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Postharvest biocontrol ability and involved mechanism of volatile organic compounds from *Serratia nematodiphila* BC-SKRU-1 against *Penicillium digitatum* tangerine fruit

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ABSTRACT

Volatile organic compounds (VOCs) emitted by microorganisms offer a promising strategy for managing green mold disease in citrus fruits caused by *Penicillium digitatum*. This study evaluated the biocontrol efficacy of VOCs produced by *Serratia nematodiphila* BC-SKRU-1 (BC-SKRU-1 VOCs) against green mold in postharvest tangerine fruits, targeting *P. digitatum* NKP4321. Our findings demonstrated that BC-SKRU-1 VOCs exhibited a broad spectrum of activity against seven plant pathogens, with the highest efficacy observed against NKP4321 (68.21%). A concentration of 10^6 CFU mL^{-1} for VOC production by BC-SKRU-1 completely inhibited the mycelial growth of NKP4321, whereas a concentration of 10^7 CFU mL^{-1} was required to fully inhibit spore germination. The application of four plates of BC-SKRU-1 VOCs at 10^8 CFU mL^{-1} significantly reduced the severity of green mold disease, with optimal biocontrol achieved through continuous fumigation during storage. Notably, 2-nonanol, identified as the predominant component among the 20 VOCs using HS-SPME/GC-MS, exhibited superior antifungal activity both *in vitro* and *in vivo*. It effectively decreased ergosterol production and disrupted plasma membrane integrity in NKP4321 cells. Furthermore, 2-nonanol altered the cellular content of non-enzymatic antioxidants (e.g., GSH, GSSG) and enzymatic antioxidants (e.g., CAT, SOD), elucidating its comprehensive antifungal mechanism. These findings highlight the potential of BC-SKRU-1 VOCs, particularly 2-nonanol, as effective biocontrol agents for enhancing postharvest fruit preservation by combating fungal pathogens.

1. Introduction

Citrus (*Citrus* spp.) is rich in vitamins, minerals, and dietary fiber, promoting human health (Liu et al., 2022; Lu et al., 2023) and widely cultivated in over 100 countries (Agustí et al., 2014; Smilanick et al., 2008). Its main postharvest disease is caused by *Penicillium digitatum* Sacc (Ferreira et al., 2020) and accounts for about 90% of postharvest losses (Cheng et al., 2020; Li et al., 2024). Controlling this green mold in citrus traditionally relies on synthetic fungicides like prochloraz, thiabendazole, pyrimethanil, imazalil, and fludioxonil (Hao et al., 2010). While effective, these chemicals raise concerns due to their environmental and health impacts (Bhatta, 2022; Fadda et al., 2021; Sánchez-Torres and Gandía, 2022). The adverse effects and the development of pathogen resistance have spurred the search for safe and eco-friendly alternatives (Koch et al., 2018; Patle et al., 2018; Zhong et al., 2021). Persistent fungicide use has also led to resistance, highlighting the need for alternative strategies (Oiki et al., 2022; Zhong et al., 2021).

The use of microbial volatile organic compounds (VOCs) for biological control has emerged as a highly effective, safe, and environmentally friendly strategy for managing various postharvest diseases (Boukaew et al., 2013, 2018, 2021, 2024; Ferreira et al., 2023; Khadiri et al., 2023; Tian et al., 2024). These VOCs, small signaling molecules produced by microorganisms, act as crucial bioactive compounds with

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notable efficacy against plant pathogens (Almeida et al., 2023; Guevara-Avendaño et al., 2019). Numerous microbial VOCs, including those produced by *Bacillus* spp. (Ferreira et al., 2023; Khadiri et al., 2023; Li et al., 2024; Liu et al., 2023), *Streptomyces* spp. (Tian et al., 2024), *Pseudomonas* spp. (Agisha et al., 2019; Ferreira et al., 2023; Qessaoui et al., 2022; Wang, Zhou, et al., 2021), *Muscodor* spp. (Saxena & Strobel, 2021; Suwannarach et al., 2016), *Ceratocystis* spp. (Li et al., 2015), yeasts such as *Wickerhamomyces* spp., *Metschnikowia* spp., *Aureobasidium* spp., and *Saccharomyces* spp. (Parafati et al., 2017), *Nodulisporium* spp. (Yeh et al., 2021), *Clavispora* spp. (Pereyra et al., 2022), and *Trichoderma* spp. (Boukaew et al., 2024; Ferreira et al., 2020; Jampílek & Kráľová, 2024), have been recognized for their effective control of postharvest diseases in citrus fruit caused by *P. digitatum* under controlled conditions. However, the VOCs of *Serratia* against *P. digitatum* have not been investigated.

Serratia, a genus classified within the Enterobacteriaceae family, encompasses 18 distinct species, showcasing a wide range of biological and ecological diversity, from beneficial to pathogenic (Lee et al., 2023) or opportunistic human pathogenic species (Grimont & Grimont, 2009). *S. nematodiphila* stands out as a valuable asset, serving not only as a biocontrol agent but also as a bio-stimulant and bio-fertilizer (Firdu et al., 2022; Gondil et al., 2017; Khoa et al., 2016; Soenens & Imperial, 2020). Some *Serratia* species, including *S. plymuthica* (Schmidt et al., 2017), *S. ureilytica*, and *S. bockelmannii* (Abreo et al., 2021), have been reported to produce VOCs with effects on plant pathogens. For instance, *S. plymuthica* PRI-2C produces sodorifen when exposed to VOCs released by the fungus *Fusarium culmorum* (Schmidt et al., 2017). Additionally, dimethyl disulfide is a VOC secreted by *Serratia*, with both *S. ureilytica* and *S. bockelmannii* capable of synthesizing it *in vitro* (Abreo et al., 2021). The presence of bacterial and exogenous dimethyl disulfide inhibits the growth of *Pythium cryptoirregulare*, reducing the incidence of damping-off in tomato seedlings caused by *P. cryptoirregulare* when treated with *S. ureilytica* (Abreo et al., 2021). Notably, the application of *Serratia* in the agricultural field is facilitated by the lack of officially published documentation regarding its pathogenic potential to humans and its environmental impact.

This study aims to investigate the impact of VOCs produced by *S. nematodiphila* BC-SKRU-1 on the control of *P. digitatum* in citrus fruits. The potential of VOCs from BC-SKRU-1 as biocontrol agents for treating gray mold and conduct relevant biological control research is explored. The objectives of this study are to (i) assess the biocontrol capacity of BC-SKRU-1 VOCs against *P. digitatum* both *in vitro* and *in vivo*, (ii) identify the VOCs using headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS), and (iii) investigate the mechanisms of inhibition by the main VOCs from BC-SKRU-1 against *P. digitatum* cells.

2. Material and methods

2.1. Sources of materials

2.1.1. Microorganisms and inoculum preparation

The antagonistic strain BC-SKRU-1 was identified as *Serratia nematodiphila* (Yossan, 2024). It was cultured on nutrient agar (NA) at 30 °C for 24 h. For a bacterial suspension, the strain was inoculated into 50 mL of nutrient broth (NB) and incubated at 30 °C for 24 h. The suspension was then adjusted with sterilized water to a final concentration of 10^5–10^8 CFU mL^{-1} using the McFarland standard.

The green mold pathogen, *Penicillium digitatum* NKP4321, was sourced from the Center of Excellence in Microbial Diversity and Sustainable Utilization at Chiang Mai University. *Rhizoctonia solani* (AG-1 IA) came from the Phatthalung Rice Research Center, while the anthracnose pathogen *Colletotrichum gloeosporioides* PSU-03, was isolated from infected chili fruits (Boukaew et al., 2021). Aflatoxin-producing *Aspergillus parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 were sourced from Prince of Songkla University and the Phitsanulok Seed Research and Development Center, respectively. *Curvularia oryzae* and *Phomopsis* sp., causing severe diseases in oil palms and durians, were obtained from the Suratthani Oil Palm Research Center and Assistant Professor Dr. Maneerat Koohapitagtam, respectively. All strains were cultured on PDA at 30 °C for 3–7 days.

Mycelial plugs of *R. solani*, *Phomopsis* sp., *C. oryzae*, and *C. gloeosporioides* PSU-03 were grown on PDA at 30 °C for three to seven days in order to conduct antifungal suppression experiments. Simultaneously, spore suspensions of *P. digitatum* NKP4321, *A. parasiticus* TISTR 3276, and *A. flavus* PSRDC-4 were cultivated on PDA at 30 °C for 5 days. After this period, spores from NKP4321, TISTR 3276, and PSRDC-4 were harvested by scraping them off the agar and suspending them in distilled water. The spore concentrations were then adjusted using a hemocytometer to achieve the required levels for subsequent experiments.

2.1.2. Tangerine fruits and fruit preparation

Tangerine fruits (*Citrus tangerina* Tanaka cv. Sai Num Phung) were purchased from a supermarket in Hat Yai, Songkhla Province, Thailand. The fruits were selected based on their uniform size and absence of damage. They were cleaned with tap water and then surface-disinfected for 3 min using commercial sodium hypochlorite at a 2% (v/v) concentration. Afterward, the tangerines were rinsed with sterile distilled water and dried. To prepare for infection experiments, each fruit was punctured twice with a sterile needle (3 mm deep × 5 mm diameter), and the tissue around the wounds was removed.

2.2. Suppressive activities of VOCs produced by S. nematodiphila BC-SKRU-1 against seven plant pathogenic fungi

The production of VOCs by strain BC-SKRU-1, referred to as BC-SKRU-1 VOCs, involved the inoculation of a NA plate (90 mm dimeter) with 100 μL of a bacterial suspension (10^7 CFU mL^{-1}) of strain BC-SKRU-1, which was then evenly spread. This culture was incubated for 24 h at 30 °C (Ling et al., 2022). Following incubation, the volatile activity of the BC-SKRU-1 culture was assessed.

The dual-culture sealed plate technique (Calvo et al., 2020) (Fig. 1) was used to assess the antifungal activity of BC-SKRU-1 VOCs against the fungal pathogens described in section 2.1.1. For *R. solani*, *Phomopsis* sp., *C. oryzae*, and *C. gloeosporioides* PSU-03, a 5-mm-diameter agar plug was positioned at the center of the PDA dishes (Petri plate; 90 mm diameter). For NKP4321, TISTR 3276, and PSRDC-4, a 5 μL spore suspension (10^5 spores mL^{-1}) was inoculated at the center of PDA dishes (Petri plate; 90 mm diameter) using an autopipette. Simultaneously, NA plates containing strain BC-SKRU-1 VOCs were prepared, and the lids of these plates were replaced with base plates containing each pathogen. The control groups contained only the NA medium. Both sets of base plates were then sealed with Parafilm and incubated at 30 °C for 3–7 days. After the incubation period, the mycelial growth of each fungus was measured. The percentage inhibition of pathogenic fungi by BC-SKRU-1 was calculated using the formula from Parizi et al. (2012): Percentage inhibition of growth = ((Control - Treatment)/Control) × 100. Each experiment included three replicates and was repeated twice.

2.3. Effect of different inoculation concentrations of S. nematodiphila BC-SKRU-1 for production of VOCs against P. digitatum NKP4321

We investigated the inhibitory effects of BC-SKRU-1 at concentrations from 10^5 to 10^8 CFU mL^{-1} on mycelial growth and spore germination of NKP4321 (Francesco et al., 2020) using the dual-culture sealed plate technique (Calvo et al., 2020) (Fig. 1). For mycelial inhibition, a 5 μL spore suspension (10^5 spores mL^{-1}) of NKP4321 was centrally inoculated on PDA dishes. Similarly, for spore germination, 100 μL of spore suspension (10^4 spores mL^{-1}) was spread on the dishes. In both setups, NA plates (90 mm dimeter) with BC-SKRU-1 VOCs were prepared with their lids replaced by base plates seeded with NKP4321. The control groups contained only the NA medium. After sealing with

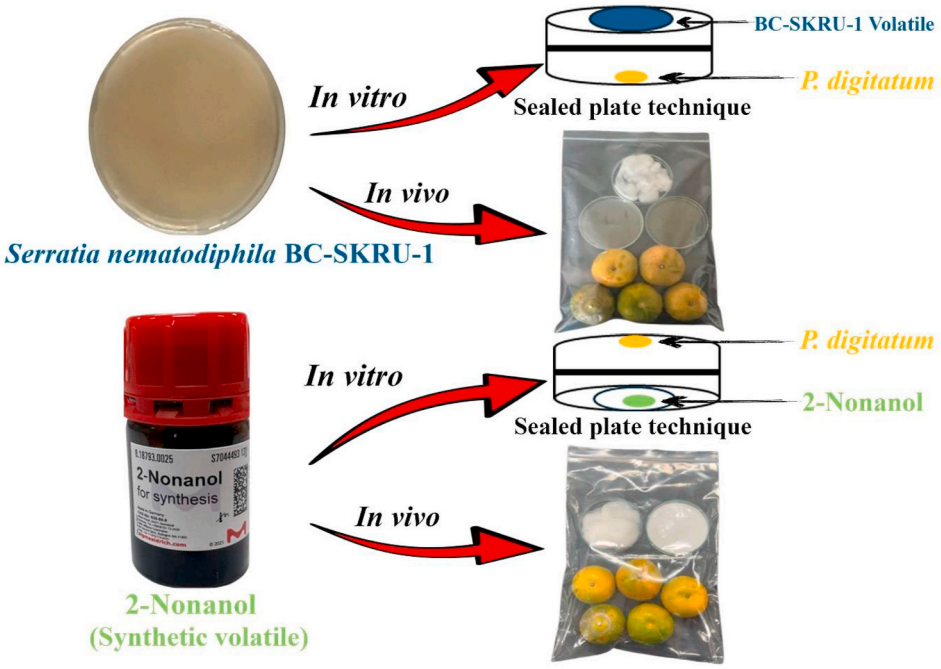


Fig. 1. Experimental setup for both *in vitro* and *in vivo* assays to evaluate the antifungal effects of emitted or synthetic 2-nonanol from *Serratia nematodiphila* BC-SKRU-1 on the growth and development of *Penicillium digitatum* NKP4321 in tangerine fruit.

Parafilm and incubating at 30 °C, mycelial growth was assessed after 7 days and spore germination after 48 h. Growth inhibition and germination rates were measured, demonstrating the antifungal efficacy of BC-SKRU-1 VOCs. Each experiment included three replicates and was repeated twice.

2.4. *In vivo biocontrol of P. digitatum NKP4321 in tangerine fruit by S. nematodiphila BC-SKRU-1 VOCs*

2.4.1. *Effect of inoculation concentration of BC-SKRU-1*

To assess the antifungal effects of BC-SKRU-1 VOCs on NKP4321 in tangerines, we modified the method of Archana et al. (2021) (Fig. 1). Each of five tangerines received two wounds, inoculated with 20 μL of a 10^5 spores mL^{-1} NKP4321 suspension and allowed to dry for 30 min (Calvo et al., 2020). The fruits were then enclosed in 1 L zip-lock bags (size: 30 × 45 cm). BC-SKRU-1 VOCs were produced at concentrations from 10^5 to 10^8 CFU mL^{-1} on NA medium in Petri dishes (90 mm dimeter) with perforated lids to release the VOCs. Control fruits were exposed only to NA medium. All treatments were stored at 30 °C and 90–95% relative humidity (Parafati et al., 2017; Perez et al., 2017; Zhang et al., 2021) for 12 days. Disease severity (DS) was measured by the average lesion diameter, and biocontrol efficacy was calculated as: [(DS of control - DS of treated)/DS of control] × 100%. This procedure was replicated three times with each replicate including five fruits (Tian et al., 2020).

2.4.2. *Effect of plate numbers of BC-SKRU-1*

The biological control efficacy of BC-SKRU-1 VOCs was tested using varying numbers of NA medium plates (90 mm dimeter), each containing 10^7 CFU mL^{-1} of BC-SKRU-1, against NKP4321 in tangerine fruits, following the method in Archana et al. (2021) (Fig. 1). Each of five fruits received two wounds inoculated with 20 μL of a 10^5 spores mL^{-1} NKP4321 suspension, then dried for 30 min (Calvo et al., 2020). Fruits were placed in 1 L zip-lock bags (size: 30 × 45 cm) with 1–4 BC-SKRU-1 VOC plates. The control fruits were exposed only to NA medium. All treatments were stored at 30 °C and 90–95% relative humidity (Parafati et al., 2017; Perez et al., 2017; Zhang et al., 2021) for 12 days. Disease severity was measured by the average lesion diameter, and biocontrol efficacy was calculated as described above. This procedure was replicated three times, with each replicate including five fruits (Tian et al., 2020).

2.4.3. *Effect of different fumigation periods of BC-SKRU-1 VOCs*

The biocontrol effects of BC-SKRU-1 VOCs for varying fumigation periods were tested against NKP4321 in tangerine fruits using the method adapted from Archana et al. (2021). Fruits received two wounds each, inoculated with 20 μL of 10^5 spores mL^{-1} of NKP4321, and dried for 30 min (Calvo et al., 2020). These were then sealed in 1 L zip-lock bags (30 × 45 cm) containing four BC-SKRU-1 VOC plates (90 mm diameter), exposed for 6 h, 12 h, 24 h, and throughout 12 days of storage. Post-treatment, BC-SKRU-1 plates were removed. The control fruits were exposed only to NA medium. All treatments were incubated at 30 °C and 90–95% humidity (Parafati et al., 2017; Perez et al., 2017; Zhang et al., 2021) for 12 days. Disease severity was measured by the average lesion diameter, and biocontrol efficacy was calculated as described above. This procedure was replicated three times, with each replicate including five fruits (Tian et al., 2020).

2.5. *Identification of S. nematodiphila BC-SKRU-1 VOCS*

To quantify the VOCs of BC-SKRU-1, we adapted the method outlined by Ling et al. (2022) with minor adjustments. Specifically, a 10 μL bacterial suspension of BC-SKRU-1 at a concentration of 10^7 CFU mL^{-1} was inoculated into the headspace above a 3 mL NA culture in a 5 mL glass flask and incubated at 30 °C for 24 h. Subsequently, the volatiles produced in the NA headspace by strain BC-SKRU-1 were captured and quantified using the purge and trap method, as described by Ling et al. (2022). Negative controls included sterile NA headspace samples only. The VOCs were collected using headspace solid-phase microextraction (HS-SPME), with gas chromatography-mass spectrometry (GC-MS) procedures and mass spectra analysis following the protocols detailed by Boukaew et al. (2024).

2.6. *In vitro evaluation of 2-nonanol on P. digitatum NKP4321*

The inhibitory effects of 2-nonanol, a VOC from *S. nematodiphila* BC-

SKRU-1, were tested against mycelial growth and spore germination of NKP4321 on PDA medium using the dual-culture sealed plate technique (Wang et al., 2022) (Fig. 1). For the mycelial growth inhibition, pure standard 2-nonanol (Sigma–Aldrich) was applied in varying amounts (0, 12.5, 25, 50, and 100 μL) onto filter paper in petri dishes. Each dish was inoculated with 10 μL of a pathogen spore suspension (10^5 spores mL^{-1}), sealed, and incubated at 30 °C for 7 days. In the control group, distilled water replaced 2-nonanol. Colony diameters were measured for mycelial growth inhibition. Each experiment included three replicates and was repeated twice.

For the spore germination inhibition, 2-nonanol's effect on NKP4321 spores was assessed similarly. However, 100 μL of spore suspension (10^4 spores mL^{-1}) was spread on PDA dishes. After 48 h of incubation, spore germination units were counted. Each experiment included three replicates and was repeated twice.

2.7. Comparing S. nematodiphila BC-SKRU-1 VOCs with 2-nonanol for green mold biocontrol in tangerine fruit

To assess the biocontrol efficacy of BC-SKRU-1 VOCs and 2-nonanol against NKP4321 infection in tangerine fruits, we followed a methodology akin to Archana et al. (2021), as detailed in section 2.4.1. Each of the five fruits received two wounds and was inoculated with NKP4321 spore suspension (20 μL of 10^5 spores mL^{-1}), followed by air-drying for 30 min (Calvo et al., 2020). The fruits were then enclosed in 1 L zip-lock bags (size: 30 × 45 cm). Four Petri dishes (90 mm diameter) containing BC-SKRU-1 VOCs and 2-nonanol at concentrations of 6.25, 12.5, 25, and 50 μL L^{-1} were prepared, alongside control dishes containing only NA medium or distilled water. All treatments were incubated at 30 °C and 90–95% humidity (Parafati et al., 2017; Perez et al., 2017; Zhang et al., 2021) for 12 days. Disease severity was measured by the average lesion diameter, and biocontrol efficacy was calculated as described above. This procedure was replicated three times, with each replicate comprising five fruits (Tian et al., 2020).

2.8. Mechanism action of 2-nonanol against P. digitatum NKP4321

The antifungal mechanism of 2-nonanol against NKP432 was studied using 20-mL test tubes filled to a final volume of 10 mL. Various concentrations of 2-nonanol (0.781, 1.563, 3.125, 6.25, 12.50, and 25 μL mL^{-1}) were introduced into the PDB medium, while the control group received no 2-nonanol. Each tube was then inoculated with a 100 μL spore inoculum containing 10^5 spores mL^{-1} of NKP432. The inoculation tubes were placed on a rotating shaker set at 150 rpm and cultured for 7 days at 30 °C. Following incubation, the culture broth was divided into two batches: one was subjected to an analysis using the approach described by Li et al. (2011) to evaluate the mycelial mats of NKP4321; the other batch was utilized to investigate the mechanism of antifungal action.

2.8.1. Determination of ergosterol content in P. digitatum NKP4321 cells treated with 2-nonanol

The quantification method described by Das et al. (2020) was used to determine the ergosterol concentration in NKP4321 cells treated with 2-nonanol. Additional procedural details were provided by Tian et al. (2012). Fungal sample preparation for ergosterol analysis followed the technique outlined by Boukaew et al. (2023). Each experiment included three replicates and was repeated twice.

2.8.2. Effects of 2-nonanol on oxidative impairment in P. digitatum NKP4321 cells

The effects of 2-nonanol on oxidative damage in NKP4321 cells were studied by preparing fungal biomass and enzyme extracts after exposure to concentrations of 1/2 MIC and MIC. Cellular reactive oxygen species (ROS) were quantified using the method described by Keston and Brandt (1965). CAT activity was measured following the protocol by Beers and Sizer (1952), and SOD levels were determined using the procedures established by Lowry et al. (1951) and Kostyuk and Potapovich (1989). The analysis of glutathione in its reduced (GSH) and oxidized (GSSG) forms, as well as the GSH/GSSG ratio, was conducted according to the methodology outlined by Hissin and Hilf (1976). All assays were carried out using the detailed procedures from Das et al. (2020). Each experiment included three replicates and was repeated twice.

2.8.3. Measurement of electrical conductivity

The methodology described by Lewis and Papavizas (1987) was modified to measure cellular leakage. To achieve a final concentration of 10^5 spores mL^{-1}, spores of NKP432 were first injected into 100 mL of PDB. The culture was then incubated for 3 day at 30 °C on a rotary shaker set to 150 rpm. Following harvesting, 2.5 g (wet weight) of the mycelia were suspended in 25 mL of sterile distilled water with 2-nonanol at concentrations of 1/4 MIC, 1/2 MIC, and MIC. The mycelia were then thoroughly rinsed with sterile distilled water. Following the protocol developed by Liu et al. (2017), these suspensions were incubated on a rotary shaker at 150 rpm and 30 °C for intervals of 0, 2, 4, 6, and 8 h. The mycelia were filtered using sterile cheesecloth after incubation. Following the necessary adjustments, the filtrates were used to quantify electrolyte loss using a conductivity meter (EC 700, APENA), according to the procedure described by Lee et al. (1998). Each experiment included three replicates and was repeated twice.

2.9. Statistical analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp). When significant differences were observed, mean separations were conducted using Tukey's test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Suppressive activities of VOCs produced by S. nematodiphila BC-SKRU-1 against seven plant pathogenic fungi

To evaluate the antifungal impact of metabolic VOCs on mycelial growth, a dual-culture sealed plate technique system was established to prevent any direct contact between BC-SKRU-1 and seven pathogenic fungi. The VOCs emitted by BC-SKRU-1 significantly ($p < 0.05$) inhibited fungal mycelial growth, with variations observed among the pathogen species (Table 1). Notably, the highest antagonistic activity of BC-SKRU-1 VOCs was against *P. digitatum* NKP4321, resulting in a substantial inhibition rate of 68.21%, followed by *A. flavus* PSRDC-4 (65.29%) and *A. parasiticus* 3276 (61.31%). Consistent inhibitory effects were observed against *Phomopsis* sp., *C. oryzae*, and *C. gloeosporioides* PSU-03 (approximately 55%). However, the inhibitory effect on *R. solani* was relatively weaker at 45.28%. Consequently, *P. digitatum* NKP4321 was selected for further testing due to its susceptibility to BC-SKRU-1 VOCs.

3.2. Effect of different inoculation concentrations of S. nematodiphila BC-SKRU-1 for the production of VOCs against P. digitatum NKP4321

The impact of varying inoculation concentrations (10^5–10^8 CFU mL^{-1}) of BC-SKRU-1 on VOC production and its effects on the mycelial growth and spore germination of NKP4321 are summarized in Table 2. The findings show that the VOCs produced by BC-SKRU-1 significantly inhibited ($p < 0.05$) the mycelial development of NKP4321. At 10^5 CFU mL^{-1}, strain BC-SKRU-1 VOCs exhibited suppressive activity against the mycelial growth of NKP4321 by more than 58%. An increased inoculum concentration of BC-SKRU-1 improved VOC-induced inhibition, and at 10^6–10^8 CFU mL^{-1}, NKP4321 growth was not significantly ($p > 0.05$) inhibited; instead, it was completely inhibited (100%).

The results of NKP4321 spore germination showed that

Table 1
Assessing the impact of volatile organic compounds (VOCs) produced by *Serratia nematodiphila* BC-SKRU-1 on the growth of the following fungi: *Rhizoctonia solani*, *Phomopsis* sp., *Curvularia oryzae*, *Aspergillus flavus* PSRDC-4, *Aspergillus parasiticus* 3276, *Colletotrichum gloeosporioides* PSU-03, and *Penicillium digitatum* NKP4321. The fungi were grown on PDA medium and incubated at 30 °C for a period of 3–7 days, depending on the specific growth rates of each fungus.

Parameters	Assessing the impact of VOCs from BC-SKRU-1 on the growth of various plant pathogenic fungi													
	R. solani		*Phomopsis* sp.		C. oryzae		A. flavus		A. parasiticus		C. gloeosporioides		P. digitatum	
	Control	VOCs assay	Control	VOCs assay	Control	VOCs assay	Control	VOCs assay	Control	VOCs assay	Control	VOCs assay	Control	VOCs assay
Radial growth (cm)	9.00 ± 0.0	4.93 ± 0.43	9.00 ± 0.0	4.00 ± 0.0	9.00 ± 0.0	4.03 ± 0.05	8.50 ± 0.0	3.00 ± 0.13	8.28 ± 0.17	3.20 ± 0.34	4.90 ± 0.66	2.15 ± 0.24	3.08 ± 0.10	0.98 ± 0.10
Inhibition of radial growth (%)	$45.28^{d} \pm 4.83$		$55.56^{c} \pm 0.12$		$55.28^{c} \pm 0.56$		$65.29^{ab} \pm 1.52$		$61.31^{b} \pm 4.30$		$55.84^{c} \pm 4.33$		$68.21^{a} \pm 3.97$	
Colony morphology														

Note: The presented data represent the mean of three replicates ± standard deviation (SD). Values within the same row that share the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

Table 2
Influence of *Serratia nematodiphila* BC-SKRU-1 concentrations (10^5–10^8 CFU mL^{-1}) on the production of volatile organic compounds (VOCs) against mycelial growth and spore germination of *Penicillium digitatum* NKP4321 on PDA medium after incubation at 30 °C for 7 days and 48 h, respectively.

Parameters	Control	BC-SKRU-1 VOCs concentration (CFU mL^{-1})			
		10^5	10^6	10^7	10^8
Radial growth (cm)	$2.60^{a} \pm 0.28$	$1.08^{b} \pm 0.05$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$
Inhibition of radial growth (%)	–	$58.65^{b} \pm 1.25$	100^{a}	100^{a}	100^{a}
Inhibition of spore germination (%)	–	$38.29^{c} \pm 1.65$	$60.76^{b} \pm 3.25$	100^{a}	100^{a}

Note: The presented data represent the mean of three replicates ± standard deviation (SD). Values within the same row that share the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

concentrations of BC-SKRU-1 (10^5–10^7 CFU mL^{-1}) significantly ($p < 0.05$) inhibited spore germination, as indicated by the production of VOCs. In this study, it was observed that the inhibitory effect of BC-SKRU-1 VOCs at 10^7 CFU mL^{-1} completely inhibited spore germination (100%) in NKP4321. Additionally, higher concentrations exhibited suppressive activity against the mycelial growth of NKP4321 (10^6 CFU mL^{-1}).

3.3. *In vivo* biocontrol of *P. digitatum* NKP4321 in tangerine fruit by *S. nematodiphila* BC-SKRU-1 VOCs

3.3.1. Effect of inoculation concentration of BC-SKRU-1

To assess the effect of VOC concentration on biocontrol efficacy against green mold disease caused by NKP4321, different inoculation concentrations of BC-SKRU-1, ranging from 10^5 to 10^8 CFU mL^{-1}, were employed. Following a twelve-day incubation period, observation of lesion diameter revealed statistically significant differences ($p < 0.05$) among the various inoculation concentrations of BC-SKRU-1 in tangerine fruit. In wounds treated with these concentrations, lesion diameter markedly reduced by 12 days post-inoculation with NKP4321 compared to the control (Fig. 2). The biocontrol activity across the inoculation concentrations demonstrated a substantial decrease in lesion diameter ranging from 4.11 cm^2 to 1.56 cm^2 compared to the control (4.33 cm^2) (Fig. 2A), with biocontrol efficacy increasing from 5.08% to 63.94% (Fig. 2B). Notably, the most effective biocontrol activity, with a reduction in lesion diameter to 1.56 cm^2 and a biocontrol efficacy of 63.94%, was observed at 10^8 CFU mL^{-1}. There was a significant difference ($p < 0.05$) in lesion diameter between inoculations at 10^5 CFU mL^{-1} and 10^6 CFU mL^{-1}, while no significant difference ($p > 0.05$) was observed between 10^7 CFU mL^{-1} and 10^8 CFU mL^{-1}. These findings underscore the intricate relationship between VOC concentration, inoculation levels, and biocontrol effectiveness, with the concentration of 10^8 CFU mL^{-1} showing promise as an optimal strategy for mitigating green mold disease caused by NKP4321. Based on the data from this study, 10^8 CFU mL^{-1} was selected for subsequent experiments.

3.3.2. Effect of plate numbers of BC-SKRU-1

To assess the impact of VOC concentration on the *in vivo* biocontrol efficacy of BC-SKRU-1, varying numbers of BC-SKRU-1 plates (ranging from 1 to 4), each with a concentration of 10^8 CFU mL^{-1}, were utilized. Following a 12-day exposure to optimal environmental conditions for fungal proliferation (30 °C and approximately 90–95% relative humidity), tangerine fruit controls inoculated with NKP4321 and not treated with VOCs exhibited lesion diameter values exceeding 4.23 cm^2 (Fig. 3). The application of different numbers of BC-SKRU-1 plates to tangerine fruit wounds resulted in statistically significant ($p < 0.05$) variations in lesion diameter values against NKP4321. Applying BC-SKRU-1 with 1–4 plates of tangerine fruit demonstrated a decrease in lesion diameter

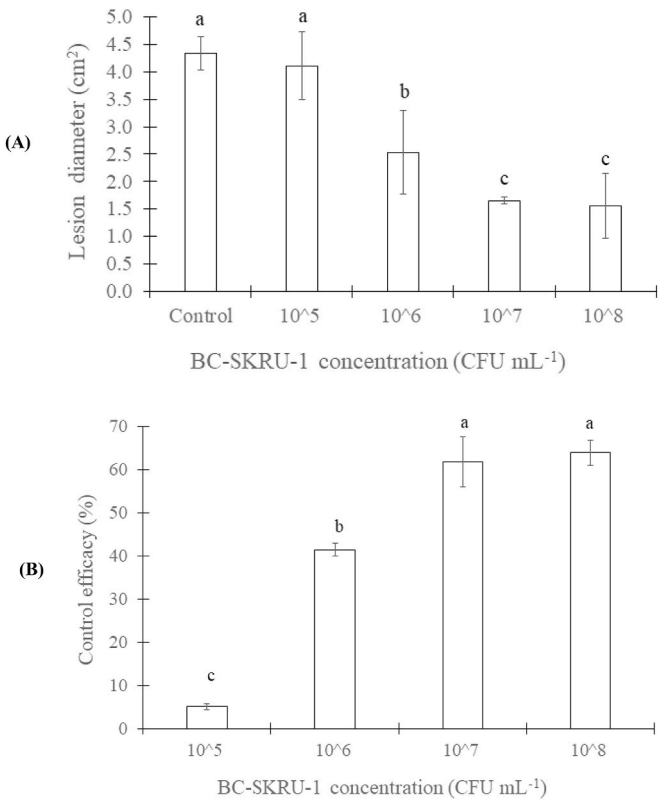


Fig. 2. Impact of *Serratia nematodiphila* BC-SKRU-1 concentration (10^5–10^8 CFU mL^{-1}) on the production of volatile organic compounds against green mold development in tangerine fruit after a 12-day storage period at 30 °C under humid conditions. **(A)** Lesion diameters and **(B)** Control efficacy. The presented data represent the mean of three replicates ± standard deviation (SD) (n = 15). Values within the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

values ranging from 4.14 cm^2 to 0.67 cm^2 (Fig. 3A), with biocontrol efficacy increasing from 2.20% to 84.10% (Fig. 3B). Notably, the most pronounced biocontrol activity was observed with the application of 4 plates of BC-SKRU-1, resulting in a remarkable reduction in lesion diameter values from 4.23 cm^2 to 0.67 cm^2 and an 84.10% biocontrol efficacy. The figures presented in Fig. 3C illustrate the curative efficacies of VOCs with varying numbers of BC-SKRU-1 plates against green mold induced by NKP4321, specifically focusing on the symptoms observed on tangerine fruits.

3.3.3. Effect of different fumigation periods of BC-SKRU-1 VOCs

The application of 4 plates of BC-SKRU-1 was utilized as the inoculum for all treatments. The fumigation periods (6, 12, 24 h, and continuous during storage (12 days)) significantly influenced ($p < 0.05$) the level of infection caused by NKP4321 (Table 3). Inoculated NKP4321 and non-fumigated BC-SKRU-1 VOCs in tangerine fruit showed disease severity, with lesion diameter values of 4.60 cm^2. Increasing the fumigation period from 6 h to continuous during storage resulted in reduced disease severity, with lesion diameter values decreasing to 3.67 cm^2–0.72 cm^2, lower than the control (4.60 cm^2), and with biocontrol efficacy increasing from 20.29% to 84.39%. However, fumigation with BC-SKRU-1 VOCs for 12 h (2.35 cm^2) did not significantly ($p > 0.05$) control NKP4321 in tangerine fruit compared to fumigation periods of 24 h (1.81 cm^2). Interestingly, fumigation with BC-SKRU-1 VOCs continuously during storage (12 days) showed the best control of green mold disease caused by NKP4321, as indicated by lesion diameter values of 0.72 cm^2 and 84.39% biocontrol efficacy.

3.4. Identification of S. nematodiphila BC-SKRU-1 VOCs and the efficacy of the main VOC, 2-nonanol, against P. digitatum NKP4321

Identification of BC-SKRU-1 VOCs and the efficacy of the main VOC, 2-nonanol, against NKP4321 are illustrated in Fig. 4 and summarized in Table 4. Comprehensive gas chromatography-mass spectrometry (GC–MS) analysis of VOCs derived from the BC-SKRU-1 revealed the presence of 20 distinct compounds, including 10 alcohols, 4 ketones, 2 acids, an alkane, pyrazine, aldehyde, and phenol. All identified compounds exhibited substantial similarity indices (SI) exceeding 90% when compared to entries in the NIST library, as illustrated in Fig. 4A and summarized in Table 4. Notably, alcohols emerged as the predominant fraction within the BC-SKRU-1 VOC profile, with 2-nonanol being the major constituent at 21.07% of the total compounds. Following closely, n-hexadecanoic acid constituted 13.18%, 2-undecanol 7.47%, 2-nonanone 6.44%, and phenol 4.64%, as depicted in Fig. 4A. This detailed analysis provides valuable insights into the composition and distribution of VOCs in BC-SKRU-1. Therefore, 2-nonanol, the major VOC of BC-SKRU-1, was selected to test its ability against NKP4321 both *in vitro* and *in vivo*.

The results, presented in Fig. 4B and C, indicate a significant ($p < 0.05$) inhibition of mycelial growth and spore germination of NKP4321 with increasing concentrations of 2-nonanol. At a lower concentration of 3.125 μL, 2-nonanol inhibited mycelial growth by 35.67% (Fig. 4B) and spore germination by 21.34% (Fig. 4C). However, at a concentration of 12.5 μL, 2-nonanol completely inhibited (100%) both mycelial growth and spore germination.

3.5. Comparing S. nematodiphila BC-SKRU-1 VOCs with 2-nonanol for green mold biocontrol in tangerine fruit

We compared four plates of BC-SKRU-1 VOCs with different concentrations of 2-nonanol (6.25, 12.5, 25, and 50 μL L^{-1}) for their effectiveness in controlling the NKP4321 infection in tangerine fruits, and the results are presented in Table 5. Tangerine fruits inoculated with NKP4321 and untreated with BC-SKRU-1 VOCs or 2-nonanol exhibited disease severity, with lesion diameter values of 4.63 cm^2. However, fumigation with BC-SKRU-1 VOCs or 2-nonanol significantly reduced ($p < 0.05$) disease severity in tangerine fruit caused by NKP4321. Fumigation with BC-SKRU-1 VOCs resulted in disease severity, with lesion diameter values of 0.68 cm^2 and an 85.24% biocontrol efficacy, which was higher than the biocontrol achieved using 2-nonanol at 6.25 μL L^{-1} (lesion diameter = 1.61 cm^2 and 65.32% biocontrol efficacy). However, it did not significantly ($p > 0.05$) control NKP4321 in tangerine fruit compared to 2-nonanol at 12.5 μL L^{-1} (lesion diameter = 0.59 cm^2 and 87.25% biocontrol efficacy). It is noteworthy that fumigation with 25 μL L^{-1} of 2-nonanol completely controlled the green mold disease caused by NKP4321, as indicated by 0 cm^2 disease severity and 100% biocontrol efficacy.

3.6. Mechanism action of 2-nonanol against P. digitatum NKP4321

Increased concentrations of 2-nonanol (0–25 μL mL^{-1}) significantly ($p < 0.05$) reduced the mycelial dry weights of NKP4321, as detailed in Table 6. At a concentration of 1.563 μL mL^{-1}, 2-nonanol inhibited over 90% of the growth of NKP4321, achieving complete inhibition (100%) at 3.125 μL mL^{-1}, as shown in Table 6A. Consequently, 1.563 μL mL^{-1} has been determined to be the minimal inhibitory concentration (MIC). The mechanisms underlying 2-nonanol's antifungal activities against NKP4321 were subsequently investigated using fungal cells at control, 1/2 MIC, and MIC doses.

3.6.1. Determination of ergosterol content in P. digitatum NKP4321 cells treated with 2-nonanol

Treatment with 2-nonanol significantly ($p < 0.05$) decreased the ergosterol content in the cell wall of NKP4321, as documented in

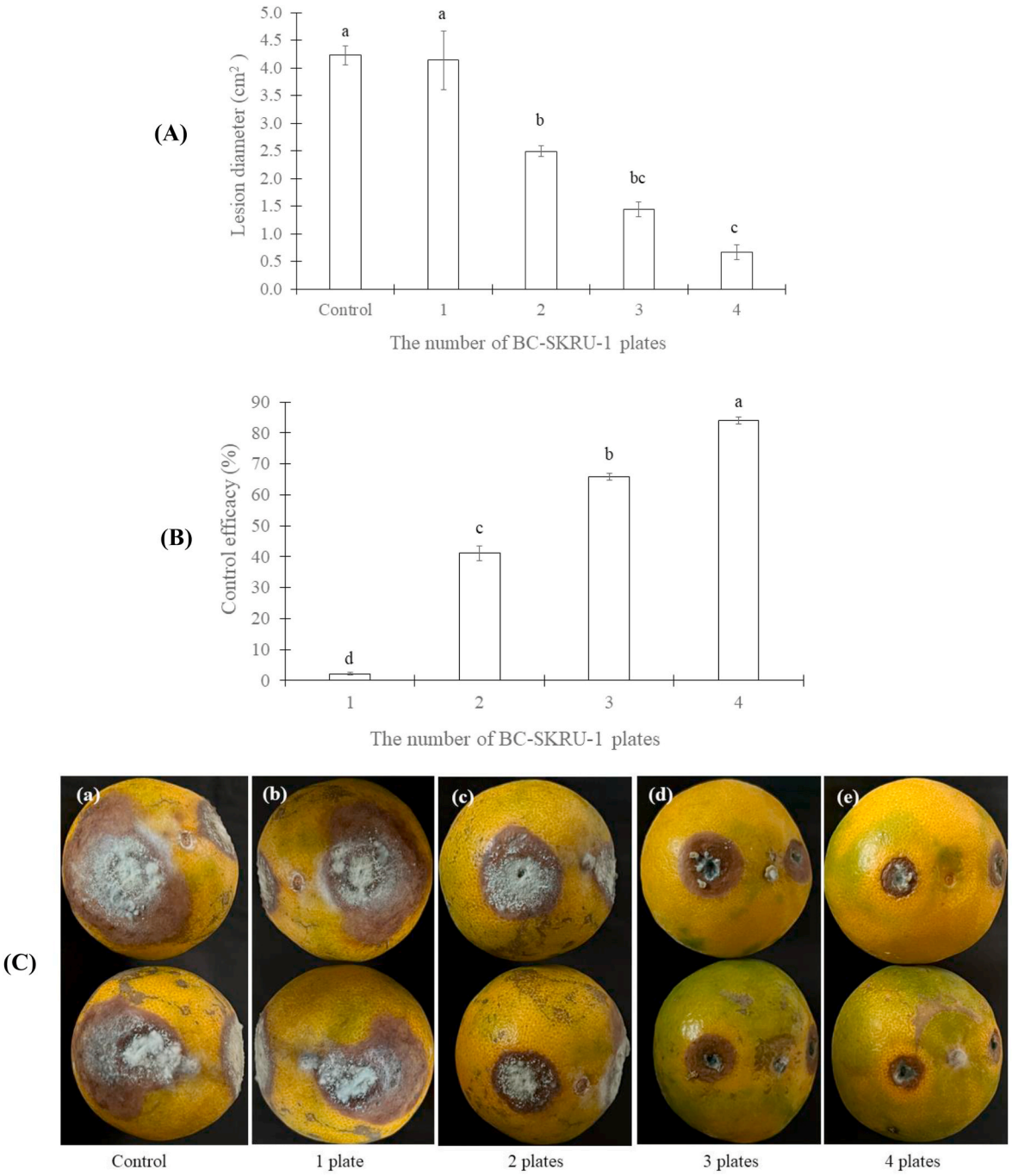


Fig. 3. Tangerine fruits were fumigated with 1, 2, 3, and 4 *Serratia nematodiphila* BC-SKRU-1 VOCs plates, respectively, and the lesion diameter **(A),** control efficacy **(B),** and disease symptoms **(C)** were calculated based on the disease severity of the fruit infected with *Penicillium digitatum* NKP4321 after a 12-day storage period at 30 °C under humid conditions. The presented data represent the mean of three replicates ± standard deviation (SD) (n = 15). Values within the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

Table 6, thereby compromising the structural integrity of the fungal cell wall. 2-nonanol significantly ($p < 0.05$) inhibited ergosterol biosynthesis in a dose-dependent manner in the NKP4321. At 1/2 MIC, the inhibition of ergosterol production was 85.06%. This inhibition increased to 88.71% when the concentration was raised to the MIC, as shown in Table 6b.

3.6.2. Effect of 2-nonanol on quantification of cellular reactive oxygen species, antioxidant enzyme activities, and cell membrane permeability of P. digitatum NKP4321

The effect of 2-nonanol on the quantification of cellular reactive oxygen species (ROS), antioxidant enzyme activities, and cell membrane permeability of NKP4321 is presented in Fig. 5. Antimicrobial agents can induce alterations in the antioxidant systems of fungal pathogens, including the generation of ROS and the activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and cellular glutathione levels. In this study, we investigated the effects of 2-nonanol on the antioxidant systems in NKP4321 cells by treating them with 0 (control), 1/2 MIC, and MIC concentrations of the compound. Treatment with 2-nonanol increased ROS levels to 2.11 μM/mg protein at 1/2 MIC and 2.70 μM/mg protein at MIC, up from 1.25 μM/mg protein in the control (Fig. 5A). There was also a significant increase ($p < 0.05$) in the activity

Table 3
Effect of various fumigation periods of volatile organic compounds (VOCs) produced by *Serratia nematodiphila* BC-SKRU-1 on green mold development induced by *Penicillium digitatum* NKP4321 in tangerine fruit following a 12-day storage period at 30 °C under humid conditions.

Parameters	Treatments				
	Inoculated NKP4321 + non fumigated BC-SKRU-1 VOCs (control)	Inoculated NKP4321 + fumigated BC-SKRU-1 VOCs for 6 h	Inoculated NKP4321 + fumigated BC-SKRU-1 VOCs for 12 h	Inoculated NKP4321 + fumigated BC-SKRU-1 VOCs for 24 h	Inoculated NKP4321 + fumigated BC-SKRU-1 VOCs during storage
Lesion diameter (cm^2)	$4.60^{a} \pm 0.40$	$3.67^{b} \pm 0.45$	$2.35^{c} \pm 0.15$	$1.81^{c} \pm 0.09$	$0.72^{d} \pm 0.07$
Control efficacy (%)	–	20.29	48.99	60.58	84.39
Disease symptoms					

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 15). Values within the same row that share the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

of the cellular antioxidant enzymes. SOD and CAT levels rose from 0.29 units/min/mg protein and 65.29 units/min/mg protein in the control to 1.14 and 73.20 units/min/mg protein at 1/2 MIC, and further to 2.11 and 116.08 units/min/mg protein at MIC (Fig. 5B and C). Additionally, GSH, GSSG, and the GSH/GSSG ratio all showed substantial alterations ($p < 0.05$) in cells treated with 2-nonanol. GSH levels increased from 0.44 μM/mg protein at 1/2 MIC to 0.74 μM/mg protein at MIC (Fig. 5D). Correspondingly, the GSH/GSSG ratio increased, while GSSG levels decreased in response to treatments at 1/2 MIC and MIC (Fig. 5E and F).

Our study also demonstrated that treatment with 2-nonanol leads to the disruption of the plasma membrane ergosterol in NKP4321 (Table 6). To validate these findings, we investigated the release of cellular content from NKP4321 upon treatment with various concentrations of 2-nonanol (at 1/4 MIC, 1/2 MIC, and MIC), by measuring the electrical conductivity of the liquid medium. Our results indicate that there was no significant alteration in relative conductivity observed in the control group over the entire 8-h measurement period. However, upon exposure to 2-nonanol at a concentration of 1/4 MIC, a discernible increase in cell content leakage from NKP4321 was observed, with the leakage content showing a progressive rise over time (Fig. 5G).

4. Discussion

This study is the first to demonstrate the antagonistic activity of VOC-producing *S. nematodiphila* BC-SKRU-1 (BC-SKRU-1 VOCs) against *P. digitatum* NKP4321 disease in postharvest tangerine fruit. The results indicate that BC-SKRU-1 VOCs and their major component, 2-nonanol, are significantly effective against NKP4321, both *in vitro* and *in vivo*. These substances likely disrupt ergosterol biosynthesis and reduce antioxidative defense molecules, contributing to their antifungal properties. Additionally, 2-nonanol compromises plasma membrane integrity.

Our study evaluated the antifungal activity of VOCs produced by BC-SKRU-1 against seven plant pathogenic fungi. The *in vitro* experiments demonstrated significant antagonistic activity, with notable growth inhibition observed in all tested fungi. Among them, *P. digitatum* NKP4321 exhibited heightened susceptibility to the VOCs, warranting further investigation into its response. We explored the effects of various inoculation concentrations, ranging from 10^5 to 10^8 CFU mL^{-1} of BC-SKRU-1 for VOC production, on NKP4321. The results revealed a dose-dependent inhibition of NKP4321 growth and spore germination. Lower concentrations, specifically 10^6 CFU mL^{-1}, were sufficient to completely inhibit mycelial growth, while higher concentrations, particularly 10^7 CFU mL^{-1}, completely suppressed spore germination. These findings underscore the potent antifungal properties of BC-SKRU-1 VOCs and their effectiveness at different concentrations.

The efficacy of VOCs was not limited to merely inhibiting fungus *in vitro* trials; rather, they also demonstrated success in controlling fungus in fruits (Archana et al., 2021; Ruiz-Moyano et al., 2020). In the current study, we fumigated tangerine fruit under controlled atmospheric conditions to examine the antifungal effectiveness of BC-SKRU-1 VOCs against green mold disease induced by NKP4321. The results showcased the prophylactic effects of VOC-mediated fumigation against green mold in tangerine fruit, significantly diminishing disease severity. Although numerous descriptive studies have documented the effectiveness of VOCs in controlling plant diseases (Liu et al., 2023; Pereyra et al., 2021, 2022; Qessaouia et al., 2022; Wang, Zhong, et al., 2021), there is a noticeable lack of research focused on screening strains for VOC production and characterizing these compounds. Furthermore, many researchers have avoided investigating the correlation between VOC concentrations and disease impacts, possibly due to the complexities involved in such studies. Nonetheless, understanding how VOC concentrations influence disease prevention, incidence, and severity is crucial for enhancing post-harvest preservation strategies for fruits and vegetables (Sharifi & Ryu, 2016). The efficacy of the treatment was dependent on the concentration of bacteria BC-SKRU-1 and the number

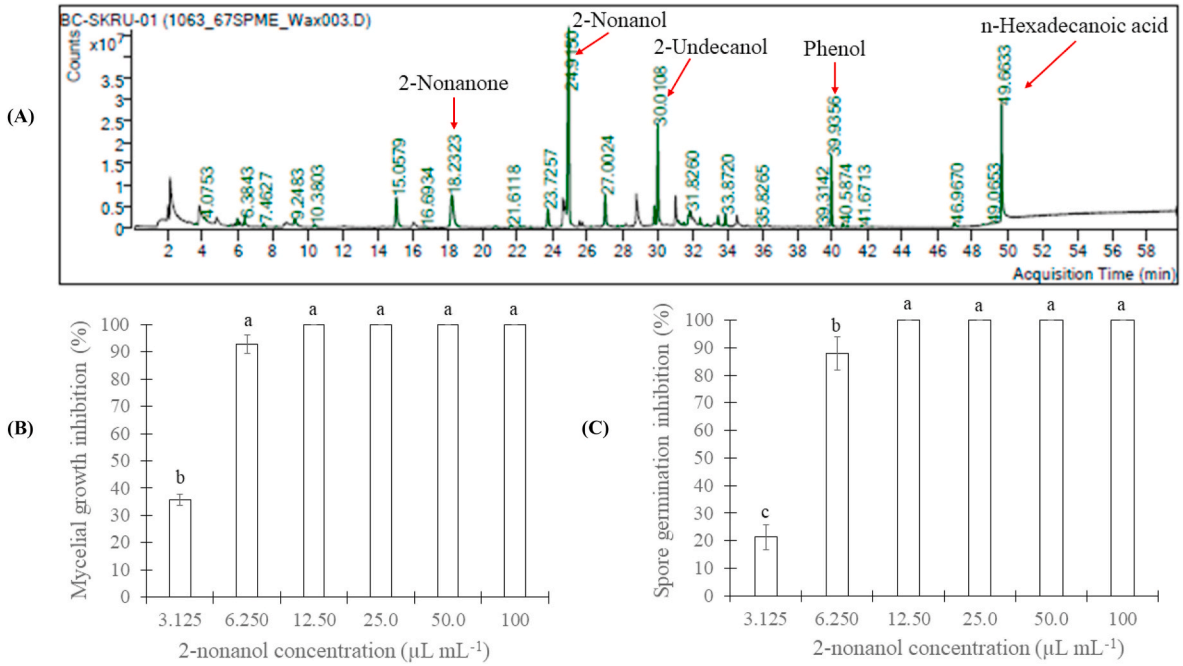


Fig. 4. (A) Mass spectra illustrating volatile organic compounds emitted by *Serratia nematodiphila* BC-SKRU-1 cultivated in nutrient agar (NA) medium after 24 h of incubation at 30 °C as detected by HS-SPME/GC-MS analysis. Influence of 2-nonanol concentration (3.125–100 μL mL^{-1}) against **(B)** mycelial growth and **(C)** spore germination of *Penicillium digitatum* NKP4321 on PDA medium after incubation at 30 °C for 7 days and 48 h, respectively. The presented data represent the mean of three replicates ± standard deviation (SD). Values with the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

Table 4
Identification of the volatile organic compounds produced by *Serratia nematodiphila* BC-SKRU-1 in nutrient agar (NA) medium after 24 h of incubation at 30 °C by SPME/GC-MS.

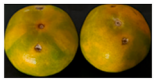
NO.	Identified compound	RT (min)	CAS Number	S (%)	Formula	RA (%)	Chemical class
1	Nonane, 4,5-dimethyl-	5.97	17302-23-7	91.00	$C_{11}H_{24}$	0.62	Alkane
2	2-Heptanone	9.25	110-43-0	91.97	$C_7H_{14}O$	0.87	Ketone
3	2-Heptanol	15.06	543-49-7	94.48	$C_7H_{16}O$	3.83	Alcohol
4	2-Nonanone	18.23	821-55-6	96.17	$C_9H_{18}O$	6.44	Ketone
5	Pyrazine, 3-ethyl-2,5-dimethyl-	21.61	13360-65-1	90.04	$C_8H_{12}N_2$	0.22	Pyrazine
6	1-Hexanol, 2-ethyl-	23.73	104-76-7	93.20	$C_8H_{18}O$	1.84	Alcohol
7	Benzaldehyde	24.52	100-52-7	93.63	C_7H_6O	0.08	Aldehyde
8	2-Nonanol	24.91	628-99-9	95.49	$C_9H_{20}O$	21.07	Alcohol
9	2-Undecanone	27.00	112-12-9	98.32	$C_{11}H_{22}O$	2.98	Ketone
10	Benzyl methyl ketone	29.81	103-79-7	95.84	$C_9H_{10}O$	1.37	Ketone
11	2-Undecanol	30.01	1653-30-1	97.18	$C_{11}H_{24}O$	7.47	Alcohol
12	Benzeneethanol, .alpha.-methyl-	31.83	698-87-3	92.53	$C_9H_{12}O$	0.86	Alcohol
13	trans-Geraniol	32.43	106-24-1	91.23	$C_{10}H_{18}O$	0.42	Alcohol
14	Benzeneethanol	33.45	60-12-8	96.84	$C_8H_{10}O$	0.59	Alcohol
15	2-Tridecanol	33.87	1653-31-2	92.28	$C_{13}H_{28}O$	0.93	Alcohol
16	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	35.83	7212-44-4	91.25	$C_{15}H_{26}O$	0.12	Alcohol
17	Phenol, 2,4-bis(1,1-dimethylethyl)-	39.94	96-76-4	98.61	$C_{14}H_{22}O$	4.64	Phenol
18	FARNESOL ISOMER B	40.59	4602-84-0	94.71	$C_{15}H_{26}O$	0.28	Alcohol
19	Tetradecanoic acid	46.97	544-63-8	93.99	$C_{14}H_{28}O2$	0.57	Acids
20	n-Hexadecanoic acid	49.66	57-10-3	74.95	$C_{16}H_{32}O2$	13.18	Acids

of BC-SKRU-1 plates utilized. As the concentration and number of plates increased, there was a significant alteration in the disease status of tangerine fruit inoculated with the pathogenic fungus NKP4321.

The study comprehensively evaluated the impact of VOC concentration on biocontrol efficacy against green mold disease caused by NKP4321 on tangerine fruit. Various concentrations of BC-SKRU-1 and different fumigation periods were tested. The results showed a clear relationship between VOC concentration, inoculation levels, fumigation periods, and biocontrol effectiveness. Higher concentrations of BC-SKRU-1, particularly at 10^8 CFU mL^{-1}, significantly reduced disease severity and increased biocontrol efficacy, suggesting this concentration as an optimal strategy for mitigating green mold disease. Continuous fumigation during storage for 12 days provided the best control, achieving an 84.39% biocontrol efficacy. These findings highlight the intricate interplay between VOC concentration, inoculation levels, fumigation periods, and biocontrol effectiveness, offering insights for practical strategies to control fungal diseases in post-harvest fruit. This study aligns with previous research demonstrating the effectiveness of VOCs in controlling mold and decay in citrus fruits (Pena et al., 2019; Pereyra et al., 2021, 2022; Wang et al., 2021a, 2021b; Yeh et al., 2021; Qessaouia et al., 2022; Liu et al., 2023).

The evidence from both *in vitro* experiments and trials on tangerine fruits convincingly demonstrates the antifungal properties of VOCs produced by BC-SKRU-1 against NKP4321. Our extensive analysis of

Table 5
Comparison biocontrol efficacy of volatile organic compounds (VOCs) produced by *Serratia nematodiphila* BC-SKRU-1 with 2-nonanol at concentrations of 6.25–50 μL L^{-1} on green mold development induced by *Penicillium digitatum* NKP4321 in tangerine fruit following a 12-day storage period at 30 °C under humid conditions.

Parameters	Treatments					
	Control	BC-SKRU-1 VOCs (four plate L^{-1})	2-nonanol concentration (μL L^{-1})			
			6.25	12.5	25	50
Lesion diameter (cm^2)	$4.63^{a} \pm 0.40$	$0.68^{c} \pm 0.03$	$1.61^{b} \pm 0.14$	$0.59^{c} \pm 0.10$	$0.0^{d} \pm 0.0$	$0.0^{d} \pm 0.0$
Control efficacy (%)	–	85.24	65.32	87.25	100	100
Disease symptoms						

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 15). Values within the same row that share the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

Table 6
Influence of 2-nonanol at concentrations of 0, 0.781, 1.563, 3.125, 6.25, 12.50, and 25 μL mL^{-1} on **(A)** percent growth inhibition and **(B)** percent inhibition of ergosterol production in *Penicillium digitatum* NKP4321 cultured in potato dextrose broth (PDB) at 30 °C for 7 days.

(A)

2-nonanol concentration (μL mL^{-1})	Mycelial dry weights (mg)	Mycelial growth inhibition (%)
0 (Control)	$198.91^{a} \pm 1.42$	–
0.781	$24.30^{b} \pm 0.28$	87.83
1.563	$18.12^{c} \pm 0.65$	90.89
3.125	$0^{d} \pm 0.00$	100
6.25	$0^{d} \pm 0.00$	100
12.50	$0^{d} \pm 0.00$	100
25	$0^{d} \pm 0.00$	100

(B)

2-nonanol concentration (μL mL^{-1})	Mean of percentage inhibition of ergosterol production ± SD
0 (Control)	$0.00^{c} \pm 0.00$
1/2 MIC	$85.06^{b} \pm 0.20$
MIC	$88.71^{a} \pm 0.22$

Note: The presented data represent the mean of three replicates ± standard deviation (SD). Values within the same row that share the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

these VOCs, utilizing HS-SPME/GC-MS, identified 2-nonanol as a significant component, prompting further investigation in antifungal experiments. Previous research has documented the efficacy of 2-nonanol against various plant pathogenic fungi, typically produced by *Bacillus* sp. (Ren et al., 2022; Song et al., 2024), *Pseudomonas* sp. (Montes-Osuna et al., 2022), and *Lactiplantibacillus plantarum* (Simone et al., 2024). However, our findings reveal 2-nonanol production in *S. nematodiphila* BC-SKRU-1, suggesting a broader range of microbial species capable of synthesizing this compound. Our investigations showed that 2-nonanol significantly inhibited mycelial growth and spore germination of NKP4321 at increasing concentrations. In tangerine fruit assays, both BC-SKRU-1 VOCs and 2-nonanol effectively mitigated disease severity caused by NKP4321, with 2-nonanol demonstrating superior performance at higher concentrations. Remarkably, at 25 μL L^{-1}, 2-nonanol achieved complete eradication of green mold disease, highlighting its robust antifungal potential. These findings underscore the importance of 2-nonanol in combating plant pathogens and highlight its promising role in sustainably controlling postharvest diseases. Additionally, recent studies by Simone et al. (2024), Ren et al. (2022), and Song et al. (2024) further support the efficacy of 2-nonanol against various postharvest fungal pathogens such as *Aspergillus niger*, *Alternaria solani*, *Botrytis cinerea*, and *Penicillium expansum*, suggesting its potential application in fruit preservation. Our results position BC-SKRU-1 as a promising biocontrol agent, with 2-nonanol playing a pivotal role in controlling NKP4321 and potentially other fungal pathogens.

Numerous studies have documented the effectiveness of VOCs produced by antagonistic microorganisms in controlling fungal diseases in fruits (Liu et al., 2023; Pena et al., 2019; Pereyra et al., 2021, 2022; Qessaouia et al., 2022; Wang, Zhong, et al., 2021; Yeh et al., 2021). These VOCs disrupt fungal growth by affecting cellular processes, such as membrane integrity, metabolic pathways, and ergosterol biosynthesis. They may also induce systemic resistance in fruits. While research has primarily focused on morphological and cellular effects, the present study investigates the mechanisms by which 2-nonanol, produced by BC-SKRU-1, inhibits NKP4321. Specifically, it examines ergosterol reduction, antioxidant defense mechanisms, and plasma membrane integrity.

Ergosterol is essential for fungi, forming a vital part of cell membranes and supporting structure, fluidity, and growth. Its critical role makes it a target for antifungal drugs (Tao et al., 2014). Our findings indicate that 2-nonanol inhibits ergosterol production in NKP4321 in a dose-dependent manner. This observation aligns with research by Song et al. (2024), who found that 2-nonanol treatment downregulated genes involved in ergosterol production in *P. expansum*. Interestingly, some genes, specifically Egr4, Egr6, and SrbA, were upregulated, suggesting a potential compensatory role in ergosterol synthesis (Tahtah et al., 2023). Consequently, we propose that 2-nonanol's antifungal effects stem from its ability to disrupt gene expression related to ergosterol synthesis in NKP4321.

In fungi, reactive oxygen species (ROS) are essential for cellular signaling, differentiation, and defense against pathogens. However, excessive ROS levels can lead to cellular damage and impact growth (Bissaro et al., 2018). Excessive intracellular accumulation of ROS serves as the primary biochemical trigger for apoptosis (Da et al., 2019). We postulated that the build-up of ROS could cause NKP4321 cells to undergo apoptosis after treatment with 2-nonanol. Our findings corroborate this hypothesis, revealing a significant increase in ROS levels when cells were exposed to 1/2 MIC and MIC concentrations of 2-nonanol compared to the control. This overproduction of ROS can lead to oxidative stress, damaging vital cellular structures such as the plasma membrane and DNA (Han et al., 2016; Matthew, 2018; Wang et al., 2020). Our findings suggest that 2-nonanol may disrupt the fungus's DNA and cell walls, allowing nutrients to seep out and triggering a number of stress reactions, thereby explaining its complex antifungal mechanism.

Catalase (CAT) and superoxide dismutase (SOD) are essential enzymes in fungal cells for managing oxidative stress (Hansberg et al., 2012; Schatzman et al., 2020). CAT breaks down hydrogen peroxide into water and oxygen, while SOD converts superoxide radicals into less reactive molecules (Ighodaro & Akinloye, 2018). These enzymes protect fungal cells from oxidative harm, preserving cellular integrity and supporting vital functions necessary for growth and reproduction. In our study, we examined the response of these enzymes in NKP4321 cells exposed to 1/2 MIC and MIC concentrations of 2-nonanol. We observed

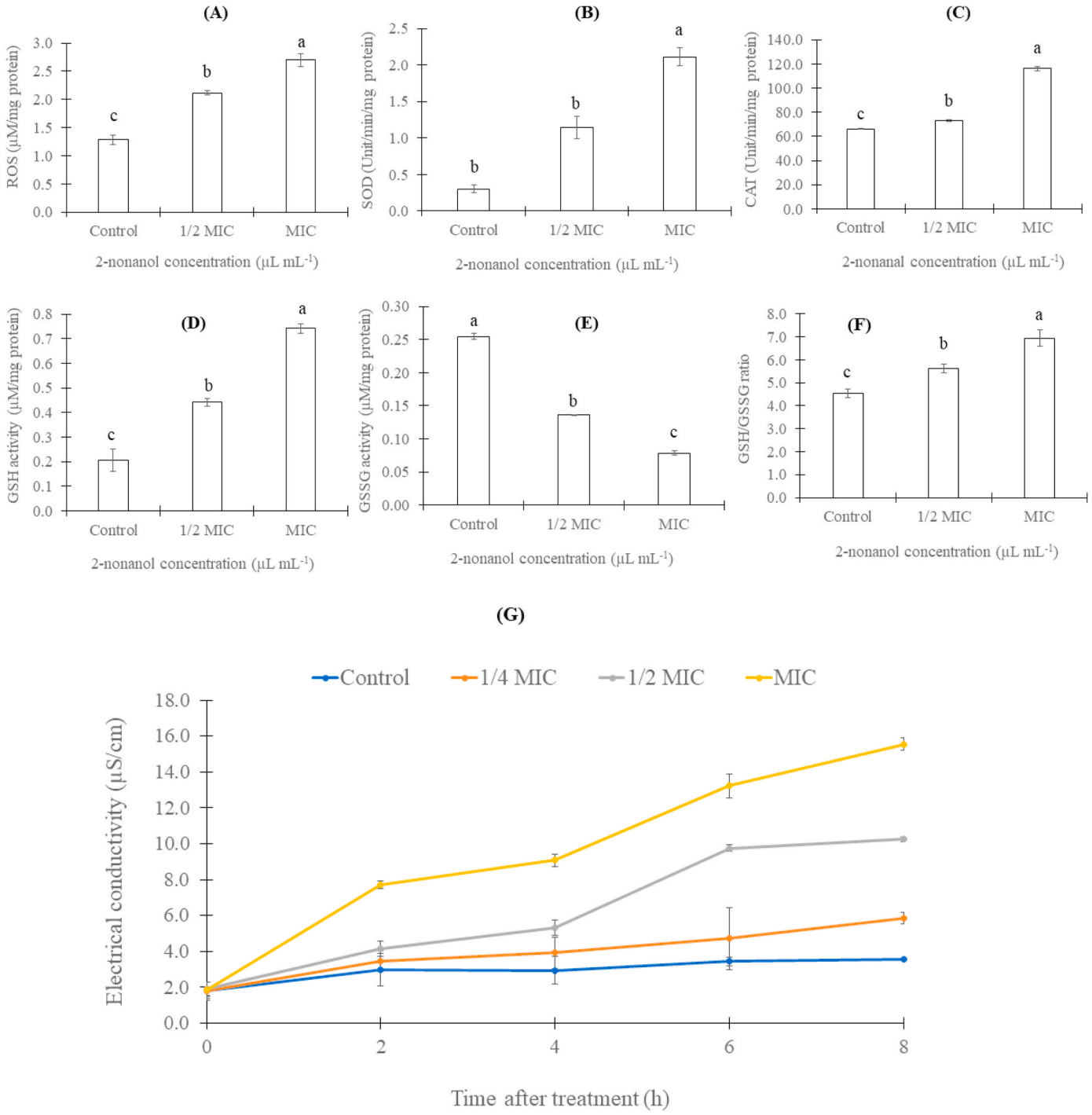


Fig. 5. The effect of 2-nonanol concentration at 1/2 MIC and MIC on the levels of reactive oxygen species (ROS) **(A)**, superoxide dismutase (SOD) activity **(B)**, catalase (CAT) activity **(C)**, reduced glutathione (GSH) activity **(D)**, oxidized glutathione (GSSG) activity **(E)**, and GSH/GSSG ratio **(F)** was assessed in *Penicillium digitatum* NKP4321 cells cultured in potato dextrose broth (PDB) at 30 °C for 7 days. **(G)** shows the effect of 2-nonanol concentration (at 1/4 MIC, 1/2 MIC, and MIC) on the cellular leakage of NKP4321. Three-day-old mycelia were treated with 2-nonanol for 0, 2, 4, 6, and 8 h in distilled water. Subsequently, the mycelia were filtered, and the filtrate solutions were used to measure electrical conductivity. The values presented are the means of three replicates (±SD). Values with the same letter are not considered significantly different, as determined by ANOVA following Tukey's HSD test at a significance level of $p > 0.05$.

increased SOD and CAT activities, indicating an adaptive response to mitigate oxidative stress induced by elevated ROS levels. Additionally, we analyzed the role of glutathione (GSH), a major cellular antioxidant. Contrary to expectations, our results showed a decrease in GSSG levels and an increase in GSH levels, with an overall rise in the GSH/GSSG ratio. This unusual increase in GSH amidst oxidative stress implies a robust antioxidative mechanism activated in response to 2-nonanol exposure. Monitoring the levels and activities of CAT, SOD, and glutathione in fungi exposed to oxidative stressors like 2-nonanol is crucial for understanding how these organisms manage oxidative stress and adapt to environmental challenges.

The plasma membrane is essential for maintaining cellular integrity, regulating exchanges, and transmitting energy and information in fungal cells (Gow et al., 2017). VOCs like organic acids target these

membranes by increasing permeability and fluidity, leading to protein conformational changes, intracellular content leakage, and cell death (Zhao et al., 2022). Our research shows that 2-nonanol significantly alters the permeability of NKP4321 plasma membrane, evidenced by measurable changes in conductivity in treated cells. This increased permeability suggests that 2-nonanol induces intracellular leakage, disrupting membrane integrity. This aligns with the effects of other membrane-targeting agents, such as *cis*-9-heptadecenoic acid from *Pseudomonas* spp., which increases membrane fluidity and kills pathogenic fungi like *B. cinerea* (Avis & Belanger, 2001). Similarly, decanoic acid compromises *Candida albicans* cell membranes, causing cytoplasmic content efflux and pathogen elimination (Bergsson et al., 2001). These findings underscore the critical role of membrane integrity in fungal viability and highlight the potential of VOCs like 2-nonanol as effective fungicidal agents. By targeting the plasma membrane, such compounds offer promising avenues for developing novel antifungal treatments that disrupt essential cellular functions in plant pathogenic fungi.

5. Conclusion

Our study has demonstrated the potent antifungal properties of BC-SKRU-1 VOCs, particularly against *P. digitatum* NKP4321, where they have been shown to inhibit mycelial growth and spore germination effectively. At an optimal concentration of 10^8 CFU mL^{-1}, these VOCs significantly reduced the severity of green mold disease in postharvest tangerine fruits, with continuous fumigation proving to be the most effective method of application. Among the twenty VOCs produced by BC-SKRU-1, 2-nonanol emerged as the most effective, inhibiting NKP4321 completely in both *in vitro* and *in vivo* experiments. The mode of action of BC-SKRU-1 VOCs includes disrupting membrane integrity and ergosterol synthesis, as well as altering intracellular levels of antioxidant molecules, thus providing a multifaceted approach to controlling fungal growth. These findings not only highlight the efficacy of 2-nonanol but also validate the use of BC-SKRU-1 VOCs as a promising biocontrol strategy for enhancing the preservation of citrus fruits against postharvest fungal pathogens.

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Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Sawai Boukaew: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Siriporn Yossan:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Wanida Petlamul:** Writing – review & editing, Funding acquisition, Data curation. **Karistsapol Nooprom:** Writing – review & editing, Data curation. **Norathep Sakphet:** Writing – review & editing, Data curation. **Krittin Chumkaew:** Writing – review & editing, Data curation. **Sirasit Srinuanpan:** Writing – review & editing. **Zhiwei Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests regarding the publication of this study.

Data availability

The authors do not have permission to share data.

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