and **(C)** colony morphology after exposure to VOCs. Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 3).

compounds that suppressed fungal growth (Fig. 3A, B). Measurement of fungal colony areas confirmed a significant reduction in mycelial growth compared with the control lacking strain KT−21. Both experimental arrangements—fungus inoculated on either side of the bacterial streak and bacterium flanking the central fungal plug—produced similar inhibitory effects, demonstrating the reproducibility of strain KT−21's antagonism

In liquid co-cultivation assays using PDB, co-incubation with strain KT−21 resulted in a marked decrease in fungal biomass relative to the untreated control. Mycelial dry weight was significantly reduced, confirming the bacterium's ability to suppress fungal growth under submerged culture conditions (Fig. 3C).

The potential ability of strain KT−21 to utilize fungal biomass was further evaluated under nutrient-limited conditions (1000 × diluted nutrient broth) by co-culturing the bacterium with *L. theobromae* mycelia. A marked increase in bacterial populations was observed (from 4.66 to 7.34 log CFU) in the presence of fungal biomass compared with the control without mycelia (Fig. 3D). CFU counts and spotting assays suggest that strain KT−21 may utilize fungal biomass as a supplementary nutrient source; however, this provides only indirect evidence and does not constitute direct proof of mycolytic activity.

3.4. Identification of strain KT−21 by genome analysis and biosynthetic gene clusters

The general genome features of strain KT−21 are summarized in Supplementary Table S1. The genome comprises a single circular chromosome of 5150,028 bp with a GC content of 37.93%. A total of 5271 coding sequences (CDSs) were predicted, with an average gene length of 811.83 bp, along with 57 tRNA and 5 rRNA genes. Seven secondary metabolite biosynthetic gene clusters were also identified. The circular genome map (Supplementary Fig. S1) shows the distribution of CDSs, RNA genes, GC content, and GC skew, with a relatively uniform gene distribution across the genome.

Phylogenomic analysis based on concatenated core genes using AutoMLST2 placed strain KT−21 within the *Bacillus aryabhattai* lineage. The strain clustered with the reference genome *B. aryabhattai* GCF_001619595, forming a well-supported monophyletic group. The maximum-likelihood phylogenomic tree (Supplementary Fig. S2) showed a consistent topology with strong bootstrap support, confirming the taxonomic identity of strain KT−21 as *B. aryabhattai*.

Further analysis using antiSMASH v6.0.1 identified seven BGCs, including terpene, siderophore, type III polyketide synthase (T3PKS), RRE-containing, and lassopeptide clusters (Table S2). Some BGCs showed similarity to known compounds, including surfactin (13%), carotenoid (50%), and paeninodin (60%), while others exhibited low similarity, suggesting potentially novel biosynthetic pathways.

3.5. Identification and profiling of VOCs produced by B. aryabhattai strain KT−21

SPME-GC–MS analysis of VOCs emitted by *B. aryabhattai* strain KT−21 identified a total of 32 compounds belonging to diverse chemical classes, including alkanes, ketones, alcohols, pyrazines, sulfur-

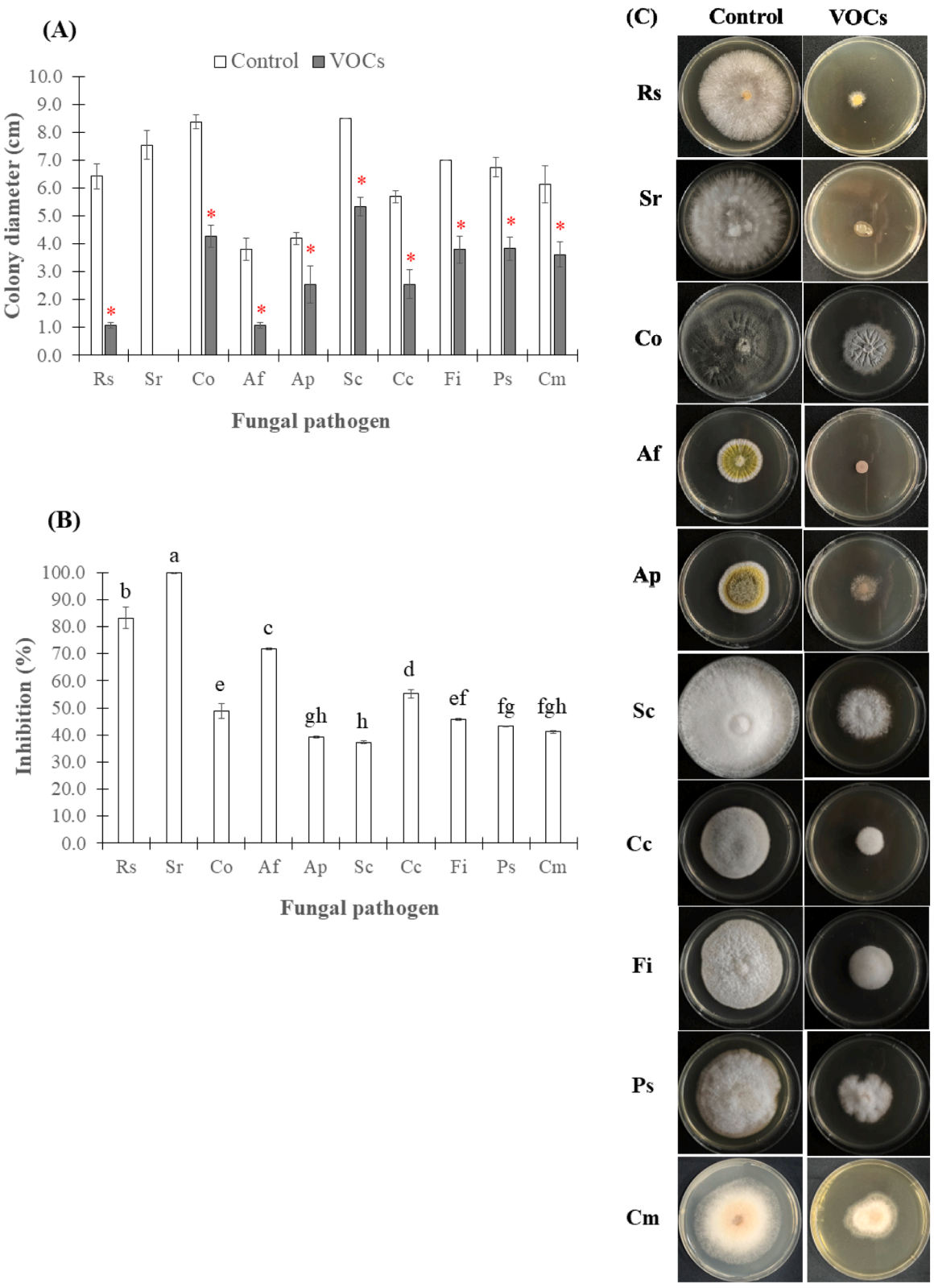

Fig. 2. Broad-spectrum antifungal activity of VOCs produced by *B. aryabhattai* strain KT−21 against ten plant pathogenic fungi. **(A)** Representative colony growth. Asterisks indicate significant differences between treated samples and the untreated control within each isolate (*$p < 0.05$; independent *t*-test). Data are expressed as mean ± SD (n = 3). **(B)** Percentage of mycelial growth inhibition of ten fungal strains. Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 3). **(C)** Colony morphology of *R. solani* (Rs), *S. rolfsii* (Sr), *C. oryzae* (Co), *A. flavus* (Af), *A. parasiticus* (Ap), *S. commune* (Sc), *C. cassiicola* (Cc), *F. incarnatum* (Fi), *P. salaccae* (Ps), and *C. musae* (Cm) after exposure to VOCs and incubation on PDA at 28 ± 2 °C for 3–7 days.

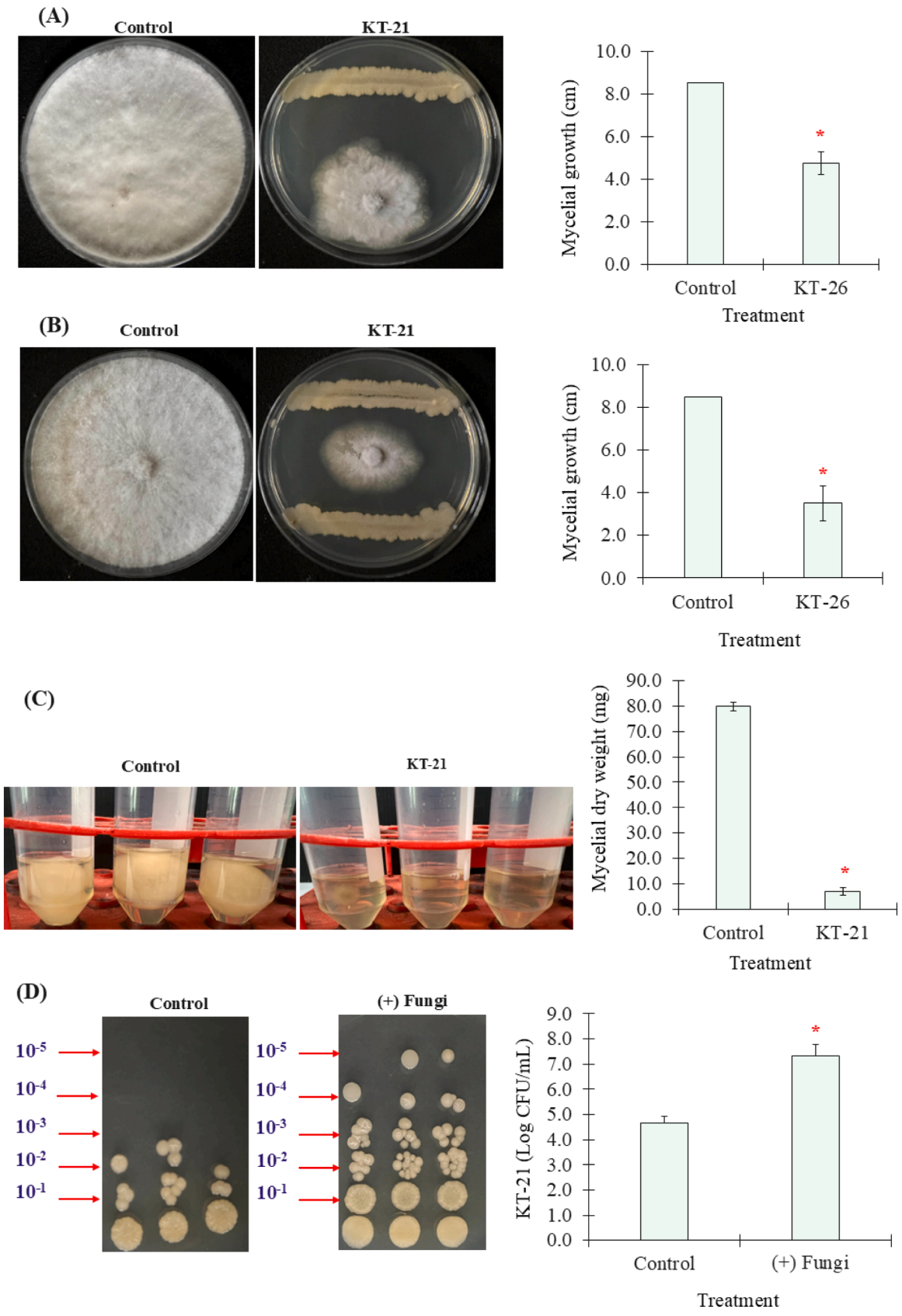

Fig. 3. *In vitro* antagonistic activity, co-cultivation, and mycolytic assays between *B. aryabhattai* strain KT−21 and *L. theobromae*. **(A)** Dual culture assay on PDA with a single bacterial streak positioned on one side of the plate and a fungal inoculation placed opposite to it, showing inhibition zone formation, **(B)** dual culture assay on PDA with two bacterial streaks positioned on opposite sides of the plate and a fungal inoculation placed at the center, **(C)** co-cultivation assay on PDB, and **(D)** mycolytic assay. Asterisks indicate significant differences between treated samples and the untreated control within each isolate (*$p < 0.05$; independent *t*-test). Data are expressed as mean ± SD (n = 3).

containing compounds, phenolics, heterocycles, terpenoids, nitriles, thiols, lactones, carboxylic acids, and aromatic hydrocarbons (Table 1). Among these, alcohols represented the dominant class, largely due to the high abundance of linalool (30.73%) and phenylethyl alcohol (10.29%). Ketones were the second most prominent group, with acetophenone (7.36%) as a major component. Alkanes were also consistently detected across multiple compounds, although generally at lower individual proportions. In addition, sulfur-containing compounds, including 2-mercaptoethanol (4.77%), contributed notably to the overall VOC profile. Other detected compounds, such as pyrazines, phenolics, heterocycles, lactones, nitriles, and carboxylic acids, were present at relatively low levels. The predominance of alcohols and ketones, together with the presence of bioactive sulfur-containing volatiles, suggests that these compounds collectively contribute to the broad-spectrum antifungal activity exhibited by *B. aryabhattai* strain KT−21.

3.6. Antifungal activity of synthetic VOCs against L. theobromae

The antifungal activity of four synthetic VOCs identified from *B. aryabhattai* strain KT−21 varied markedly depending on compound type and concentration (Table 2 and Fig. S3, Supplementary data), with distinct effects on both mycelial growth and fungal viability of *L. theobromae*. Phenylethyl alcohol exhibited the weakest activity, causing only slight inhibition even at the highest concentration tested (22.92% at 60 μL), and did not affect fungal viability. Acetophenone showed a clear dose-dependent effect, reducing mycelial growth by 69.79% at 10 μL and achieving complete inhibition at ≥ 40 μL. However, fungal regrowth after subculturing indicated a fungistatic mode of action. Similarly, 2-mercaptoethanol displayed strong antifungal activity, with 74.22% inhibition at 10 μL and complete suppression of mycelial growth at ≥ 20 μL. Despite this, fungal viability was retained, as evidenced by regrowth, indicating a fungistatic effect.

In contrast, linalool was the most potent compound. It reduced mycelial growth by 27.09% and 47.14% at 2.5 and 5 μL, respectively, and achieved complete inhibition at 10 μL. Notably, fungicidal activity was observed at concentrations ≥ 20 μL (Fig. S3), where no fungal regrowth occurred following transfer to fresh medium. Based on its superior antifungal performance, linalool was selected for further investigation, and its IC_{50} against *L. theobromae* mycelial growth was determined to be 5.27 μL per sealed Petri dish (Fig. S4, Supplementary data).

3.7. Control efficacy of B. aryabhattai strain KT−21 VOCs against nutmeg black rot caused by L. theobromae

Disease development of *L. theobromae* on nutmeg fruit was evaluated based on lesion diameter and biocontrol efficacy following exposure to strain KT−21 VOCs at 10^6, 10^7, and 10^8 CFU/mL (Fig. 4). Severe disease symptoms were observed in the positive control, with a mean lesion diameter of 3.25 cm, whereas no symptoms developed in the negative control (0 cm) (Fig. 4A). All strain KT−21 VOC treatments significantly reduced lesion development compared with the positive control ($p < 0.05$) (Fig. 4B). Lesion diameters decreased to 1.25 cm and 1.18 cm at 10^6 and 10^7 CFU/mL, respectively, with no significant ($p > 0.05$) difference between these two concentrations. Notably, strain KT−21 VOCs at 10^8 CFU/mL resulted in the greatest reduction, with a lesion diameter of 0.55 cm, which was significantly lower than the other treatments.

Consistent with these results, biocontrol efficacy increased with concentration, reaching 61.31%, 64.05%, and 82.86% at 10^6, 10^7, and 10^8 CFU/mL, respectively (Fig. 4C). The highest efficacy was observed at 10^8 CFU/mL, which was significantly greater than that at 10^6 and 10^7 CFU/mL. These findings demonstrate that strain KT−21 VOCs effectively suppress *L. theobromae* on nutmeg fruit in a concentration-

Table 1
Chemical composition of VOCs produced by *B. aryabhattai* strain KT−21, analyzed using SPME-GC–MS after cultivation on nutrient agar at 36°C for 24 h.

No.	Retaining time (min)	Chemicals	Group	CAS NO.	Molecular formula (MF)	Peak area Ratio (%) ± SD
1	1.8693	Heptane, 2,4-dimethyl-	Alkane	2213–23–2	C_9H_20	1.16 ± 0.01
2	2.1264	Octane, 4-methyl-	Alkane	2216–34–4	C_9H_20	0.51 ± 0.14
3	4.4885	Dodecane	Alkane	112–40–3	$C_{12}H_{26}$	3.07 ± 0.07
4	5.1402	Disulfide, dimethyl	Sulfur compound	624–92–0	$C_2H_6S_2$	0.69 ± 0.02
5	5.4294	Nonane, 5-butyl-	Alkane	17312–63–9	$C_{13}H_{28}$	0.14 ± 0.02
6	5.5884	Nonane, 4,5-dimethyl-	Alkane	17302–23–7	$C_{11}H_{24}$	1.12 ± 0.07
7	5.7259	Dodecane, 4,6-dimethyl-	Alkane	61141–72–8	$C_{14}H_{30}$	0.39 ± 0.07
8	7.6684	4-Methyl−2-hexanone	Ketone	999023–90–2	$C_7H_{14}O$	1.56 ± 0.01
9	8.7484	2-Heptanone	Ketone	110–43–0	$C_7H_{14}O$	0.09 ± 0.02
10	9.8287	1-Butanol, 3-methyl-	Alcohol	123–51–3	$C_5H_{12}O$	0.95 ± 0.04
11	11.5319	3-Heptanone, 5-methyl-	Ketone	541–85–5	$C_8H_{16}O$	0.18 ± 0.01
12	12.0104	Decane, 5-ethyl−5-methyl-	Alkane	17312–74–2	$C_{13}H_{28}$	0.66 ± 0.05
13	14.5508	Pyrazine, 2,5-dimethyl-	Pyrazine	123–32–0	$C_6H_8N_2$	1.22 ± 0.07
14	14.7275	Pyrazine, 2,6-dimethyl-	Pyrazine	108–50–9	$C_6H_8N_2$	1.34 ± 0.14
15	15.9095	Dimethyl trisulfide	Sulfur compound	3658–80–8	$C_2H_6S_3$	0.12 ± 0.07
16	21.1589	Pyrazine, 3-ethyl−2,5-dimethyl-	Pyrazine	13360–65–1	$C_8H_{12}N_2$	0.16 ± 0.07
17	27.6247	Ethanone, 1-(2-thiazolyl)-	Ketone	24295–03–2	C_5H_5NOS	0.65 ± 0.06
18	27.7836	Phenylethyl alcohol	Alcohol	60–12–8	$C_8H_{10}O$	10.29 ± 0.17
19	28.1902	Acetophenone	Ketone	98–86–2	C_8H_8O	7.36 ± 0.07
20	28.9818	L-α-Terpineol	Alcohol	10482–56–1	$C_{10}H_{18}O$	1.55 ± 0.78
22	29.7747	Naphthalene	Aromatic hydrocarbon	91–20–3	C10H8	1.07 ± 0.07
21	33.1767	Linalool	Alcohol	78–70–6	$C_{10}H_{18}O$	30.73 ± 0.62
23	33.3070	Benzonitrile, 2-methyl-	Nitrile	529–19–1	C_8H_7N	1.22 ± 0.02
24	35.9999	p-Cresol	Phenolic compound	106–44–5	C_7H_8O	0.72 ± 0.21
25	36.9999	γ-Methyl-γ-decalactone	Lactone	7011–83–8	$C_{10}H_{18}O_2$	0.39 ± 0.14
26	38.0213	Acenaphthylene	Aromatic hydrocarbon	208–96–8	$C_{12}H_8$	0.20 ± 0.07
27	38.1338	1-(6-Methyl−3-pyridinyl)ethanone	Heterocycle	42972–46–3	C_8H_9NO	0.15 ± 0.06
28	40.7712	Phenol, 4-(1,1-dimethylpropyl)-	Phenolic compound	80–46–6	$C_{11}H_{16}O$	0.68 ± 0.07
29	41.1979	2-mercaptoethanol	Thiol	60–24–2	C_2H_6OS	4.77 ± 0.21
30	41.8889	Indole, 3-methyl-	Heterocycle	83–34–1	C_9H_9N	0.21 ± 0.07
31	42.9923	Benzeneacetic acid	Carboxylic acid	103–82–2	$C_8H_8O_2$	0.23 ± 0.14
32	43.4458	Chloroxylenol	Phenolic compound	88–04–0	C_8H_9ClO	0.32 ± 0.07

Data are presented as mean ± SD of two independent HS-SPME–GC–MS analyses (n = 2).

Table 2
Effect of different concentrations of four major purified volatile organic compounds (VOCs)—acetophenone, phenylethyl alcohol, 2-mercaptoethanol, and linalool—identified from *B. aryabhattai* strain KT−21 on the mycelial growth and viability of *L. theobromae* after 3 days of incubation at 28 ± 2 °C.

Concentration (μL per sealed Petri dish)	Pure VOCs											
	Acetophenone			Phenylethyl alcohol			2-Mercaptoethanol			Linalool		
	Colony diameter (cm)	Inhibition (%)	Viability	Colony diameter (cm)	Inhibition (%)	Viability	Colony diameter (cm)	Inhibition (%)	Viability	Colony diameter (cm)	Inhibition (%)	Viability
Control	$6.40^{a} \pm 0.00$	-	-	$6.40^{a} \pm 0.00$	-	-	$6.40^{a} \pm 0.00$	-	-	$6.40^{a} \pm 0.00$	-	-
2.5	$6.40^{a} \pm 0.00$	$0.00^{d} \pm 0.00$	-	$6.40^{a} \pm 0.00$	$0.00^{b} \pm 0.00$	-	$5.38^{b} \pm 0.34$	$15.89^{d} \pm 5.32$	-	$4.67^{b} \pm 0.29$	$27.09^{c} \pm 4.51$	-
5.0	$6.40^{a} \pm 0.00$	$0.00^{d} \pm 0.00$	-	$6.23^{a} \pm 0.29$	$2.60^{b} \pm 4.51$	-	$3.28^{c} \pm 1.10$	$48.70^{c} \pm 1.21$	-	$3.38^{c} \pm 0.20$	$47.14^{b} \pm 3.16$	-
10	$1.93^{b} \pm 0.12$	$69.79^{c} \pm 1.81$	-	$5.67^{ab} \pm 0.14$	$11.46^{ab} \pm 2.25$	-	$1.65^{d} \pm 0.18$	$74.22^{b} \pm 2.82$	-	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	A
20	$1.32^{c} \pm 0.05$	$79.43^{b} \pm 5.54$	-	$5.67^{ab} \pm 0.63$	$11.46^{ab} \pm 1.83$	-	$0.00^{e} \pm 0.00$	$100^{a} \pm 0.00$	A	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	D
40	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	A	$5.42^{b} \pm 0.14$	$15.36^{b} \pm 2.26$	-	$0.00^{e} \pm 0.00$	$100^{a} \pm 0.00$	A	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	D
60	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	A	$4.93^{b} \pm 0.24$	$22.92^{a} \pm 3.69$	-	$0.00^{e} \pm 0.00$	$100^{a} \pm 0.00$	A	$0.00^{d} \pm 0.00$	$100^{a} \pm 0.00$	D

Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 3). Mycelial plugs exhibiting 100% growth inhibition were transferred to fresh PDA plates and incubated for an additional 2 days to assess viability. The symbol "–" indicates treatments where viability assessment was not applicable due to partial or no growth inhibition. Fungal viability was classified as A (regrowth, alive) and D (no regrowth, dead) according to Section 2.7.

dependent manner, with optimal performance at 10^8 CFU/mL.

3.8. Comparative efficacy of B. aryabhattai strain KT−21 VOCs and linalool against nutmeg black rot caused by L. theobromae

Significant differences in disease severity and biocontrol efficacy were observed among treatments ($p < 0.05$) (Fig. 5). The positive control exhibited severe disease symptoms, with a lesion diameter of 3.33 cm, whereas no symptoms were observed in the negative control (Fig. 5A). Treatment with strain KT−21 VOCs significantly reduced lesion development to 1.75 cm, which was comparable to linalool at 2 ×IC_{50} (1.75 cm) (Fig. 5B). Linalool exhibited a clear concentration-dependent effect. Lesion diameter decreased from 2.68 cm at IC_{50} to 1.75 cm at 2 × IC_{50}, and was further reduced to 0.55 cm and 0.28 cm at 3 ×IC_{50} and 4 ×IC_{50}, respectively. The lowest lesion diameter was observed at 4 ×IC_{50}, indicating the strongest antifungal activity.

A similar trend was observed for biocontrol efficacy. Strain KT−21 VOCs achieved 83.41% efficacy, which was comparable to linalool at 3 ×IC_{50} (83.45%), but lower than that of 4 ×IC_{50} (91.71%). In contrast, linalool at IC_{50} and 2 ×IC_{50} showed substantially reduced efficacy (19.31% and 47.55%, respectively) (Fig. 5C). These results demonstrate that strain KT−21 VOCs provide a level of disease suppression comparable to linalool at 3 ×IC_{50}, while linalool at 4 ×IC_{50} exhibited superior antifungal performance.

3.9. Effects of linalool on L. theobromae and nutmeg fruit quality

3.9.1. On L. theobromae development

The effect of linalool on *L. theobromae* infection in nutmeg fruit was assessed based on lesion diameter and biocontrol efficacy (Fig. 6). Severe symptoms were observed in the positive control, with a lesion diameter of 3.38 cm, whereas no symptoms developed in the negative control (Fig. 6A, B). Simultaneous application of linalool markedly suppressed infection, resulting in the smallest lesion diameter (0.23 cm) and the highest biocontrol efficacy (93.33%) (Fig. 6C).

When linalool was applied after pathogen inoculation, lesion development increased progressively with delayed application. Lesion diameters reached 1.03, 1.88, and 2.25 cm at 1, 2, and 3 days post-inoculation, respectively, while biocontrol efficacy decreased from 69.52% to 45.00% and 32.98%. These results indicate that linalool is most effective at the early stage of infection, and its efficacy declines as disease progresses.

3.9.2. On nutmeg fruit quality

Table 3 shows significant differences ($p < 0.05$) among treatments for all fruit quality parameters after five days of incubation. The positive control exhibited the greatest deterioration, with a higher pH (3.73) and markedly reduced total soluble solids (1.03 °Brix), titratable acidity (0.49%), total phenolic content (138.76 μg GAE/mL), and antioxidant activity (40.18%) compared to the negative control.

In contrast, linalool treatments significantly preserved fruit quality. The simultaneous application was the most effective, maintaining pH (2.91), titratable acidity (1.46%), total phenolic content (351.63 μg GAE/mL), and antioxidant activity (84.34%), which were closest to the negative control. Delayed applications (+1, +2, and +3 days) also improved all parameters compared to the positive control; however, their effectiveness gradually declined with increasing delay, particularly in total phenolic content and antioxidant activity.

3.10. Ultrastructural analysis of L. theobromae by SEM and TEM

Ultrastructural features of *L. theobromae* observed by SEM and TEM are shown in Fig. 7. SEM revealed clear morphological differences between control and treated hyphae of *L. theobromae*. In the control group (Fig. 7A–B), fungal hyphae exhibited normal morphology, characterized by smooth, cylindrical, and intact surfaces with uniform thickness and well-defined structure. No signs of deformation or surface damage were observed. In contrast, treated hyphae (Fig. 7C–D) displayed marked morphological alterations, including distortion, irregular shapes, and uneven surfaces. Pronounced shrinkage, collapse of hyphal structures, and surface wrinkling were evident. Notably, the hyphal surface exhibited the presence of pores and perforations, indicating severe structural damage, with some regions showing fragmentation.

Consistent with these surface alterations, TEM further revealed significant internal ultrastructural damage. In the control, hyphal cells maintained normal cellular organization, including an intact cell wall, closely appressed plasma membrane, dense cytoplasm, and well-preserved organelles (Fig. 7E–F). In contrast, treated hyphae exhibited progressive cellular disruption. At moderate magnification, the cytoplasm appeared disorganized and less electron-dense, accompanied by

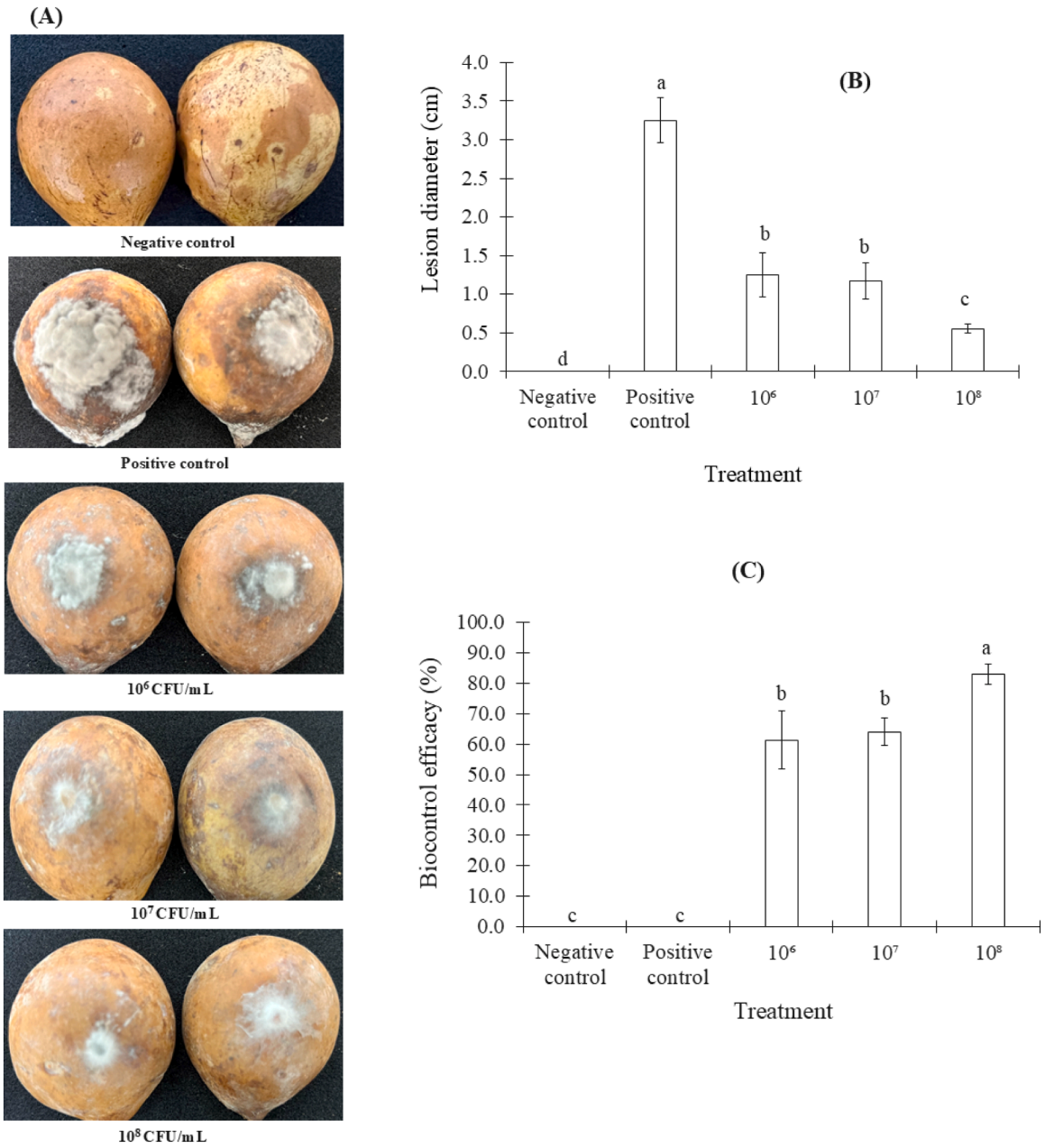

Fig. 4. Effect of *B. aryabhattai* strain KT−21 concentration (10^6–10^8 CFU/mL) on VOC-mediated suppression of *L. theobromae* development in nutmeg fruit after 5 days of storage at 28 ± 2 °C under humid conditions. **(A)** Disease symptoms, **(B)** lesion diameter, and **(C)** control efficacy. Means followed by different letters indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 18). ***Note:*** The negative control consisted of non-inoculated and untreated fruits, while the positive control consisted of fruits inoculated with the pathogen only.

abnormal vacuolation (Fig. 7G). At higher magnification, severe damage was observed, including disruption of intracellular organelles, enlargement of vacuoles, and loss of cytoplasmic integrity. In some regions, cytoplasmic contents were degraded or depleted, indicating cellular collapse (Fig. 7H). Together, these observations demonstrate that linalool treatment induces both surface deformation and internal ultrastructural damage, ultimately leading to loss of cellular integrity in fungal hyphae.

4. Discussion

Microbial VOCs have emerged as important mediators of interkingdom interactions, particularly in postharvest systems where spatial separation limits the effectiveness of diffusible metabolites. Unlike soluble antifungal compounds, VOCs can act over distances and function as biofumigants under confined conditions, offering a mechanistic advantage in suppressing fungal pathogens (Ulloa et al., 2023; Wang et al., 2024). This non-contact mode of inhibition is a key advantage of VOCs compared with conventional antifungal metabolites, as it allows suppression of pathogens without direct interaction, which is particularly suitable for postharvest systems where fumigation-like conditions can be applied (Weisskopf et al., 2021).

In addition, bacterial VOCs differ from plant-derived essential oils in their ecological origin, temporal production, and adaptive roles, as they are actively synthesized in response to microbial competition and environmental cues. In this study, *B. aryabhattai* strain KT−21 exhibited antagonistic activity against *L. theobromae*, and several bioactive VOCs were identified. Among the major VOCs detected, only linalool belongs to the terpene group. Genome annotation revealed the presence of multiple biosynthetic gene clusters; however, specific genes or pathways

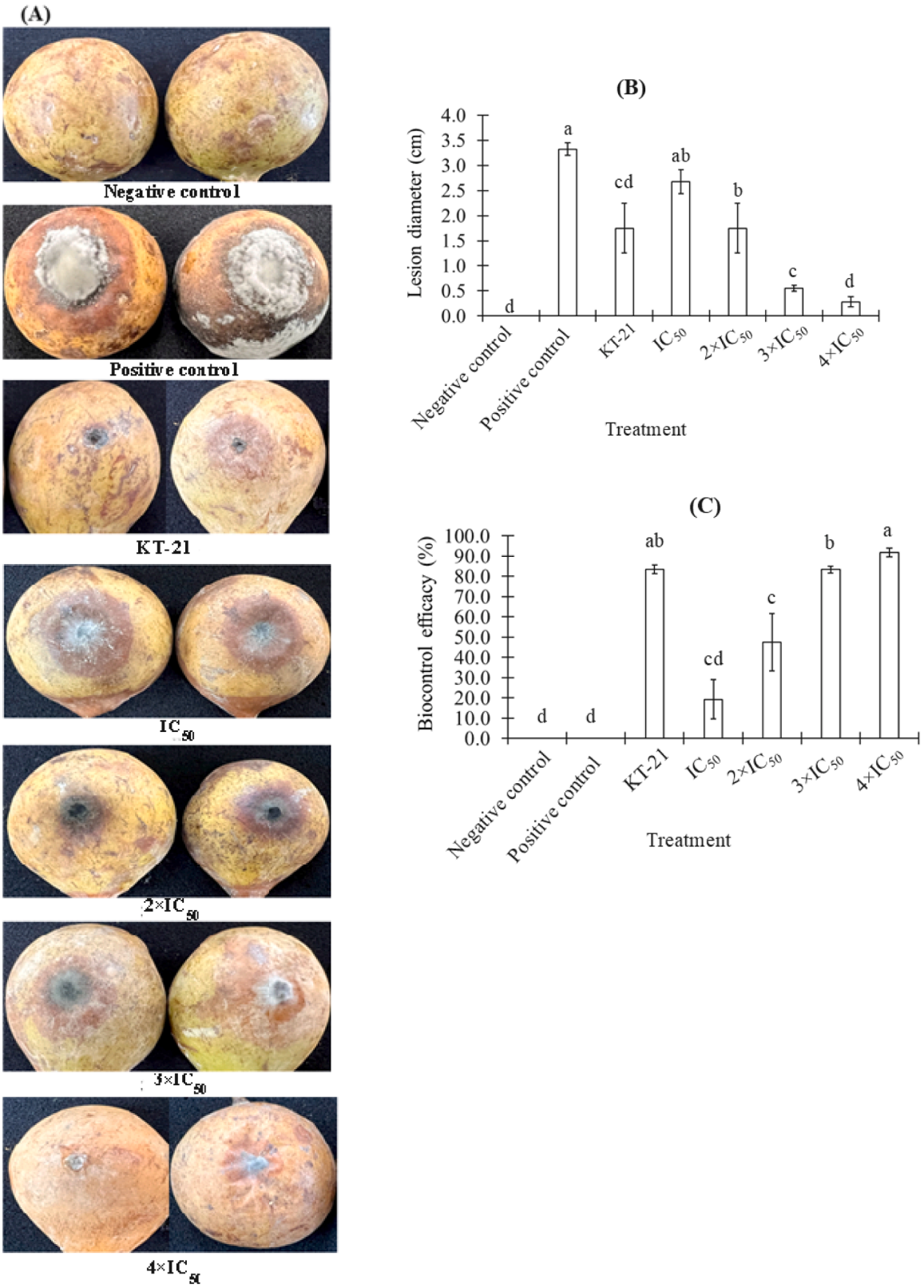

Fig. 5. Comparative effects of VOCs produced by *B. aryabhattai* strain KT−21 and linalool treatments (at IC_{50}, 2 ×IC_{50}, 3 ×IC_{50}, and 4 ×IC_{50}) against *L. theobromae* development in nutmeg fruit after 5 days of storage at 28 ± 2 °C under humid conditions. **(A)** Disease symptoms, **(B)** lesion diameter, and **(C)** control efficacy. Means followed by different letters indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 18). ***Note:*** The negative control consisted of non-inoculated and untreated fruits, while the positive control consisted of fruits inoculated with the pathogen only.

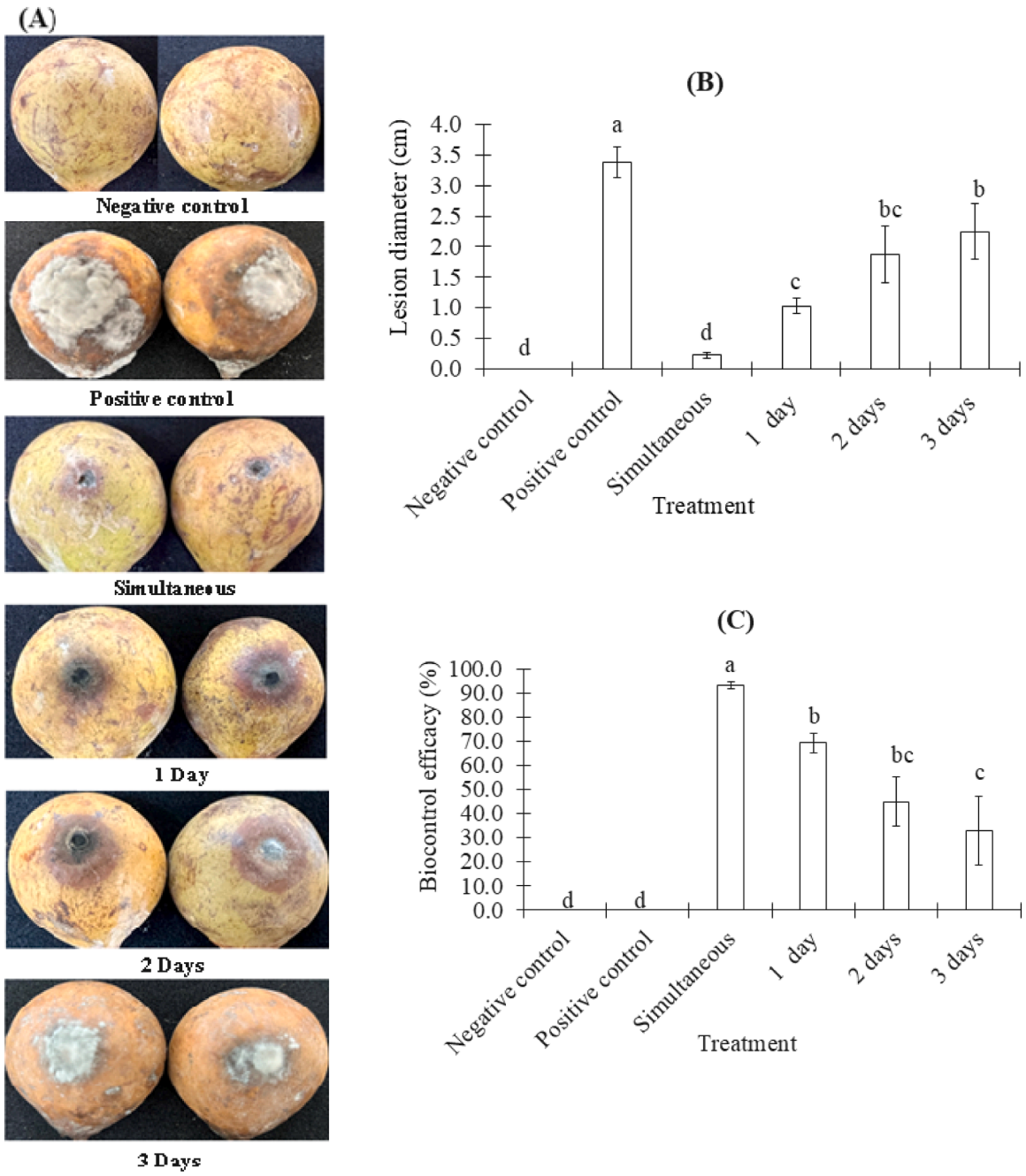

Fig. 6. Effect of linalool application timing on *L. theobromae* development in nutmeg fruit. Fruits were treated with 4 $\times IC_{50}$ of linalool either simultaneously with pathogen inoculation or at 1, 2, or 3 days after inoculation. Disease development was assessed after 5 days of storage at 28 ± 2 °C under humid conditions. **(A)** Disease symptoms, **(B)** lesion diameter, and **(C)** control efficacy. Means followed by different letters indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 18). ***Note:*** The negative control consisted of non-inoculated and untreated fruits, while the positive control consisted of fruits inoculated with the pathogen only. Linalool treatments were applied at 0 (simultaneous), 1, 2, and 3 days after inoculation.

responsible for the biosynthesis of the identified VOCs were not determined in this study, and the relationship between predicted gene clusters and VOC production remains speculative. The antifungal effects were further supported by additional assays exploring potential modes of action. The efficacy of VOCs, particularly linalool, was evaluated in nutmeg fruit, where disease suppression and improved postharvest quality were observed, indicating their potential for postharvest disease management.

Strain KT−21 demonstrated broad-spectrum inhibition across taxonomically diverse fungi, suggesting that its VOCs may affect conserved cellular processes essential for fungal survival. This broad-spectrum activity is consistent with previous reports indicating that *Bacillus* spp. are among the most widely used biocontrol agents due to their ability to produce diverse secondary metabolites and rapidly colonize ecological niches, particularly fruit wound sites where infection is initiated (Droby et al., 2016). Consistent with previous reports, VOCs from *P. fluorescens* disrupted membrane integrity and respiration in *Botrytis cinerea* (Yue et al., 2023), while VOCs from *P. chlororaphis* impaired redox balance and energy metabolism (Wang et al., 2025), reducing the likelihood of resistance development compared with single-target fungicides.

A major finding of this study is the predominance of linalool within the VOC profile of strain KT−21. Traditionally considered a plant

Table 3
Effects of linalool on the quality of nutmeg fruit inoculated with *L. theobromae* after 5 days of incubation at 28 ± 2 °C under humid conditions.

Parameters	Treatment					
	Negative control	Positive control	Simultaneous	1 Day	2 Days	3 Days
pH	$2.88^{b} \pm 0.012$	$3.73^{a} \pm 0.00$	$2.91^{b} \pm 0.01$	$2.93^{b} \pm 0.01$	$2.91^{b} \pm 0.01$	$2.90^{b} \pm 0.01$
Total soluble solids (°Brix)	$4.22^{a} \pm 0.02$	$1.03^{b} \pm 0.05$	$1.00^{b} \pm 0.00$	$1.00^{b} \pm 0.00$	$1.03^{b} \pm 0.05$	$1.03^{b} \pm 0.05$
Titratable acidity (% citric acid)	$2.07^{a} \pm 0.02$	$0.49^{e} \pm 0.02$	$1.46^{b} \pm 0.02$	$1.18^{c} \pm 0.03$	$1.14^{cd} \pm 0.02$	$1.10^{d} \pm 0.02$
Total phenol content (μg GAE/mL)	$1137^{a} \pm 46.97$	$138.76^{d} \pm 2.43$	$351.63^{b} \pm 15.47$	$230.72^{c} \pm 2.25$	$246.70^{c} \pm 14.07$	$220.77^{c} \pm 10.70$
% Radical scavenging activity	$90.03^{a} \pm 0.05$	$40.18^{e} \pm 0.73$	$84.34^{b} \pm 1.14$	$62.58^{c} \pm 1.86$	$48.34^{d} \pm 0.77$	$47.60^{d} \pm 1.23$

Note: Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$). Data are presented as mean ± SD (n = 18). The negative control consisted of non-inoculated and untreated fruits, while the positive control consisted of fruits inoculated with the pathogen only. Linalool treatments were applied at 0 (simultaneous), 1, 2, and 3 days after inoculation.

monoterpene, linalool has been increasingly identified as a microbial metabolite with potential biocontrol functions. The detection of multiple chemical classes of VOCs in strain KT−21, including alcohols, ketones, sulfur-containing compounds, and heterocycles, is consistent with previous studies showing that *Bacillus*-derived VOCs comprise diverse groups with distinct biological activities (Li et al., 2015; Grahovac et al., 2023). For instance, VOCs from *S. philanthi* RM−1–138 contained linalool as the most abundant compound, effectively inhibiting the growth and sclerotia formation of *Sclerotium rolfsii* (Boukaew et al., 2025). Similarly, co-cultures of *Burkholderia vietnamiensis* and *Trichoderma harzianum* released linalool among key volatiles (Li et al., 2024b). Mechanistically, terpene-derived VOCs such as linalool exert antifungal activity primarily through membrane-targeting effects.

It should be noted that the concentrations of individual synthetic VOCs tested in this study represent controlled bioassay exposure levels rather than their relative abundance in the natural VOC profile of strain KT−21. Therefore, the observed inhibitory effects reflect intrinsic compound activity under experimental conditions and should not be directly interpreted as proportional contributions to the *in situ* antifungal activity of strain KT−21 VOCs.

These findings are strongly supported by previous ultrastructural and physiological studies showing that VOCs from *Bacillus* spp. can disrupt cell wall integrity, increase membrane permeability, and cause leakage of intracellular contents, ultimately leading to fungal cell collapse (Ling et al., 2022; Grahovac et al., 2023). Recent studies have also demonstrated that VOCs can reduce ergosterol content and interfere with mitochondrial energy metabolism (Zhao et al., 2022; Yue et al., 2023). In addition, VOC-induced oxidative stress has been reported as a critical mechanism, where accumulation of reactive oxygen species (ROS) damages proteins, lipids, and nucleic acids, contributing to fungal cell death (Zhao et al., 2022). The ultrastructural damage observed in this study aligns with these mechanisms.

Although linalool plays a dominant role, the antifungal activity of strain KT−21 is likely multicomponent rather than strictly synergistic. However, the current study does not provide direct experimental evidence of synergistic interactions among VOCs, and therefore such interactions remain speculative. Such multifunctional behavior is a characteristic feature of *Bacillus* spp., which can combine competition for nutrients and space, biofilm formation, and production of antimicrobial metabolites to suppress pathogens effectively (Fira et al., 2018; Caulier et al., 2019). Moreover, the marked increase in bacterial population in the presence of fungal biomass suggests a potential capacity to utilize fungal-derived nutrients; however, this represents only indirect evidence and does not confirm mycolytic activity, which has been reported as an adaptive strategy in nutrient-limited environments (Wang and Kuzyakov, 2024; AboDalam et al., 2026).

From an applied perspective, VOCs produced by strain KT−21 reduced disease development on nutmeg fruit. This is consistent with previous studies demonstrating that VOC fumigation can effectively control postharvest diseases without direct contact, making it a promising alternative to synthetic fungicides (Ling et al., 2022). Evidence from plant-derived linalool shows similar protective effects (Shen et al., 2022; Xu et al., 2026). In addition, VOCs from *Bacillus* have been reported to enhance fruit resistance by increasing antioxidant enzyme activities (e.g., CAT, POD, SOD) and promoting accumulation of phenolic compounds, which contribute to delayed senescence and improved fruit quality (Ling et al., 2023). It should be noted that linalool itself possesses antioxidant properties, which may partially contribute to the observed DPPH radical scavenging activity and should be considered when interpreting the results. However, the concurrent preservation of total phenolic content suggests that the observed effects are not solely due to the direct antioxidant activity of linalool, but also involve maintenance of fruit physiological status.

At lower inoculum levels (10^{6}–10^{7} CFU/mL), strain KT−21 VOCs showed no significant difference in lesion reduction, indicating a plateau in biocontrol efficacy. This suggests that the antagonistic activity of VOCs reaches a saturation threshold, beyond which further increases in bacterial density do not proportionally enhance disease suppression. Such a response may be attributed to limitations in volatile diffusion and effective concentration within the sealed system, as well as spatial and ecological constraints commonly observed in microbial biocontrol interactions. Therefore, 10^{6} CFU/mL may already be sufficient to achieve effective disease suppression under practical postharvest conditions, whereas higher inoculum (10^{8} CFU/mL) is required to obtain maximal efficacy.

Finally, VOC production is known to be influenced by multiple factors, including nutrient composition, environmental conditions, and microbial interactions (Weisskopf et al., 2021). Temperature is also an important factor influencing VOC production and composition, which may affect biocontrol efficacy under different experimental conditions. In this study, different incubation temperatures were applied *in vitro* and *in vivo*, which should be considered when interpreting the results. Therefore, optimizing culture conditions and understanding regulatory pathways will be essential for improving the consistency and efficacy of strain KT−21 VOCs in practical applications. Further validation under commercial storage conditions and evaluation of sensory impacts remain necessary.

5. Conclusion

In this study, *B. aryabhattai* strain KT-21 produced a linalool-dominant VOC profile that suppressed the growth of multiple fungal pathogens. The strain exhibited a combination of volatile-mediated inhibition, direct antagonism, and mycolytic activity, indicating a multimechanistic mode of action. Genomic analysis confirmed terpene biosynthetic gene clusters, linking observed VOC production to metabolic potential. *In vivo* assays on nutmeg fruit showed that strain KT-21 VOCs and linalool reduced disease severity while maintaining key fruit quality attributes, including phenolic content and antioxidant activity. These results provide initial evidence that strain KT-21 can produce a linalool-dominant and synergistic VOC blend, highlighting a microbial source of bioactive terpenes for postharvest disease management. Future

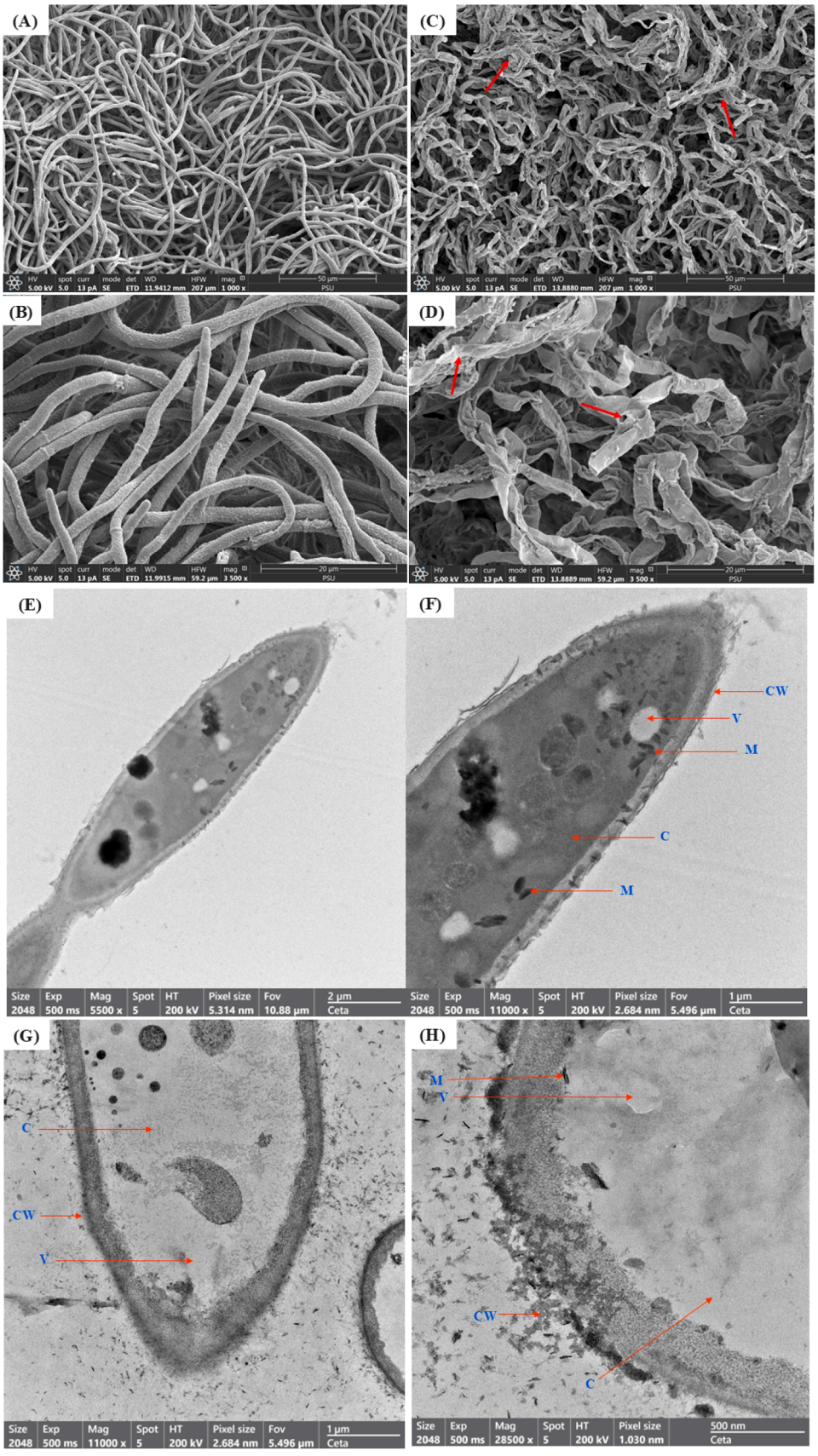

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Fig. 7. Ultrastructural alterations in *L. theobromae* induced by linalool, as revealed by SEM and TEM analyses. SEM images show normal, smooth, and intact hyphal morphology in the control (**A, B** at 1000 × and 3500 ×), whereas linalool-treated hyphae (**C, D**; 1000 × and 3500 ×) exhibit surface deformation, shrinkage, and structural collapse. TEM observations further confirmed these effects, with control hyphae (**E, F**; 5500 × and 11000 ×) displaying well-preserved ultrastructure, including intact cell walls, dense cytoplasm, and organized intracellular components. In contrast, treated hyphae (**G, H**; 11000 × and 22500–28500 ×) show severe cellular damage, characterized by cytoplasmic disorganization, abnormal vacuolation, disruption of organelles, and cell wall degradation. CW, cell wall; C, cytoplasm; V, vacuole; M, mitochondria.

studies should address VOC optimization, performance under commercial storage, and interactions with native microbial communities to support practical application of strain KT-21 as a biocontrol agent.

CRediT authorship contribution statement

Sirasit Srinuanpan: Writing – review & editing. **Zhiwei Zhang:** Writing – review & editing. **Julalak Chuprom:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Sawai Boukaew:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kanokphorn Sangkharak:** Writing – review & editing. **Jackrit Anantasaran:** Writing – review & editing, Formal analysis. **Wanida Petlamul:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Benjamas Cheirsilp:** Writing – review & editing, Funding acquisition. **Norathep Sakphet:** Writing – review & editing, Formal analysis.

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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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2026.114544.

Data availability

Data will be made available on request.

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