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Volatile organic compounds from *Streptomyces philanthi* RM-1-138: Biocontrol of *Sclerotium rolfsii* and their impact on chili pepper seed germination

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ABSTRACT

Streptomyces species have been recognized in agriculture both as biological control agents and as plant growth-promoting bacteria. In the present study, we aimed to investigate volatile organic compounds (VOCs) produced in wheat seed culture (WSC) of *S. philanthi* RM-1-138 (RM-1-138 VOCs) and its major component L-linalool against *Sclerotium rolfsii* and their effects on seed germination in chili pepper. Our *in vitro* sealed plate bioassay demonstrated that 30 g per air space of WSC of RM-1-138 and L-linalool at 20 μL per air space effectively killed *S. rolfsii* mycelia and sclerotia. *In vitro* bioprotection assays using chili pepper as a model revealed that RM-1-138 VOCs (30 g per air space) and L-linalool (40 μL per air space) achieved 100 % survival of chili pepper seedlings infected by *S. rolfsii*, a significantly higher survival rate compared to the pot bioassay experiments, which achieved only 60 % and 55 % survival, respectively. However, our seed germination experiments found that RM-1-138 VOCs and L-linalool were detrimental to chili pepper seed germination and growth. Specifically, seeds with radicles approximately 1 mm in size exposed to these VOCs exhibited a strong negative impact on stem length, root length, and fresh weight compared to the control. Microscopy images confirmed that both VOCs were highly toxic to cotyledon production, causing hypocotyls and radicles to turn dark brown and subsequently rot. While RM-1-138 VOCs and L-linalool demonstrate significant potential for controlling *S. rolfsii* growth, further studies are needed to explore strategies, such as combining these VOCs with plant growth-promoting bacteria or chemical elicitors, to mitigate their toxicity and enhance seed germination and root development in chili peppers.

1. Introduction

Sclerotium rolfsii Sacc. (Teleomorph: *Athelia rolfsii*) is one of the most devastating soil-borne phytopathogens, causing root and stem rot diseases in economically important crops such as chili pepper (*Capsicum annuum* L.), sugar beet (*Beta vulgaris* L.), tomato (*Solanum lycopersicum*), peanut (*Arachis hypogaea* L.), snap bean (*Phaseolus vulgaris* L.), pumpkin (*Cucurbita pepo* L.), and cannabis (*Cannabis sativa* L.) [1–7]. These diseases are prevalent globally, with *S. rolfsii* capable of infecting over 500 species across 100 plant families worldwide [8]. *S. rolfsii*, a soil-borne fungus that predominantly causes root and stem rot in chili pepper, is a major concern [9]. Thriving at temperatures between 25 and 40 °C, it produces large quantities of sclerotia that can persist in the soil for years, making it a significant pathogen worldwide [10]. Recently, root and stem rot caused by *S. rolfsii* has become a significant threat to pepper production [11].

Controlling *S. rolfsii* is challenging due to its ability to attack plants from the soil, its wide host range, and its capacity to survive in the soil for extended periods. While chemical control methods sometimes yield good results, improper use of fungicides poses health and environmental risks, affects food safety, and can lead to fungal resistance [12–14]. Developing efficient and environmentally friendly antagonistic

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microbes offers a promising alternative method for disease control.

Biological control using microorganisms has emerged as a promising alternative to disease management [15–19]. Streptomycetes are increasingly being recognized in agriculture both as biological control agents [20–22] and as plant growth-promoting bacteria (PGPB) [21,23–25]. They exhibit significant antagonistic potential against a wide range of fungal pathogens [22,26]. Several species of *Streptomyces* have been employed to manage soilborne diseases. For instance, *S. diastaticus* subsp. *ardesiacus* controls *Fusarium* wilt and bacterial wilt in tomato plants caused by *Fusarium oxysporum* and *Ralstonia solanacearum* [22]. *S. carpaticus* EGY-S7 combats *Fusarium* wilt in tomatoes caused by *F. oxysporum* f. sp. *lycopersici* [27], while *S. cellulosae* addresses stem, root, and pod rot in peanuts caused by *S. rolfsii* [28]. *Streptomyces* sp. RP1A-12 manages stem rot in peanuts due to *S. rolfsii* [29], and *Streptomyces* sp. H4 controls *Fusarium* wilt in bananas caused by *F. oxysporum* f. sp. *cubense*. Additionally, *S. araujoniae* S-03 targets *Rhododendron* root rot caused by *Phytophthora cinnamomi* [30], while *S. mycarofaciens* SS-2-243 and *S. philanthi* RL-1-178 address *Sclerotium* root and stem rot and *Ralstonia* wilt in chili peppers caused by *S. rolfsii* and *R. solanacearum* [31]. *Streptomyces* bacteria can effectively suppress various plant fungal and bacterial diseases through multiple mechanisms. These mechanisms include the production of both volatile and non-volatile antibiotics [30,32–34], the secretion of cell wall-degrading enzymes [30], hyperparasitism of pathogenic organisms [35], the promotion of plant growth [21,23–25,33], and the induction of systemic resistance in the host plant [36].

PGPB provides an eco-friendly alternative to reduce chemical usage while enhancing the productivity of economically important crops. The release of VOCs by PGPB has become a promising biotechnological approach to increase biomass accumulation in model plants [37]. Beneficial *Streptomyces* enhance plant growth by promoting the development of robust root systems and improving the ability of host plants to acquire nutrients and water from the soil [38]. For example, VOCs from *Streptomyces* increase shoot fresh weight, shoot length, root fresh weight, root length, hypocotyl diameter, and chlorophyll content in *Phaseolus vulgaris* seedlings [39]. VOCs from *Streptomyces* sp. TOR3209 increase the fresh weight of tobacco seedlings [40]. VOCs from several *Streptomyces* isolates significantly enhanced plant shoots and root biomass in *Arabidopsis thaliana* seedlings [41]. Additionally, VOCs from *Streptomyces* PM5 have been shown to promote growth and modulate metabolism in tomato plants [23]. However, the effects of VOCs from strain RM-1-138 on being as plant growth promoting bacteria have not yet been investigated.

Previous studies on *S. philanthi* RM-1-138 have demonstrated their ability to produce non-volatile substances that inhibit the growth of *S. rolfsii* and *R. solanacearum*, which cause *Sclerotium* root and stem rot and *Ralstonia* wilt in chili peppers, respectively [31]. Additionally, VOCs from *S. philanthi* RM-1-138 grown in WSC have been shown to inhibit the growth of *Rhizoctonia solani* and *Colletotrichum gloeosporioides*, responsible for rice sheath blight and anthracnose, respectively [42,43]. A total of 36 VOCs have been identified in the volatiles of strain RM-1-138, including alcohols, alkenes, aromatic hydrocarbons, sulfides, ketones, esters, and alkanes, with L-linalool being the most abundant [42].

We hypothesize that the VOCs produced by *S. philanthi* RM-1-138 will act as effective biocontrol agents against *S. rolfsii*, reducing its growth and pathogenicity. Additionally, these VOCs, including key components such as L-linalool, may influence chili pepper seed germination, either promoting or inhibiting seedling growth depending on concentration and exposure.

The study aims to provide updated information on the efficacy of VOCs produced by *S. philanthi* RM-1-138 and L-linalool, against *S. rolfsii*, as well as their impact on chili pepper seed germination. Specifically, the objectives are to: (i) evaluate the ability of these VOCs and L-linalool to inhibit the growth of *S. rolfsii* both *in vitro* and on chili pepper plants, as the antifungal effects of RM-1-138 VOCs on various plant pathogens are known, but their activity against *S. rolfsii* has not yet been explored; (ii)

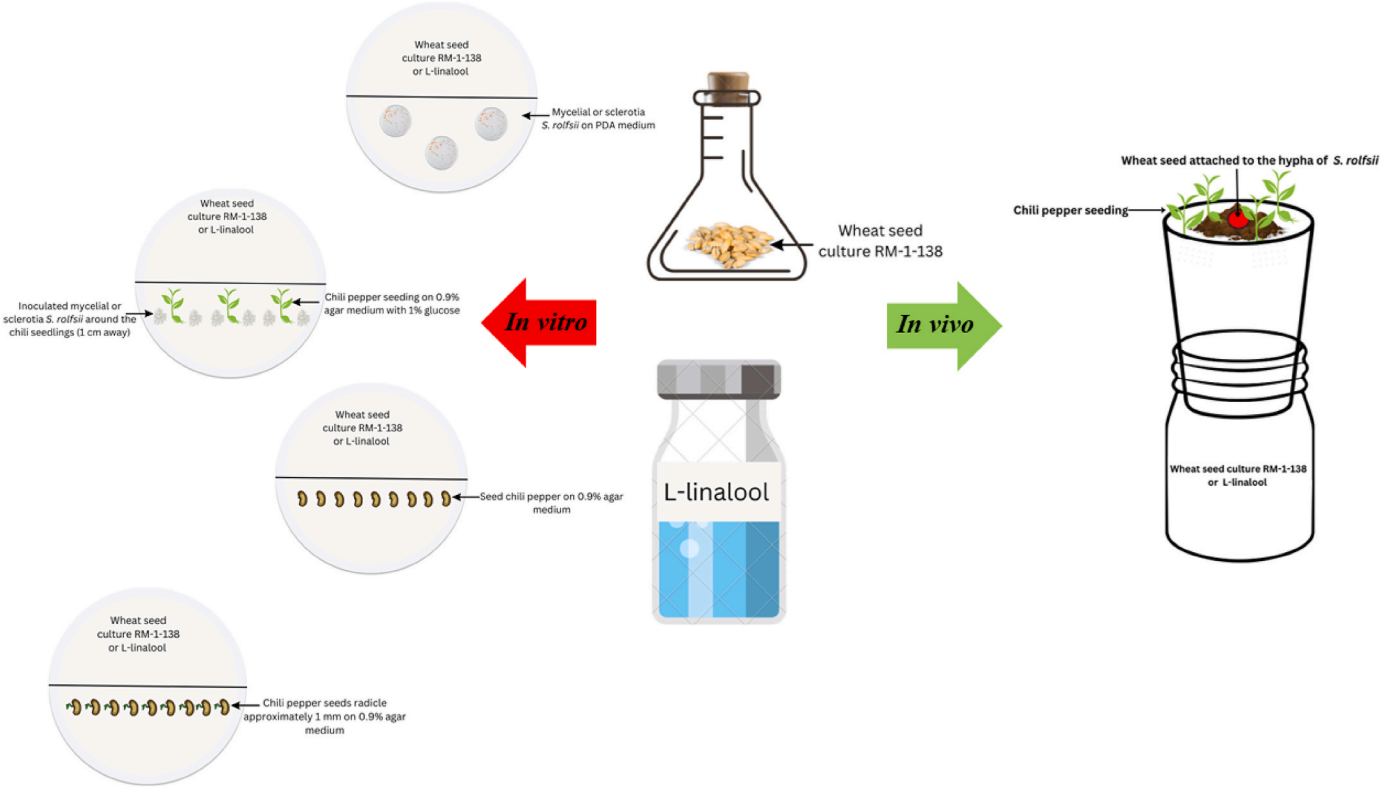

Fig. 1. Experimental setup for both *in vitro* and *in vivo* assays to evaluate the antifungal effects of emitted or synthetic L-linalool from *S. philanthi* RM-1-138 against root and stem rot caused by *S. rolfsii* and their effects on seed germination in chili pepper.

assess the effects of these VOCs and L-linalool on chili pepper seed germination and seedling growth, recognizing that while *Streptomyces* species are often considered plant growth-promoting bacteria, it is crucial to determine whether the VOCs from RM-1-138 and L-linalool have beneficial or detrimental effects on chili pepper development.

2. Materials and methods

2.1. Sources of materials

2.1.1. Microorganisms and growth conditions

The strain RM-1-138 used in this research was previously isolated from the rhizosphere soil of chili pepper plants in southern Thailand [31]. It was cultivated on glucose yeast-malt extract agar (GYM; Himedia®, India) at 30 °C for 10 days. Spores were harvested from ten-day-old GYM cultures, suspended in 10 mL of water, and quantified using a hemocytometer. The inoculum was then prepared by diluting the spores in sterilized distilled water to achieve a concentration of 10^7 spores mL^{-1}.

Sclerotium rolfsii was obtained from the Mycorrhiza and Mycotechnology Laboratory, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand. The fungal strain was cultivated on potato dextrose agar (PDA; Himedia®, India) and maintained at 30 °C for 3 days to obtain mycelium for use. Additionally, it was incubated for 10 days to induce sclerotia production before being utilized in this study.

2.1.2. Pure commercial chemical volatile

Pure technical-grade L-linalool (Sigma-Aldrich, ≥95 % Kosher, FG) was used to investigate its antifungal effects. L-linalool is known for its antifungal activity. It is also a major component of strain RM-1-138, as identified through headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) [42].

2.1.3. Chili pepper

The chili pepper cultivar 'Super Hot 2 F1' (EAST-WEST SEED Co., Ltd.) was chosen for this study. The preparation of chili pepper seeds for experimental investigation followed the method described by Marzouk et al. [44]. Chili pepper seeds were disinfected with 2 % sodium hypochlorite for 10 min and then rinsed five times with sterile distilled water. The disinfected seeds were incubated on sterile, moist filter paper at 30 °C with a 16-h photoperiod for 5 days to promote germination. The seedlings underwent a second round of disinfection, involving a 3-min soak in 1 % sodium hypochlorite, followed by a 5-s rinse in 70 % ethanol, and then washed five times with sterile distilled water. The disinfected seedlings were aseptically divided into small fragments (0.1 cm in length) consisting of cotyledons, stems, and roots for use in the experiment.

2.2. Preparation of VOCs from S. philanthi RM-1-138

A spore suspension of strain RM-1-138 (10^7 spores mL^{-1}) was inoculated onto autoclaved wheat seeds in 250 ml conical flasks at a rate of 5 mL per 100 g of wheat seeds. The inoculated flasks were then incubated at 30 °C for 14 days before being used in this study [42]. Following this incubation period, the RM-1-138 VOC activity of the strain's WSC was investigated.

2.3. Growth inhibition of S. rolfsii by RM-1-138 VOCs and L-linalool

2.3.1. Effect of RM-1-138 VOCs and L-linalool on mycelial growth of S. rolfsii

We investigated the effects of RM-1-138 VOCs and L-linalool on the mycelial growth of *S. rolfsii* using the sealed plate technique (Fig. 1), as described by Calvo et al. [45]. Each experiment involved placing three small Petri dishes (50 × 15 mm, D × H) inside larger Petri dishes (140 × 20 mm, D × H). The smaller dishes were filled with 5 mL of PDA inoculated with a 0.5 cm diameter fungal plug taken from the periphery of an actively growing *S. rolfsii* culture. The larger Petri dishes contained varying concentrations (0–50 g per airspace) of wheat seed culture of strain RM-1-138 or L-linalool concentrations (0–100 μL per airspace). Control treatments included equivalent amounts of autoclaved wheat seeds or sterilized distilled water. After sealing with parafilm to facilitate gas exchange while preventing direct contact, we monitored mycelial growth until it reached the edge of the control plate. The hyphal diameter was measured, and we calculated the percentage of hyphal growth inhibition using the formula: Percentage inhibition (%) = [(Dc × Dt)/Dc] × 100, where Dc represents the hyphal growth of the fungus on the control plate and Dt represents the mycelial growth on the test plate. Each treatment was conducted in triplicate and replicated twice.

2.3.2. Effect of RM-1-138 VOCs and L-linalool on sclerotia germination of S. rolfsii

We investigated the impact of RM-1-138 VOCs and L-linalool on sclerotia germination of *S. rolfsii* using the sealed plate technique, as described by Calvo et al. [45], following procedures similar to those in section 2.3.1. However, we utilized sclerotia production in *S. rolfsii*. Each experiment involved placing three small Petri dishes (50 × 15 mm, D × H) inside larger Petri dishes (140 × 20 mm, D × H). The smaller dishes were filled with 5 mL of PDA inoculated with sclerotia of *S. rolfsii* (10 sclerotia per plate). The larger Petri dishes contained varying concentrations (0–50 g per airspace) of WSC of strain RM-1-138 or L-linalool concentrations (0–100 μL per airspace). Control treatments included equivalent amounts of autoclaved wheat seeds or sterilized distilled water. After sealing with parafilm to allow gas exchange while preventing direct contact, we assessed sclerotia germination after 5 days of incubation. Toxicity of sclerotia germination was quantified as the percentage of sclerotia germinated. Each treatment was performed in triplicate, with each replicate consisting of 10 sclerotia, and the entire experiment was repeated twice.

To determine the efficacy of RM-1-138 VOCs and L-linalool in suppressing or eradicating the tested phytopathogen, mycelial plugs or sclerotia from PDA agar plates showing complete growth inhibition (100 %) were transferred to fresh PDA agar plates and incubated at 30 °C for three days. Absence of growth indicated eradication of the pathogen by RM-1-138 VOCs or L-linalool.

2.4. Biological control potential of RM-1-138 VOCs and L-linalool against S. rolfsii in chili pepper both in vitro and in vivo assays

2.4.1. In vitro experiment assay

For *in vitro* bioprotection assays, two types of *S. rolfsii* inoculum were investigated: mycelial *S. rolfsii* and sclerotia *S. rolfsii* (Fig. 1).

For this experiment with mycelial inoculation of *S. rolfsii*, two-compartment Petri dishes (140 × 20 mm, D × H) were used to physically separate the seedlings and VOCs while allowing gas exchange between compartments, following a modified method based on Marzouk et al. [44]. In one compartment of the Petri dishes, 20 mL of 0.9 % agar plus 1 % glucose was poured. Ten disinfected, uniform 5-day-old germinated seedlings, prepared as previously described, were then placed on the agar medium and allowed to grow for 7 days. Then, two 2-mm diameter fungal plugs from the periphery of an actively growing culture of *S. rolfsii* were placed around the chili seedlings (1 cm away) on the Petri dish plates. In the other compartment of the Petri dish, *S. philanthi* inoculum ranging from 0 to 50 g per airspace or L-linalool concentrations ranging from 0 to 100 μL per airspace were added. The dishes were sealed with parafilm to facilitate gas exchange while preventing direct contact. Control treatments included equal amounts of autoclaved wheat seeds or sterilized distilled water. Each setup was then incubated at 30 °C under a 12-h light/12-h dark photoperiod, as described by Tahir et al. [46]. After 5 days of inoculation, the number of surviving seedlings was counted. The experiments included three replicates per treatment, with each replicate consisting of 10 chili seedlings.

Table 1
Effect of volatile organic compounds (VOCs) from *S. philanthi* RM-1-138 (RM-1-138 VOCs) and L-linalool on mycelial growth of *S. rolfsii* (3 days) and sclerotia production (10 days) at 30 °C.

Treatment	Concentration	Mycelial growth (cm)		Inhibition (%)	No. of sclerotia/plates	Viability [a]
		In control	In RM-1-138 VOCs or L-linalool			
Wheat seed culture of RM-1-138 (g per air space)	0 g	5.0 ± 0.0	–	–	137.42^{a} ± 32.05	–
	10 g	5.0 ± 0.0	4.13^{a} ± 0.21	17.4^{c} ± 1.56	67.57^{b} ± 12.05	A
	20 g	5.0 ± 0.0	3.30^{a} ± 0.05	34.0^{b} ± 2.34	11.75^{c} ± 1.70	A
	30 g	5.0 ± 0.0	0.0^{b} ± 0.0	100^{a} ± 0.0	0.0^{d} ± 1.60	D
	40 g	5.0 ± 0.0	0.0^{b} ± 0.0	100^{a} ± 0.0	0.0^{d} ± 0.0	D
	50 g	5.0 ± 0.0	0.0^{b} ± 0.0	100^{a} ± 0.0	0.0^{d} ± 0.0	D
L-linalool (μL per air space)	0 μL	5.0 ± 0.0	–	–	127.33^{a} ± 23.01	–
	20 μL	5.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	0.0^{b} ± 0.0	D
	40 μL	5.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	0.0^{b} ± 0.0	D
	60 μL	5.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	0.0^{b} ± 0.0	D
	80 μL	5.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	0.0^{b} ± 0.0	D
	100 μL	5.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	0.0^{b} ± 0.0	D

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 significantly different, as determined by ANOVA followed by Tukey's HSD test at a significance level of $p > 0.05$.

[a] The viability of mycelial growth of *S. rolfsii* was determined 5 days after exposure to VOCs from *S. philanthi* RM-1-138 or L-linalool, followed by their transfer to a fresh PDA medium plate. The treatment with only 100 % VOC inhibited mycelial growth of *S. rolfsii* completely. A = alive, D = dead.

For this experiment involving inoculation with sclerotia *S. rolfsii*, the design was similar to the inoculation with mycelial *S. rolfsii*. However, two sclerotia of *S. rolfsii* were deposited on the Petri dish plates around the chili seedlings (1 cm away). The incubation and observation of surviving seedlings were conducted as described earlier. The experiments included three replicates per treatment, with each replicate consisting of 10 chili seedlings.

2.4.2. *In pot experiment assay*

2.4.2.1. Preparation of S. rolfsii inoculum. A 5 mm diameter mycelial plug was taken from a 3-day-old *S. rolfsii* colony grown on a PDA medium. It was then inoculated into flasks containing autoclaved wheat seeds at a rate of 1 an agar mycelial plug per 100 g of wheat seeds. The flasks were then incubated at 30 °C for an additional 7 days to cultivate the inoculum with wheat seeds.

2.4.2.2. Effect of RM-1-138 VOCs and L-linalool on Sclerotium root and stem rot in chili pepper seedlings. The effectiveness of VOCs from different inoculum sizes of RM-1-138 and L-linalool in controlling root and stem rot during the growth stage was assessed in tissue culture jars. We followed a method based on Tahir et al. [46] with modifications. In summary, WSC of strain RM-1-138, ranging from 0 to 50 g per airspace, or L-linalool concentrations ranging from 0 to 100 μL per air space, were placed in the treatment tissue culture jars (10 × 20 cm, D × H). After 10 days of growth, chili pepper seedlings were selected and transplanted into plastic pots (6 × 3 cm, D × H) containing autoclaved soil (a mixture of sand, clay, and organic matter). They were allowed to grow for an additional 10 days at a density of 4 chili seedlings per pot. Wheat seeds harboring *S. rolfsii* were inoculated in the center of each pot, positioned 2 cm away from the base of each pepper seedling (modified method from Qiu et al. [47]). These pots were then enclosed inside glass jars and sealed with parafilm to prevent the escape of strain RM-1-138 VOCs or L-linalool. Additionally, ten small holes (2 mm) were made at the bottom of each pot to expose the seedlings to the VOCs. Control treatments included equal amounts of autoclaved millet grain or sterilized distilled water. The jars containing the pots were placed under greenhouse conditions, and the number of surviving chili peppers was counted after 5 days of inoculation. Each treatment was replicated three times, with 5 pots per replicate (each pot containing 4 chili seedlings).

2.5. *Determination of the effect of RM-1-138 VOCs and L-linalool on chili pepper seed germination and plant growth*

To evaluate the effects of RM-1-138 VOCs and L-linalool, on chili pepper seed germination and plant growth, two distinct experimental systems were designed (Fig. 1).

2.5.1. *Effect on chili seed germination*

The effects of strain RM-1-138 VOCs and L-linalool on chili seed germination and growth parameters were investigated using two-compartment Petri dishes (140 × 20 mm, D × H), following the sealed plate technique described by Calvo et al. [45]. In one compartment of the Petri dish, 20 mL of 0.9 % agar was deposited [44]. Fifteen disinfected chili pepper seeds, prepared as previously described, were then placed on an agar medium. Next, *S. philanthi* inoculum, ranging from 0 to 50 g culture per air space, or L-linalool, at concentrations ranging from 0 to 100 μL per air space, was added to the other compartment of the Petri dish. The dishes were sealed with parafilm to allow gas exchange while preventing direct contact. Control treatments included equal amounts of autoclaved wheat seeds or sterilized distilled water. Each setup was incubated at 30 °C under a 12-h light/12-h dark photoperiod as described by Tahir et al. [46]. After 7 days, the percentage of germinated seeds, root and shoot lengths, and fresh weight were measured. The experiments included three replicates per treatment, with each replicate consisting of 15 chili seeds.

To determine the viability of chili pepper seeds after exposure to RM-1-138 VOCs or L-linalool, seeds (100 % inhibition) were transferred to a fresh 0.9 % agar medium plate and incubated continuously at 30 °C for 20 days. Complete inhibition of germination indicated seed death if no growth was observed.

2.5.2. *Effect on chili pepper plant growth*

To confirm the effect of RM-1-138 VOCs and L-linalool on chili pepper plant growth parameters, we investigated the growth stage of germinated chili pepper seeds. The bioassay utilized a modified method based on Marzouk et al. [44], employing two-compartment Petri dishes (140 × 20 mm, D × H) and the sealed plate technique described by Calvo et al. [41]. In one compartment of the Petri dish, 20 mL of 0.9 % agar was deposited [45]. Five disinfected chili pepper seeds, each with a radicle approximately 1 mm in size (5 days old) and prepared as previously described, were then placed on the agar medium. Next, the *S. philanthi* inoculum, ranging from 0 to 50 g culture per airspace, or L-linalool, at concentrations ranging from 0 to 100 μL per airspace, was added to the other compartment of the Petri dish. The dishes were sealed

Table 2
Effect of volatile organic compounds (VOCs) from *S. philanthi* RM-1-138 and L-linalool on sclerotia germination of *S. rolfsii* after 5 days at 30 °C.

Treatment	Concentration	No. sclerotia germination (% germination)		Viability [a]
		Untreated with RM-1-178 VOCs or L-linalool	Treated with RM-1-178 VOCs or L-linalool	
Wheat seed culture of RM-1-138 (g per air space)	0 g	10 ± 0 (100)	–	ND
	10 g	10 ± 0 (100)	7.0 ± 0.20[a] (70)	ND
	20 g	10 ± 0 (100)	0[b] (0)	D
	30 g	10 ± 0 (100)	0[b] (0)	D
	40 g	10 ± 0 (100)	0[b] (0)	D
	50 g	10 ± 0 (100)	0[b] (0)	D
L-linalool (μL per air space)	0 μL	10 ± 0 (100)	–	ND
	20 μL	10 ± 0 (100)	0 (0)	D
	40 μL	10 ± 0 (100)	0 (0)	D
	60 μL	10 ± 0 (100)	0 (0)	D
	80 μL	10 ± 0 (100)	0 (0)	D
	100 μL	10 ± 0 (100)	0 (0)	D

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 30). Values within the same row that share the same letter are not significantly different, as determined by ANOVA followed by Tukey's HSD test at a significance level of $p > 0.05$.

[a] The viability of sclerotia of *S. rolfsii* was determined 5 days after exposure to VOCs from *S. philanthi* RM-1-138 or L-linalool, followed by their transfer to a fresh PDA medium plate. The treatment with only 100 % VOC inhibited sclerotia of *S. rolfsii* completely. D = dead, ND = not detected.

with parafilm to allow gas exchange while preventing direct contact. Control treatments included equal amounts of autoclaved wheat seeds or sterilized distilled water. Each setup was incubated at 30 °C under a 12-h light/12-h dark photoperiod as described by Tahir et al. [46]. After 7 days, the root and shoot lengths, and fresh weight were measured. The experiments included three replicates per treatment, with each replicate consisting of five germinated seedlings, and the entire experiment was replicated twice.

The morphological effects of RM-1-138 VOCs and L-linalool on chili pepper growth were observed using a Leica microscope at the Office of Scientific Instrument and Testing, Prince of Songkla University.

2.6. Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test to determine statistically significant differences between treated samples and the untreated control, with a significance threshold set at $p < 0.05$.

3. Results

3.1. Effect of VOCs from S. philanthi RM-1-138 and L-linalool on mycelial growth and sclerotia germination of S. rolfsii

The effects of RM-1-138 VOCs and L-linalool on the mycelial growth of *S. rolfsii* and sclerotia production are detailed in Table 1. Increasing the inoculum of WSC of RM-1-138 significantly ($p < 0.05$) affected mycelial growth and sclerotia production of *S. rolfsii*. In the control, *S. rolfsii* grew to 5.0 cm and produced 137.42 sclerotia per plate, showing significantly higher growth and sclerotia production compared to the treatments with VOCs in WSC of RM-1-138. However, exposure to RM-1-138 VOCs in WSC at concentrations of 10–50 g per air space reduced *S. rolfsii* growth from 5.0 cm to 0.0 cm, with inhibition rates ranging from 17.4 % to 100 %. Sclerotia production was also significantly reduced from 137.42 to 0.0 sclerotia per plate. Notably, at 30 g per air space, growth was completely (100 %) inhibited. However, the inhibition at 40 and 50 g per air space was not significantly different ($p > 0.05$) from the control. To further assess the fungicidal effects of RM-1-138 VOCs, mycelium showing complete (100 %) inhibition on PDA agar plates was transferred to fresh PDA plates after reaching 0.5 cm of growth. These experiments confirmed that RM-1-138 VOCs at 30–50 g per air space effectively killed *S. rolfsii*.

Table 3
In vitro sealed plate bioassay presenting the survival percentage of chili pepper seedlings after inoculation with *S. rolfsii* mycelia or sclerotia and exposure to volatile organic compounds from *S. philanthi* RM-1-138 and L-linalool at 30 °C for 5 days.

Treatment	Concentration	Percent Survival	
		Inoculated with mycelia of S. rolfsii	Inoculated with sclerotia of S. rolfsii
Wheat seed culture of RM-1-138 (g per air space)	0 g	0.0[d] ± 0.0	0.0[d] ± 0.0
	10 g	50.00[c] ± 3.87	43.33[c] ± 5.77
	20 g	83.33[b] ± 5.77	86.67[b] ± 7.56
	30 g	100[a] ± 0.0	100[a] ± 0.0
	40 g	100[a] ± 0.0	100[a] ± 0.0
	50 g	100[a] ± 0.0	100[a] ± 0.0
L-linalool (μL per air space)	0 μL	0.0[c] ± 0.0	0.0[c] ± 0.0
	20 μL	86.67[b] ± 2.78	83.33[b] ± 5.32
	40 μL	100[a] ± 0.0	100[a] ± 0.0
	60 μL	100[a] ± 0.0	100[a] ± 0.0
	80 μL	100[a] ± 0.0	100[a] ± 0.0
	100 μL	100[a] ± 0.0	100[a] ± 0.0

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 30). Values within the same row that share the same letter are not significantly different, as determined by ANOVA followed by Tukey's HSD test at a significance level of $p > 0.05$.

The effects of L-linalool VOCs on the mycelial growth of *S. rolfsii* and sclerotia production are presented in Table 1. In the control, *S. rolfsii* grew to 5.0 cm and produced 127.33 sclerotia per plate, showing significantly higher growth and sclerotia production compared to treatments with L-linalool VOCs. Treatment with L-linalool at concentrations ranging from 20 to 100 μL per air space resulted in complete (100 %) inhibition of *S. rolfsii* growth, with no significant ($p > 0.05$) difference observed between the concentrations. Furthermore, all tested concentrations of L-linalool were able to kill *S. rolfsii*, indicating its fungicidal effect. Based on these results, we conclude that L-linalool in RM-1-138 VOCs is likely the main factor responsible for inhibiting the growth of *S. rolfsii*.

Table 2 presents the effect of RM-1-138 VOCs and L-linalool on the germination of *S. rolfsii* sclerotia. In the control treatment, *S. rolfsii* sclerotia exhibited 100 % germination on PDA medium, which was higher than the germination observed when exposed to RM-1-138 VOCs and L-linalool. Significant inhibition ($p < 0.05$) of sclerotia germination was observed when exposed to the trapped atmosphere of RM-1-138 VOCs at concentrations ranging from 10 to 50 g per air space of wheat seed culture. At 20 g per air space, sclerotia germination was completely inhibited and killed, which was a lower concentration than the 30 g per air space required to inhibit mycelial growth. L-linalool at all tested concentrations (20–100 μL per air space) completely (100 %) inhibited and killed *S. rolfsii*.

3.2. Biological control potential of RM-1-138 VOCs and L-linalool against S. rolfsii in chili peppers both in vitro and in vivo assays

3.2.1. In vitro experiment assay

An *in vitro* sealed plate bioassay was conducted to evaluate the survival percentage of chili pepper seedlings after inoculation with *S. rolfsii* mycelia or sclerotia and exposure to VOCs from strain RM-1-138 and L-linalool. The results, summarized in Table 3, demonstrate significant inhibition ($p < 0.05$) of *S. rolfsii* in chili pepper seedlings by VOCs from strain RM-1-138 and L-linalool compared to the control, which showed 0 % survival. The highest survival rate (100 %) was observed when

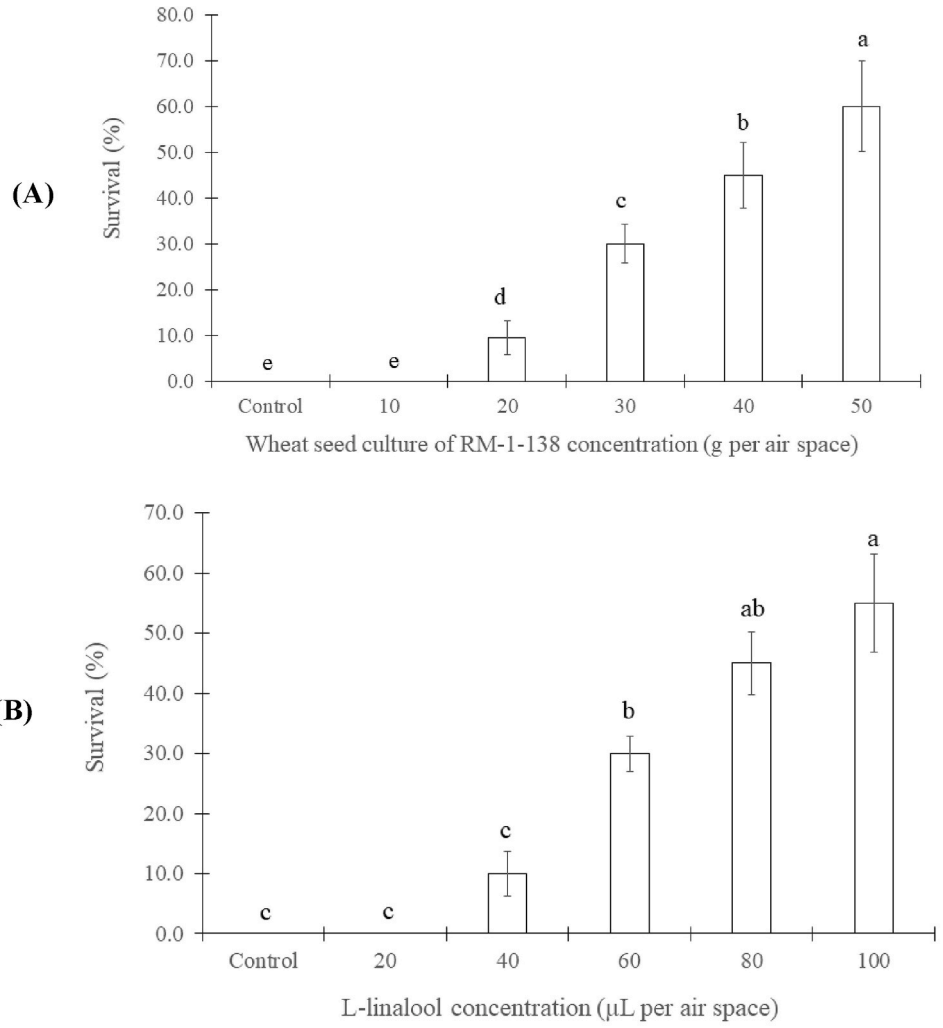

Fig. 2. *In vivo* plastic pots bioassay showing the survival percentage of chili pepper seedlings after inoculation with *S. rolfsii* and exposure to volatile organic compounds from *S. philanthi* RM-1-138 **(A)** and L-linalool **(B)** at 30 °C for 5 days. **(C)** shows disease symptoms in the control **(a)**, with the application of 50 g per air space of wheat seed culture of RM-1-138 **(b)**, and with the application of 100 μL per air space of L-linalool **(c)**. The data represent the mean of three replicates, with 5 pots per replicate (each pot containing 4 chili seedlings) ± standard deviation (SD) (n = 60). Values with the same letter do not differ significantly ($p > 0.05$) according to Tukey's multiple range test.
Note: Chili pepper seedlings were grown for 20 days before being tested in the experiment.

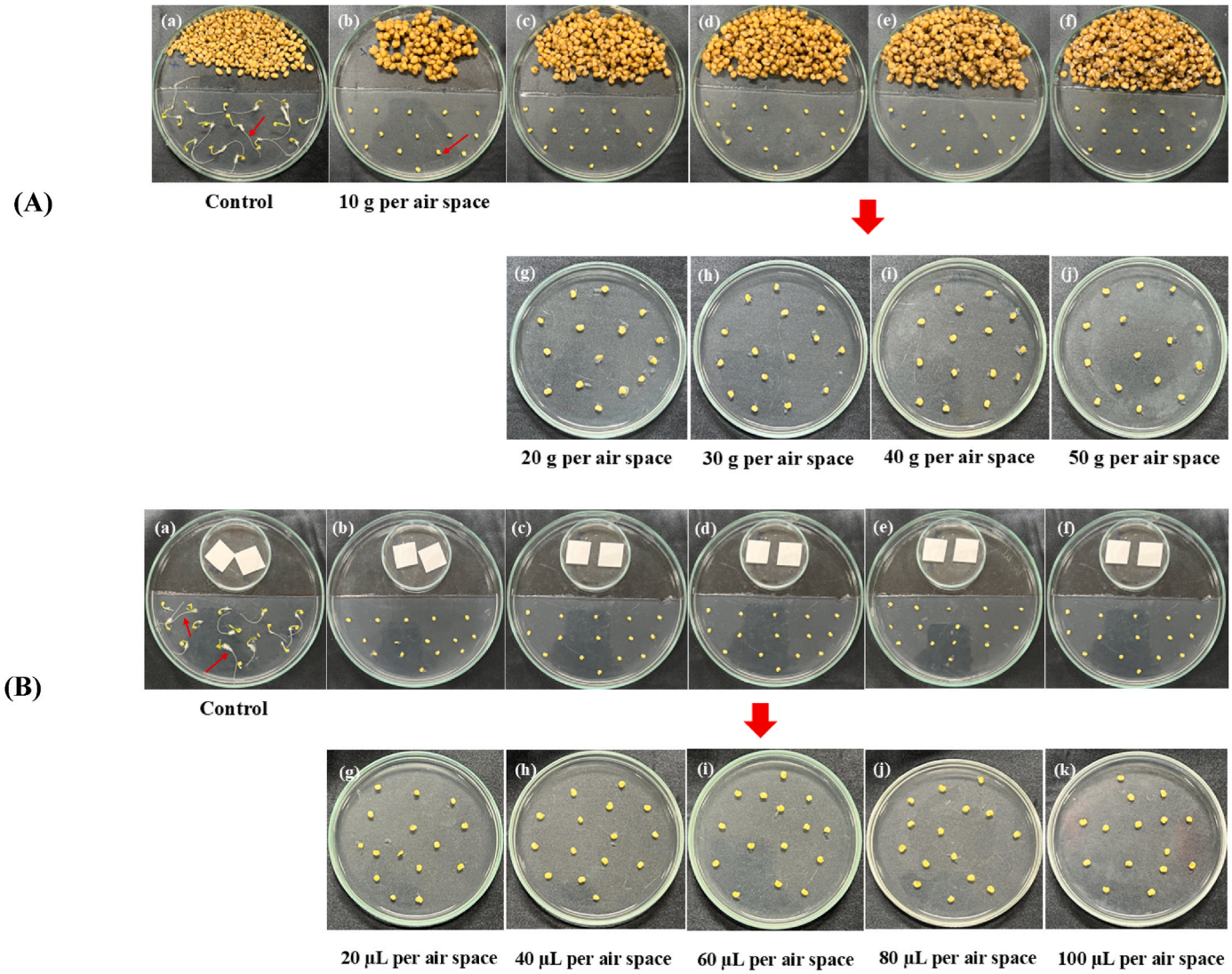

Fig. 3. Effect of volatile organic compounds (VOCs) produced in different wheat seed cultures (0–50 g per air space) by **(A)** *S. philanthi* RM-1-138 on chili pepper seed germination **(a–f)** and chili seed death **(g–j)**, and **(B)** L-linalool (0–100 µL per air space) on chili pepper seed germination **(a–f)** and chili seed death **(g–k)** after incubation at 30 °C.
Note: Seed germination was determined after 7 days. Seed death was determined 20 days after exposure to VOCs from *S. philanthi* RM-1-138 or L-linalool, followed by their transfer to a fresh 0.9 % agar medium plate.

seedlings were fumigated with 30 g per airspace of WSC of strain RM-1-138. Similarly, L-linalool provided effective control, achieving 100 % survival at 40 µL per airspace after inoculation with *S. rolfsii* mycelia or sclerotia.

3.2.2. *In pot experiment assay*

The biocontrol efficacy of VOCs from strain RM-1-138 and L-linalool against *S. rolfsii* in chili pepper seedlings was assessed using a plastic pots bioassay. After five days, chili pepper seedlings inoculated with *S. rolfsii* without the application of RM-1-138 VOCs or L-linalool showed 0 % survival (Fig. 2A–a). In contrast, seedlings treated with RM-1-138 VOCs (Fig. 2A, b) and L-linalool (Fig. 2B and c) demonstrated significantly ($p < 0.05$) higher survival rates compared to the control. The highest survival rate (60.0 %) was observed when seedlings were fumigated with 50 g of WSC of strain RM-1-138 (Fig. 2A). Similarly, L-linalool provided effective control, achieving 55 % survival at a concentration of 100 µL per airspace after inoculation with *S. rolfsii* (Fig. 2B). The biocontrol ability of RM-1-138 VOCs or L-linalool on *Sclerotium* root and stem rot symptoms was confirmed in the plastic pots bioassay, as illustrated in Fig. 2C.

3.3. *Determination of the effect of RM-1-138 VOCs and L-linalool on chili pepper seed germination and plant growth*

Chili pepper seeds were fumigated with varying concentrations of RM-1-138 WSC (10–50 g per airspace, Fig. 3A) and L-linalool (20–100 µL per airspace, Fig. 3B) in an agar medium plate bioassay for seven days. The results on seed parameters (germination, stem length, root length, and fresh weight) of chili pepper seeds are summarized in Table 4 and Fig. 3. In the control treatment, chili pepper seeds exhibited 100 % germination, a stem length of 20.11 mm, a root length of 42.67 mm, and a fresh weight of 22.22 mg per seedling. Fumigation with WSC of strain RM-1-138 significantly affected ($p < 0.05$) chili pepper seed parameters (Fig. 3A). At 10 g per airspace of WSC of RM-1-138, seed germination reduced to 17.78 %, and stem length decreased to 3.22 mm compared to the control (Table 4). Increasing the concentration of WSC of strain RM-1-138 to 20 g per airspace completely inhibited (100 %) chili pepper seed germination (Fig. 3A–c-f). The viability of chili pepper seeds was assessed 20 days after exposure to RM-1-138 VOCs. Transferring the seeds to a fresh agar medium plate revealed that RM-1-138 VOCs at concentrations up to 20 g per airspace could kill the chili

Table 4
Effect of volatile organic compounds from *S. philanthi* RM-1-138 and L-linalool on seed germination, stem length, root length and fresh weight of chili pepper seeds after incubated 30 °C in for 7 days.

Treatment	Concentration	Parameters				Viability[a]
		Percentage of seed germination	Stem length (mm)	Root length (mm)	Fresh weight (mg seedling^{-1})	
Wheat seed culture of RM-1-138 (g per air space)	0 g	$100^{a} \pm 0.0$	$20.11^{a} \pm 1.60$	$42.67^{a} \pm 4.06$	$22.22^{a} \pm 2.87$	ND
	10 g	$17.78^{b} \pm 1.38$	$3.22^{b} \pm 0.60$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	ND
	20 g	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	30 g	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	40 g	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	50 g	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
L-linalool (µL per air space)	0 µL	$100^{a} \pm 0.0$	$18.22^{a} \pm 2.65$	$38.11^{a} \pm 5.54$	$18.89^{a} \pm 2.40$	ND
	20 µL	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	40 µL	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	60 µL	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	80 µL	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D
	100 µL	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	D

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 45). Values within the same row that share the same letter are not significantly different, as determined by ANOVA followed by Tukey's HSD test at a significance level of $p > 0.05$.

[a] The viability of chili pepper seeds was determined 15 days after exposure to VOCs from *S. philanthi* RM-1-138 or L-linalool, followed by their transfer to a fresh 0.8 % agar medium plate. The treatment with only 100 % VOC inhibited chili pepper seed germination completely. D = dead, ND = not detected.

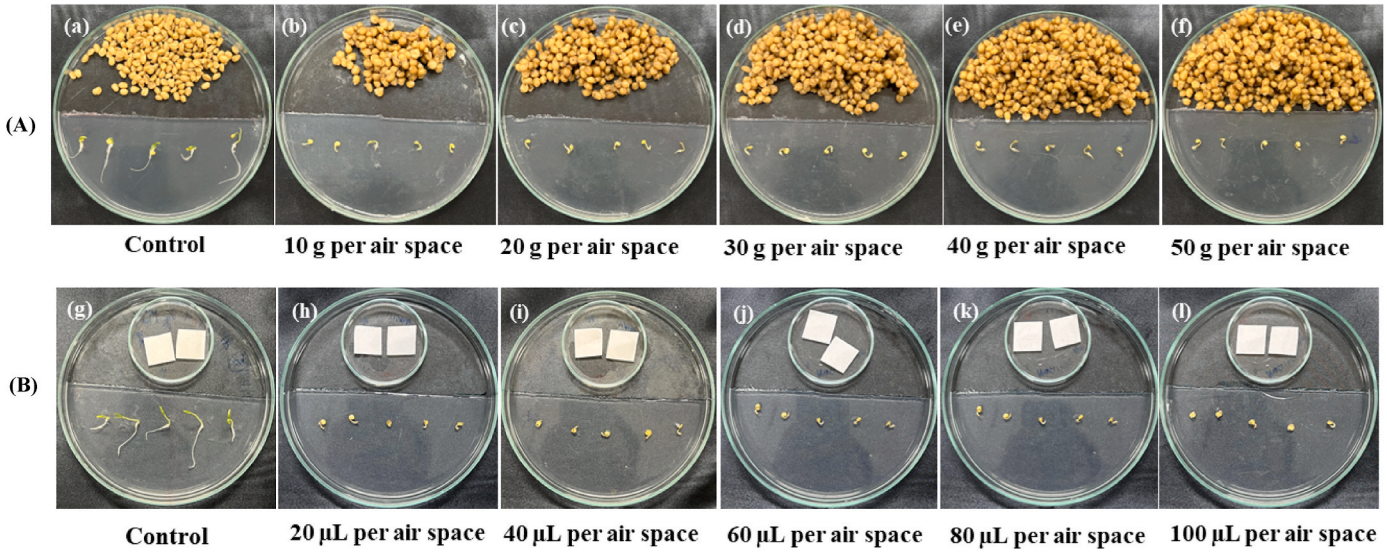

Fig. 4. Effect of volatile organic compounds (VOCs) produced in different wheat seed cultures (0–50 g per air space) by **(A)** *S. philanthi* RM-1-138 **(a–f)**, and **(B)** L-linalool (0–100 µL per air space) **(g–l)** on chili pepper growth in 0.9 % agar medium after 7 days of incubation at 30°C
Note: Chili seeds, which had radicles appearing and growing to about 1 mm in size, were used in the experiment.

pepper seeds, as shown in Fig. 3A–g-j. Table 4 and Fig. 3B present the effect of L-linalool on chili pepper seed parameters. The results were similar to those observed with RM-1-138 VOCs. At the lowest concentration of 20 µL per airspace of L-linalool, chili pepper seeds were killed (Fig. 3B–g-k).

The impact of RM-1-138 VOCs (Fig. 4A) and L-linalool (Fig. 4B), on the growth of chili pepper seeds with approximately 1 mm radicles before treatment was investigated. The results, summarized in Table 5 and Fig. 4, indicate a significant effect ($p < 0.05$) on chili pepper growth development when treated with RM-1-138 VOCs or L-linalool compared to the control treatment. In the RM-1-138 VOCs treatment at a concentration of 10 g per air space, the chili pepper seedlings exhibited reduced growth metrics: a stem length of 4.1 mm, a root length of 5.00 mm, and a fresh weight of 4.82 mg per seedling. These values were significantly ($p < 0.05$) lower than those observed in the control group, which had a stem length of 16.80 mm, a root length of 32.00 mm, and a fresh weight of 15.08 mg per seedling (Table 5 and Fig. 4A–a). Notably, when the concentration of RM-1-138 reached at least 20 g per air space, it inhibited chili pepper growth development and caused seed rot (Fig. 4A, b-f). Table 5 and Fig. 4B details the effects of L-linalool on chili pepper growth. Similar to RM-1-138 VOCs, L-linalool at the lowest tested concentration of at least 20 µL per air space resulted in no development of the chili pepper seeds, which subsequently rotted (Fig. 4B–h-l).

Microscopy images showing the morphological effects on chili pepper growth in untreated samples (Fig. 5A–a, b) and those treated with RM-1-138 VOCs (Fig. 5B, c, d) and L-linalool (Fig. 5C–e, f) are presented in Fig. 5. In the control treatment, normal growth was observed with green cotyledons, green hypocotyls, and white radicles (Fig. 5A–a, b). After treatment with RM-1-138 VOCs (Fig. 5B, c) and L-linalool (Fig. 5C–e), chili pepper seeds failed to develop cotyledons. Additionally, the hypocotyls and radicles darkened, turned brown, and eventually rotted (Fig. 5B–d; Fig. 5C–f). These findings indicate that RM-1-138 VOCs and L-linalool are highly toxic to chili pepper seed germination and plant growth.

Table 5
Effect of volatile organic compounds from *Streptomyces philanthi* RM-1-138 and L-linalool on chili pepper growth after incubated 30 °C in for 7 days.

Treatment	Concentration	Stem length (mm)	Root length (mm)	Fresh weight (mg seedling^{-1})
Wheat seed culture of RM-1-138 (g per air space)	0 g	16.80^{a} ± 0.84	32.00^{a} ± 2.00	15.08^{a} ± 3.79
	10 g	4.1^{b} ± 0.55	5.00^{b} ± 0.00	4.82^{b} ± 2.95
	20 g	RS^{c}	RS^{c}	RS^{c}
	30 g	RS^{c}	RS^{c}	RS^{c}
	40 g	RS^{c}	RS^{c}	RS^{c}
	50 g	RS^{c}	RS^{c}	RS^{c}
L-linalool (μL per air space)	0 μL	16.60^{a} ± 1.67	29.60^{a} ± 3.83	19.08^{a} ± 1.12
	20 μL	RS^{b}	RS^{b}	RS^{b}
	40 μL	RS^{b}	RS^{b}	RS^{b}
	60 μL	RS^{b}	RS^{b}	RS^{b}
	80 μL	RS^{b}	RS^{b}	RS^{b}
	100 μL	RS^{b}	RS^{b}	RS^{b}

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 significantly different, as determined by ANOVA followed by Tukey's HSD test at a significance level of $p > 0.05$.
Chili seeds, which had radicles appearing and growing to about 1 mm in size, were used in the experiment.
RS = Rotten Seeding.

4. Discussion

Different VOCs from *Streptomyces* species have been profiled for their antifungal properties [32,34,48,49]. Specifically, *S. philanthi* RM-1-138, when cultivated in wheat seeds, has been reported to produce VOCs with strong antifungal effects against *R. solani* [42] and *C. gloeosporioides* [43]. The VOCs produced by *S. philanthi* RM-1-138, including L-linalool as the most abundant compound [42], have shown to protect chili pepper seedlings in both *in vitro* and pot experiments. However, they also appear to be toxic to seed germination and plant growth. This suggests that while these VOCs have potential protective effects, their use may need to be carefully managed due to their negative impact on plant development.

The antifungal effect of these VOCs seems to be indirect, as no direct contact between the bacteria and the pathogen is required. Our results indicate that the VOCs from RM-1-138, including L-linalool, inhibited the hyphal growth and sclerotia formation of *S. rolfsii* in a sealed plate assay. The inhibition was persistent, as growth did not resume even when the pathogen was transferred to fresh PDA agar plates. These findings suggest that the VOCs produced by RM-1-138 could be a potential method for controlling *S. rolfsii*. The fungicidal activity of these VOCs is attributed to mechanisms such as cell wall disruption, alteration of membrane permeability, distortion of hyphae through vacuolization, and downregulation of ergosterol production genes, which collectively induce changes in fungal growth and pathogenicity [42,50–52]. This observation suggests that the VOCs from strain RM-1-138 exert a bacteriostatic effect, preventing the pathogen from recovering and resuming growth after initial inhibition. This evidence underscores the potent antifungal activity of the VOCs produced by strain RM-1-138, highlighting their potential as a biocontrol agent against *S. rolfsii*. The irreversible inhibition of both hyphal growth and sclerotia formation emphasizes the efficacy of these compounds in managing fungal pathogens and suggests promising applications for sustainable agricultural practices.

Similar findings have been reported by other researchers. Ayed et al.

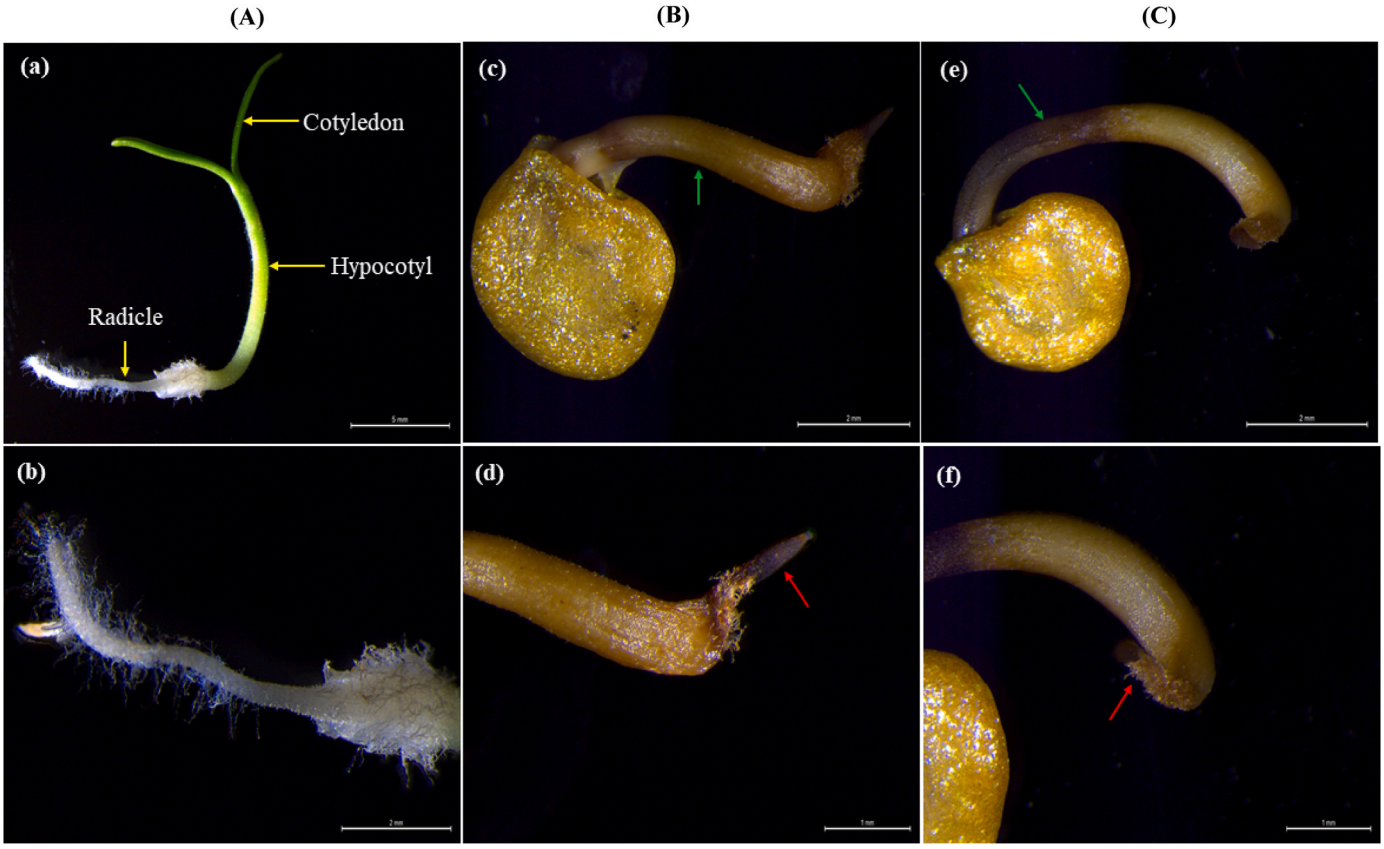

Fig. 5. Microscopy image showing the morphological effect on chili pepper after treatment with volatile organic compounds (VOCs) produced in wheat seed cultures by *S. philanthi* RM-1-138 and L-linalool after 7 days of incubation at 30 °C. **(A)** Untreated with RM-1-138 VOCs or L-linalool **(a, b)**, **(B)** treated with RM-1-138 VOCs **(c, d)**, and **(C)** treated with L-linalool **(e, f)**.

[53] demonstrated that the VOCs of *S. lydicus* significantly inhibited various plant pathogens, including *S. rolfsii*, *Phoma medicaginis*, *Fusarium solani*, *F. oxysporum*, and *R. solani*. Wu et al. [54] found that the VOCs produced by *S. albulus* NJZJSA2 inhibited the mycelial growth of *Sclerotinia sclerotiorum* and *F. oxysporum*, and completely inhibited the germination of *S. sclerotiorum* sclerotia and *F. oxysporum* conidia. Li et al. [55] reported that the VOCs produced in wheat seed culture by *S. globisporus* JK-1 suppressed the mycelial growth of various plant pathogens, especially *B. cinerea* and *S. sclerotiorum*. Additionally, Gong et al. [56] identified that 2-ethyl-5-methylpyrazine and dimethyl disulfide produced by *S. setonii* WY228 significantly inhibited the mycelial growth and spore germination of *Ceratocystis fimbriata*. These studies collectively highlight the broad-spectrum antifungal properties of VOCs produced by various *Streptomyces* species, reinforcing the potential of these compounds in developing effective and eco-friendly biocontrol strategies against a wide range of plant pathogens.

The findings demonstrated a strong inhibitory effect of these VOCs on the growth of *S. rolfsii in vitro*, which warranted further investigation into their potential application for protecting chili pepper seedlings in both *in vitro* and *in pot* experiments models. The VOCs produced by strain RM-1-138 and L-linalool provided complete protection to chili pepper seedlings from *S. rolfsii* infection. This suggests that these compounds may inhibit the pathogen's ability to establish an infection or affect its growth processes. Possible mechanisms could include direct antifungal activity, such as the inhibition of mycelial growth or sclerotia formation, as observed in previous assays. Additionally, these VOCs may trigger plant defense responses, increasing the seedlings' resistance to *S. rolfsii* infection. However, further studies are needed to explore these potential mechanisms in more detail. In pot bioassays, VOCs from strain RM-1-138 (60 % survival) and L-linalool (55 % survival) reduced *Sclerotium* root and stem rot compared to the control, although the protection was lower than in the *in vitro* assay (100 % survival). This difference may be due to the natural evaporation of the substances in the pot experiments, unlike the sealed plate bioassay, which is a closed system where VOCs remain concentrated.

Although VOCs produced by strain RM-1-138 have been explored for their potential to control plant pathogens [42,43], their plant growth-promoting benefits on chili pepper have not been previously studied. In other plants, VOCs have shown promising PGPB effects. For instance, Marzouk et al. [44] reported that VOCs from *B. subtilis* TRC7 increased hypocotyl length, radicle length, fresh weight, and vigor in tomato seedlings. Similarly, VOCs from *Streptomyces* enhanced shoot and root growth in *Phaseolus vulgaris* seedlings [39], increased fresh weight in tobacco seedlings [40], and promoted biomass in *Arabidopsis thaliana* seedlings [41]. Furthermore, VOCs from *Streptomyces* PM5 improved growth and metabolism in tomato plants [23]. In contrast to these beneficial effects, our study found that VOCs produced by strain RM-1-138 and L-linalool were detrimental to chili pepper seed germination and growth. Our results showed that VOCs from RM-1-138 and L-linalool killed the chili pepper seeds. Specifically, chili pepper seeds with radicles approximately 1 mm in size exposed to these VOCs exhibited a strong negative impact on stem length, root length, and fresh weight compared to the control. Microscopy images showed that both VOCs inhibited cotyledon development, and the hypocotyls and radicles darkened and rotted. These observations suggest that while the VOCs affect *S. rolfsii*, they also impact seedling growth, highlighting the need to assess their effects on plant development. These findings align with previous studies showing that the culture broth of strain RM-1-138 strongly inhibited chili pepper seed germination and seedling growth under laboratory conditions [31]. Our results show that both the culture broth [31] and VOCs produced by strain RM-1-138 have phytotoxic effects on chili pepper. This differs from the plant growth-promoting effects seen in other studies, suggesting that VOC activity may vary depending on the plant species and concentration used.

To address the issue of phytotoxicity, further research should focus on finding a concentration of VOCs that is effective in controlling pathogens but has less impact on plant growth. One possible approach could be to use lower concentrations of VOCs or combine them with other plant growth-promoting bacteria or chemical elicitors to reduce toxicity while maintaining their antibacterial effects. It would also be useful to explore different application methods, such as targeted or controlled release, to minimize plant exposure to higher concentrations of VOCs. These strategies could help ensure that the biocontrol benefits are achieved without compromising plant health. More studies are needed to better understand the mechanisms behind the observed toxicity and to assess whether these VOCs can be safely used in different crops or conditions.

5. Conclusion

Our study suggests that VOCs from RM-1-138 and L-linalool have potential for managing root and stem rot in chili peppers caused by *S. rolfsii*. These compounds inhibited *S. rolfsii* mycelial growth and sclerotia formation, and bioprotection assays demonstrated complete seedling survival under *in vitro* conditions. However, their effectiveness was lower in pot experiments, likely due to differences in VOC retention. Additionally, our findings indicate that RM-1-138 VOCs and L-linalool negatively affect chili pepper seed germination and growth, inhibiting cotyledon development and impairing stem and root formation. While these VOCs show significant potential for controlling *S. rolfsii*, further studies are needed to explore strategies—such as combining them with plant growth-promoting bacteria or chemical elicitors—to mitigate toxicity and enhance seed germination and root development in chili peppers.

CRediT authorship contribution statement

Sawai Boukaew: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Siriporn Yossan:** Writing – review & editing. **Krittin Chumkaew:** Writing – review & editing. **Chaiyasit Punfujinda:** Writing – review & editing. **Jirayu Buatong:** Writing – review & editing. **Sophon Boonlue:** Writing – review & editing. **Amornrat Chumthong:** Writing – review & editing. **Wanida Petlamul:** Writing – review & editing.

Availability of data and materials

Not applicable.

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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.

References

[1] R. Dixit, L. Agrawal, S.P. Singh, Prateeksha, P.C. Singh, V. Prasad, P.S. Chauhan, *Paenibacillus lentimorbus* induces autophagy for protecting tomato from *Sclerotium rolfsii* infection, Microbiol. Res. 215 (2018) 164–174, https://doi.org/10.1016/j.micres.2018.07.008.

[2] A.P. Sridharan, S. Thankappan, G. Karthikeyan, S. Uthandi, Comprehensive profiling of the VOCs of *Trichoderma longibrachiatum* EF5 while interacting with *Sclerotium rolfsii* and *Macrophomina phaseolina*, Microbiol. Res. 236 (2020) 1264, https://doi.org/10.1016/j.micres.2020.126436.

[3] D. Song, R. Wen, K. Chen, F. Wen, D. Xing, Z. Wu, Application of thifluzamide to stem rot in peppers: infection and control mechanisms of *Sclerotium rolfsii*, Pestic. Biochem. Physiol. 200 (2024) 105846, https://doi.org/10.1016/j.pestbp.2024.105846.

[4] R.H. Yi, G.G. Long, K.Y. Li, X.Y. Wang, Y. Huang, D. Li, First report of southern blight of *Manglietia decidua* caused by *Sclerotium rolfsii* in China, Plant Dis. 105 (2021) 1217, https://doi.org/10.1094/PDIS-10-20-2194-PDN, 1217.

[5] Y. He, P. Du, T. Zhao, F. Gao, M. Wang, J. Zhang, L. He, K. Cui, L. Zhou, Baseline sensitivity and bioactivity of tetramycin against *Sclerotium rolfsii* isolates in Huanghuai peanut-growing region of China, Ecotoxicol. Environ. Saf. 238 (2022) 113580, https://doi.org/10.1016/j.ecoenv.2022.113580.

[6] S. Jia, C. Song, H. Dong, X. Yang, X. Li, M. Ji, J. Chu, Evaluation of efficacy and mechanism of *Bacillus velezensis* CB13 for controlling peanut stem rot caused by *Sclerotium rolfsii*, Front. Microbiol. 14 (2023) 1111965, https://doi.org/10.3389/fmicb.2023.1111965.

[7] W. Seemakram, J. Paluka, T. Suebrasri, C. Lapjit, S. Kanokmedhakul, F. T. Schevenels, S. Boonlue, Fungicidal metabolites from *Lasiodiplodia brasiliensis* suppress sclerotium disease in cannabis, Rhizosphere 29 (2024) 100853, https://doi.org/10.1016/j.rhisph.2024.100853.

[8] J. You, T. Tang, F. Wang, T. Mao, B. Yuan, J. Guo, X. Guo, Y. Duan, J. Huang, Baseline sensitivity and control efficacy of strobilurin fungicide pyraclostrobin against *Sclerotium rolfsii*, Plant Dis. 105 (2021) 3503–3509, https://doi.org/10.1094/PDIS-01-21-0176-RE.

[9] N. Jabeen, A. Javaid, A. Shoaib, I.H. Khan, Management of southern blight of bell pepper by soil amendment with dry biomass of Datura metel, J. Plant Pathol. 103 (2021) 901–913, https://doi.org/10.1007/s42161-021-00874-6.

[10] Z.K. Punja, The biology, ecology and control of *Sclerotium rolfsii*, Annu. Rev. Phytopathol. 23 (1985) 97–127.

[11] A. Javaid, A. Ali, I.H. Khan, M.F.H. Ferdosi, Leaves of *Chenopodium album* as source of natural fungicides against *Sclerotium rolfsii*, Arab. J. Chem. 16 (2023) 104677, https://doi.org/10.1016/j.arabjc.2023.104677.

[12] E. Koch, J.O. Becker, G. Berg, R. Hauschild, J. Jehle, J. Köhl, K. Smalla, Biocontrol of plant diseases is not an unsafe technology!, J. Plant Dis. Prot. 125 (2018) 121–125, https://doi.org/10.1007/s41348-018-0158-4.

[13] A.G.Z. Cobos, Y.R. Bykbaev, F.P. Farfán, P.C. Perez, Evaluation of pesticide contamination risks and sustainable practices in Ecuadorian agriculture, Sci. Rep. 14 (2024) 26034, https://doi.org/10.1038/s41598-024-76733-y.

[14] T. Zhong, Z. Wang, M. Zhang, X. Wei, J. Kan, Z. Zalán, K. Wang, M. Du, Volatile organic compounds produced by *Pseudomonas fluorescens* ZX as potential biological fumigants against gray mold on postharvest grapes, Biol. Control 163 (2021) 104754, https://doi.org/10.1016/j.biocontrol.2021.104754.

[15] S.F.S. Ab Rahman, E. Singh, C.M.J. Pieterse, P.M. Schenk, Emerging microbial biocontrol strategies for plant pathogens, Plant Sci. 267 (2018) 102–111, https://doi.org/10.1016/j.plantsci.2017.11.012.

[16] S. Yossan, J. Kumla, N. Suwannarach, W. Petlamul, S. Srinuanpan, S. Dueramae, S. Boukaew, Exploration of antimicrobial activities and mechanisms of biocontrol agent *Serratia nematodiphila* BC-SKRU-1 against *Penicillium digitatum* in tangerine, Food Control 168 (2025) 110929, https://doi.org/10.1016/j.foodcont.2024.110929.

[17] S. Boukaew, J. Chuprom, J. Buatong, S. Sornprasit, S. Wijitsopa, K. Nooprom, R. Boonhok, Effective control of snake fruit (*Salacca zalacca*) rot using *Trichoderma asperelloides* SKRU-01: a safe approach to preserving fruit quality, Int. J. Food Microbiol. 430 (2025) 111037, https://doi.org/10.1016/j.ijfoodmicro.2024.111037.

[18] S. Boukaew, K. Chumkaew, S. Srinuanpan, Mechanism and effectiveness of natamycin produced by *Streptomyces philanthi* RL-1–178 in controlling green mold disease caused by *Penicillium digitatum* on postharvest Mandarin fruit (*Citrus reticulata* Blanco), J. Agric. Food Res. 19 (2025) 101585, https://doi.org/10.1016/j.jafr.2024.101585.

[19] S. Boukaew, K. Chumkaew, J. Kumla, N. Suwannarach, W. Petlamul, K. Nooprom, J. Chuprom, Preserving snake fruit (*Salacca zalacca*) Voss) quality: effective control of *Peniophora salaccae* SKRU002 with fungicides and biological agents, J. Agric. Food Res. 18 (2024) 101475, https://doi.org/10.1016/j.jafr.2024.101475.

[20] C. Wang, Z. Wang, X. Qiao, Z. Li, F. Li, M. Chen, Y. Wang, Y. Huang, H. Cui, Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1, FEMS Microbiol. Lett. 341 (2013) 45–51, https://doi.org/10.1111/1574-6968.12088.

[21] Z.R. Suárez-Moreno, D.M. Vinchira-Villarraga, D.I. Vergara-Morales, L. Castellanos, F.A. Ramos, C. Guarnaccia, G. Degrassi, V. Venturi, N. Moreno-Sarmiento, Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens, Front. Microbiol. 10 (2019) 290, https://doi.org/10.3389/fmicb.2019.00290.

[22] X. Zheng, J. Wang, Z. Chen, H. Zhang, Z. Wang, Y. Zhu, B. Liu, A *Streptomyces* sp. strain: isolation, identification, and potential as a biocontrol agent against soilborne diseases of tomato plants, Biol. Control 136 (2019) 104004, https://doi.org/10.1016/j.biocontrol.2019.104004.

[23] M.P. Dias, M.S. Bastos, V.B. Xavier, E. Cassel, L.V. Astarita, E.R. Santarém, Plant growth and resistance promoted by *Streptomyces* spp. in tomato, Plant Physiol, Biochemist 118 (2017) 479–493, https://doi.org/10.1016/j.plaphy.2017.07.017.

[24] B.T. Mathew, Y. Torky, A. Amin, A.-H.I. Mourad, M.M. Ayyash, A. El-Keblawy, A. Hilal-Alnaqbi, S.F. AbuQamar, K.A. El-Tarabily, Halotolerant marine rhizosphere-competent actinobacteria promote *Salicornia bigelovii* growth and seed production using seawater irrigation, Front. Microbiol. 11 (2020) 552, https://doi.org/10.3389/fmicb.2020.00552.

[25] K.A. El-Tarabily, G.A. Ramadan, A.A. Elbadawi, A.H. Hassan, S. Tariq, E. W. Ghazal, M.I.A. Gamar, S.F. AbuQamar, The marine endophytic polyamine-producing *Streptomyces mutabilis* UAE1 isolated from extreme niches in the *Arabian gulf* promotes the performance of mangrove (*Avicennia marina*) seedlings under greenhouse conditions, Front, Mar. Sci. 8 (2021) 710200, https://doi.org/10.3389/fmars.2021.710200.

[26] Z. Wang, C. Gao, J. Yang, R. Du, F. Zeng, H. Bing, B. Xia, Y. Shen, C. Liu, Endophytic *Streptomyces* sp. NEAU-ZSY13 from the leaf of *Perilla frutescens*, as a promising broad spectrum biocontrol agent against soil-borne diseases, Front. Microbiol. 14 (2023) 1243610, https://doi.org/10.3389/fmicb.2023.1243610.

[27] M.S. Abdel-Aziz, M.A. Ghareeb, A.A. Hamed, E.M. Rashad, E.R. El-Sawy, I.M. Saad, K.M. Ghoneem, Ethyl acetate extract of *Streptomyces* spp. isolated from Egyptian soil for management of *Fusarium oxysporum*: the causing agent of wilt disease of tomato, Biocatal. Agric, Biotechnol. 37 (2021) 102185, https://doi.org/10.1016/j.bcab.2021.102185.

[28] G. Abo-Zaid, A. Abdelkhalek, S. Matar, M. Darwish, M. Abdel-Gayed, Application of bio-friendly formulations of chitinase-producing *Streptomyces cellulosae* actino 48 for controlling peanut soil-borne diseases caused by *Sclerotium rolfsii*, J. Fungi 7 (2021) 167, https://doi.org/10.3390/jof7030167.

[29] S. Jacob, R.R. Sajjalaguddam, H.K. Sudini, *Streptomyces* sp. RP1A-12 mediate control of peanut stem rot caused by *Sclerotium rolfsii*, J. Integr. Agric. 17 (2018) 892–900, https://doi.org/10.1016/S2095-3119(17)61816-1.

[30] Z. Sun, Q. Wang, Y. Li, Z. Shen, X. Han, P. Chen, L. Shao, B. Fan, Y. Zhao, Secondary metabolites from *Streptomyces araujoniae* S-03 show biocontrol potential against *Rhododendron* root rot caused by *Phytophthora cinnamomic*, Forests 14 (2023) 2054, https://doi.org/10.3390/f14102054.

[31] S. Boukaew, S. Chuenchit, V. Petcharat, Evaluation of *Streptomyces* spp. for biological control of *Sclerotium* root and stem rot and *Ralstonia* wilt of chili pepper, BioControl 56 (2011) 365–374, https://doi.org/10.1007/s10526-010-9336-4.

[32] L. Cuervo, S. Álvarez-García, J.A. Salas, C. Méndez, C. Olano, M.G. Malmierca, The volatile organic compounds of *Streptomyces* spp.: an in-depth analysis of their antifungal properties,, Microorganisms 11 (2023) 1820, https://doi.org/10.3390/microorganisms11071820.

[33] M.F. Ruiz-Cisneros, J. de Jesús Ornelas-Paz, D.A. Pérez-Corral, G.I. Olivas-Orozco, D.I. Berlanga-Reyes, O.J. Cambero-Campos, M.O. Estrada-Virgen, S. Ordaz-Silva, M.Á. Salas-Marina, C. Rios-Velasco, *Streptomyces* strains inhibit the growth of *Fusarium kuroshium* and *Fusarium solani* and promote the growth of *Arabidopsis thaliana*, Biocontrol Sci. Technol. 34 (2024) 469–498, https://doi.org/10.1080/09583157.2024.2351825.

[34] M. Xing, T. Sun, T. Liu, Z. Jiang, P. Xi, Effectiveness of volatiles emitted by *Streptomyces abikoensis* TJGA-19 for managing litchi downy blight disease, Microorganisms 12 (2024) 184, https://doi.org/10.3390/microorganisms12010184.

[35] J. Köhl, R. Kolnaar, W.J. Ravensberg, Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy, Front. Plant Sci. 10 (2019) 845, https://doi.org/10.3389/fpls.2019.00845.

[36] M.S. Ngalimat, E.M. Hata, D. Zulperi, S.I. Ismail, M.R. Ismail, N.A.I. Mohd Zainudin, N.B. Saidi, M.T. Yusof, Induction of systemic resistance in rice plants against *Burkholderia glumae* infection by bioformulation of *Streptomyces* spp. under greenhouse conditions, Biol. Control 184 (2023) 105286, https://doi.org/10.1016/j.biocontrol.2023.105286.

[37] O.A.C. Almeida, N.O. de Araujo, A.T.N. Mulato, G.F. Persinoti, M.L. Sforça, M. J. Calderan-Rodrigues, J.V. de Castro Oliveira, Bacterial volatile organic compounds (VOCs) promote growth and induce metabolic changes in rice, Front. Plant Sci. 13 (2023) 1056082, https://doi.org/10.3389/fpls.2022.1056082.

[38] E.H. Verbon, L.M. Liberman, Beneficial microbes affect endogenous mechanisms controlling root development, Trends Plant Sci. 21 (2016) 218–229, https://doi.org/10.1016/j.tplants.2016.01.013.

[39] D.A. Pérez-Corral, J. de Jesús Ornelas-Paz, G.I. Olivas, C.H. Acosta-Muñiz, M.Á. Salas-Marina, D.I. Berlanga-Reyes, D.R. Sepulveda, Y. Mares-Ponce de León, C. Rios-Velasco, Growth promotion of *Phaseolus vulgaris* and *Arabidopsis thaliana* seedlings by *Streptomycetes* volatile compounds, Plants 11 (2022) 875, https://doi.org/10.3390/plants11070875.

[40] Y. He, W. Guo, J. Peng, J. Guo, J. Ma, X. Wang, C. Zhang, N. Jia, E. Wang, D. Hu, Z. Wang, Volatile organic compounds of *Streptomyces sp.* TOR3209 stimulated tobacco growth by up-Regulating the expression of genes related to plant growth and development, Front. Microbiol. 13 (2022) 891245, https://doi.org/10.3389/fmicb.2022.891245.

[41] V. Cordovez, V.J. Carrion, D.W. Etalo, R. Mumm, H. Zhu, G.P. van Wezel, J. M. Raaijmakers, Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil, Front. Microbiol. 6 (2015) 1081, https://doi.org/10.3389/fmicb.2015.01081.

[42] S. Boukaew, A. Plubrukarn, P. Prasertsan, Effect of volatile substances from *Streptomyces philanthi* RM-1–138 on growth of *Rhizoctonia solani* on rice leaf, BioControl 58 (2013) 471–482, https://doi.org/10.1007/s10526-013-9510-6.

[43] S. Boukaew, W. Petlamul, R. Bunkrongcheap, T. Chookaew, T. Kabbua, A. Thippated, P. Prasertsan, Fumigant activity of volatile compounds of *Streptomyces philanthi* RM-1–138 and pure chemicals (acetophenone and phenylethyl alcohol) against anthracnose pathogen in postharvest chili fruit, Crop Prot. 103 (2018) 1–8, https://doi.org/10.1016/j.cropro.2017.09.002.

[44] T. Marzouk, M. Chaouachi, A. Sharma, S. Jallouli, R. Mhamdi, N. Kaushik, N. Djébali, Biocontrol of *Rhizoctonia solani* using volatile organic compounds of solanaceae seed-borne endophytic bacteria, Postharvest Biol. Technol. 181 (2021) 111655, https://doi.org/10.1016/j.postharvbio.2021.111655.

[45] H. Calvo, I. Mendiara, E. Arias, A.P. Gracia, D. Blanco, M.E. Venturini, Antifungal activity of the volatile organic compounds produced by *Bacillus velezensis* strains against postharvest fungal pathogens, Postharvest Biol. Technol. 166 (2020) 111208, https://doi.org/10.1016/j.postharvbio.2020.111208.

[46] H.A.S. Tahir, Q. Gu, H. Wu, W. Raza, A. Safdar, Z. Huang, F.U. Rajer, X. Gao, Effect of volatile compounds produced by *Ralstonia solanacearum* on plant growth promoting and systemic resistance inducing potential of Bacillus volatiles, BMC Plant Biol. 17, 133, https://doi.org/10.1186/s12870-017-1083-6.

[47] Z.L. Qiu, S.D. Liu, X.G. Li, J. Zhong, J.Z. Zhu, Identification and mechanism characterization of *Streptomyces griseoaurantiacus* XQ-29 with biocontrol ability against pepper southern blight caused by *Sclerotium rolfsii*, Pestic. Biochem. Physiol. 202 (2024) 105956, https://doi.org/10.1016/j.pestbp.2024.105956.

[48] S. Boukaew, B. Cheirsilp, P. Prasertsan, S. Yossan, Antifungal effects of volatile organic compounds produced by *Streptomyces salmonis* PSRDC-09 against anthracnose pathogen in postharvest chili fruit, Appl. Microbiol. 131 (2021) 1452–1463, https://doi.org/10.1111/jam.15037.

[49] X. Li, L. Zhang, Y. Zhao, J. Feng, Y. Chen, K. Li, M. Zhang, D. Qi, D. Zhou, Y. Wei, W. Wang, J. Xie, Biocontrol potential of volatile organic compounds produced by *Streptomyces corchorusii* CG-G2 to strawberry anthracnose caused by *Colletotrichum gloeosporioides*, Food Chem. 437 (2024) 137938, https://doi.org/10.1016/j.foodchem.2023.137938.

[50] A. Giorgio, A.D. Stradis, P.L. Cantore, N.S. Iacobellis, Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia sclerotiorum*, Front. Microbiol. 6 (2015) 1056, https://doi.org/10.3389/fmicb.2015.01056.

[51] S. Tyagi, K.-J. Lee, P. Shukla, J.-C. Chae, Dimethyl disulfide exerts antifungal activity against *Sclerotinia minor* by damaging its membrane and induces systemic resistance in host plants, Sci. Rep. 10 (2020) 6547, https://doi.org/10.1038/s41598-020-63382-0.

[52] L. Weisskopf, S. Schulz, P. Garbeva, Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions, Nat. Rev. Microbiol. 19 (2021) 391–404, https://doi.org/10.1038/s41579-020-00508-1.

[53] A. Ayed, L. Kalai-Grami, I.B. Slimene, M. Chaouachi, H. Mankai, I. karkouch, N. Djebali, S. Elkahoui, O. Tabbene, F. Limam, Antifungal activity of volatile organic compounds from *Streptomyces* sp. strain S97 against *Botrytis cinerea*, Biocontrol Sci. Technol. 31 (2021) 1330–1348, https://doi.org/10.1080/09583157.2021.1947982.

[54] Y. Wu, J. Yuan, E. Yaoyao, W. Raza, Q. Shen, Q. Huang, Effects of volatile organic compounds from *Streptomyces albulus* NJZJSA2 on growth of two fungal pathogens, J. Basic Microbiol. 55 (2015) 1104–1117, https://doi.org/10.1002/jobm.201400906.

[55] Q. Li, P. Ning, L. Zheng, J. Huang, G. Li, Tom Hsiang Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit, Biol. Control 61 (2012) 113–120, https://doi.org/10.1016/j.biocontrol.2011.10.014.

[56] Y. Gong, J.-Q. Liu, M.-J. Xu, C.-M. Zhang, J. Gao, C.-G. Li, K. Xing, S. Qin, Antifungal volatile organic compounds from *Streptomyces setonii* WY228 control black spot disease of sweet potato, Appl. Environ. Microbiol. 88 (2022) e02317–e02321, https://doi.org/10.1128/aem.02317-21.