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RESEARCH PAPER

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Impact of environmental factors on *Streptomyces* spp. metabolites against *Botrytis cinerea*

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Abstract

Botrytis cinerea is an economically important disease on numerous vegetables including tomato. From our previous studies, a spore suspension of *Streptomyces philanthi* RL-1-178 and RM-1-138 and *Streptomyces mycarofaciens* SS-2-243 showed strong inhibition against *B. cinerea*. In this study, the efficacy of their antifungal metabolites against *B. cinerea* was investigated after enhancing the production through the optimum culture medium and environmental conditions (temperature, light/dark cycle). In vitro studies indicated that glucose yeast-malt (GYM) agar and incubation at 28°C were optimal for growth and mass spore production of all three *Streptomyces* strains. Moreover, light/dark conditions had a positive effect on the growth and spore production of *S. philanthi* RM-1-138 and RL-1-178 but not on *S. mycarofaciens* SS-2-243. Both strains of *S. philanthi* possessed an antifungal activity against *B. cinerea* (100% inhibition) while *S. mycarofaciens* showed different results on PDA (83% inhibition) and GYM (88% inhibition) at the optimum incubation temperature at 21°C. The antifungal compounds from *S. philanthi* RM-1-138 exhibited the highest protection efficacy against *B. cinerea* on tomato leaves (82.89% and 0.33 cm^2 lesion areas symptoms). The antifungal compounds RM-1-138, identified by GC-MS, were greatly altered based on components concentration under various temperatures and light/dark conditions. The anti-*B. cinerea* of *S. philanthi* RM-1-138 was established at a higher level in several metabolic compounds in the dark condition (11 and 32 antifungal compounds after incubation at 21°C and 28°C, respectively) than in the light condition (11 and 19 antifungal compounds after incubation at 21°C and 28°C, respectively). At 21°C, the dominant component was acetic acid (67.41% and 68.77% in light and dark conditions, respectively) while at 28°C, benzeneacetamide (43.58% in light) and propanamide (20.68% in the dark) were dominant. The results clearly demonstrated the significant influence of environmental factors on the production of antifungal metabolites of *Streptomyces* spp.

KEYWORDS
biological control, *Botrytis cinerea*, environmental conditions, *Streptomyces* spp., tomato leaves

1 | INTRODUCTION

The necrotrophic fungi *Botrytis cinerea* Pers. (teleomorph *Botryotiniafuckeliana* [de Bary] Whetzel) is the world's most dangerous fungal pathogen that contributes to economically implicated diseases in a variety of fruits and vegetables [1,2]. Biocontrol is an environmentally friendly and efficient alternative to chemical fungicide management of these pathogens [3,4]. Species of *Streptomyces* are potential biocontrol agents since they are ubiquitous in the environment, and can produce secondary metabolites such as enzyme inhibitors and antibiotics with diverse biological activities, including the ability to inhibit plant pathogenic fungi [5]. Several species of *Streptomyces* have been isolated and used to control plant pathogens in various crops, such as *B. cinerea* [6–10].

The influence of culture media and temperature on the growth of antagonistic *Streptomyces* spp. and microbial plant pathogens are important parameters. It is widely accepted that the temperature has a profound influence on the growth and production of bioactive compounds in *Streptomyces* species [11], as well as the development of disease in pathogen *B. cinerea*. Several studies showed the growth of *Streptomyces* species depend on the culture medium and temperature [12,13]. The medium containing 3% starch, 0.75% peptone, 0.025% yeast extract, 1% (w/v) soybean meal and minor/trace elements, initial pH of 6.5, and incubation at 28°C was the best condition for growth and antifungal activity of *S. platensis* 3–10 against *Plasmodiophora brassicae* [14]. GYM culture medium, containing 0.4% glucose, 0.4% yeast extract, and 1.0% malt extract, with an initial pH of 7.5 and a temperature of 30°C were found to be optimum for both cell growth and antifungal activity of *S. philanthi* RM-1-138 against *Rhizoctonia solani* [15]. The medium consisting of 3.0% glucose, 3.5% corn starch, 2.5% soybean meal, 1.2 mM $MgCl_2$, and 5.9 mM glutamate, and the temperature of 28°C was found as the most suitable condition for growth and antifungal activity of *S. rimosus* AG-P1441 against *Phytophthora capsici* [16]. Soyabean meal medium influenced the production of antibacterial compounds of *Streptomyces* spp. [17].

On the other hand, *B. cinerea* requires several specific conditions for their growth and reproduction. It is well known that growth and sporulation are important phases during the life of fungi, which is considerably influenced by external growth factors [18]. Among the external growth factors, nutrition is one of the determinants that has been previously proved by several workers in many fungal pathogens using different culture media sources [19–23]. Potato dextrose agar and incubation temperature ranging from 18°C to 22°C serve as the most conducive condition for the growth of *B. cinerea* [9,24–26].

Light conditions play a significant role in the growth and pigment production of *Streptomyces* spp. [17,27] and during disease development and pathogenicity of *B. cinerea* [26,28,29]. In *Streptomyces*, carotenoid production is a widespread metabolic activity, which occurs in a constitutive, light-dependent, or cryptic manner [27]. Light could enhance pigment production in the growing mycelia of *S. phaeopurpureus* 5125, *S. salmonicida* 5472, and *S. fulvissimus* compared to being cultivated in a dark condition [30]. The pigmented antibiotic of *S. coelicolor* A3 is produced under light induction [31]. Light can cause linkage between the population of diverse fungi, and the circadian clock of one fungal phytopathogen is linked to the pathogen's virulence program [32]. *B. cinerea* is a light-responsive strain that can actively sense any light conditions to fine-tune its development and pathogenicity [29]. Therefore, light is an essential developmental signal for *B. cinerea* as it triggers the exclusive formation of conidia, whereas constant darkness initiates the sole formation of sclerotia [28].

Under these circumstances, the present research work aimed to study the growth conditions of antagonistic bacteria and pathogenic fungi in different cultivating conditions. The objectives of this study are (i) to study the influences of culture media, temperature, and light/dark conditions on the growth of the three strains of antagonistic bacteria *Streptomyces* spp. and pathogenic fungi of *B. cinerea*, (ii) to evaluate the inhibitory ability of the three strains of *Streptomyces* spp. on the growth of *B. cinerea* in vitro and in tomato leaves, and (iii) to identify the chemical composition of the antifungal compounds of the selected strain of *Streptomyces* spp. grown at different temperatures with light and dark conditions using gas chromatography–mass spectrometry (GC-MS).

2 | MATERIALS AND METHODS

2.1 | Microorganisms

The antagonistic strain SS-2-243 of *Streptomyces mycarofaciens* and the strains RM-1-138 and RL-1-178 of *S. philanthi* were isolated from the rhizosphere of chili peppers in Southern Thailand [33] and kept on 20% glycerol at −20°C as a stock culture. They were subcultured freshly on glucose yeast-malt (GYM) agar and incubated at 28°C for 10 days before used. Spore suspensions of each strain of *Streptomyces* spp. were prepared by removing the spores from a 10-day-old culture and suspending them in sterile distilled water, then adjusting the spore concentration with sterile distilled water to 10^7 spores per milliliter using a hemocytometer.

A strain of *B. cinerea* had been isolated from infected tomato fruits with the typical symptom of gray mold in Chiang Rai province, Thailand, and identified by its morphological characteristics by Prof. Kevin David Hyde. It was obtained from the Center of Excellence in Fungal Research, Mae FahLuang University, Thailand. The fungal pathogen were maintained on potato dextrose agar (PDA) slant and kept at 4°C. The strain was sub-cultured freshly on PDA and incubated at 21°C for 3 days before used.

2.2 | The influence of culture media, temperature, and light/dark conditions on the growth of *Streptomyces* spp., and *B. cinerea*

The previous study of Boukaew et al. [33] has shown that GYM medium and incubation at 28°C were the best conditions for the growth of the three strains of *Streptomyces* spp. However, the growth condition on PDA medium and 21°C were not investigated. A 10^7 spores ml^{-1} of each strain of *Streptomyces* spp. were streaked individually on GYM agar and PDA, and incubated at 21°C and 28°C, respectively. To evaluate their ability in growing at different media and temperatures, the growth and pigment of spore mass were qualitatively determined after 14 days of incubation. Each treatment had three replicates.

The influence of light/dark condition on the growth of *Streptomyces* spp. was also explored. A 10^7 spores ml^{-1} of each strain of *Streptomyces* spp. was streaked on the selected medium and incubated in the light (photoperiod of light-dark (L:D) at 14:10 h) and dark (24 h) growth chambers at 21°C for 14 days. Samples were taken at a time interval to evaluate the ability of *Streptomyces* spp. to grow and produce pigment qualitatively in light/dark conditions.

A 5-mm-diameter mycelial plug, excised from a 3-day-old *B. cinerea* colony, was transferred to the center of GYM and PDA plate media and incubated at 21°C and 28°C. The ability of pathogenic fungi to grow at different media and temperatures was determined after 3 days of incubation. Growth was measured based on the diameter of mycelium produced (in centimeters). Each treatment had three replicates.

2.3 | The influence of culture media and temperature on antifungal activity of *Streptomyces* spp. against *B. cinerea*

S. philanthi RM-1-138 and RL-1-178 and *S. mycarofaciens* SS-2-243 were evaluated for their antagonistic properties against the pathogenic *B. cinerea* using a dual culture technique [33]. For each strain of *Streptomyces* spp., a streak of spore suspension at 10^7 spores ml^{-1} was deposited on one side of a GYM or PDA media in Petri dishes. Plates were then incubated in a growth chamber for 7 days at 21°C and 28°C.

A 5-mm-diameter mycelial plug, excised from a 3-day-old sample of the pathogenic fungi colony, was transferred to the center of each plate previously inoculated with the *Streptomyces* strains. As a control, a mycelial plug of the pathogenic fungi was placed on a GYM and PDA plate without any *Streptomyces* strain. The dual culture plates were incubated in a growth chamber at 21°C and 28°C for 3 days, after which the mycelial growth diameter of the pathogenic fungi was measured and compared to that of the control. Three replicates were conducted for each *Streptomyces* strain-*B. cinerea* individually. The colony size in each treatment was measured and the percentage inhibition of mycelial growth was calculated by the following formula: Percentage inhibition = ([Control − Treatment]/Control) × 100 [7,9].

2.4 | In vivo antagonistic activity of culture filtrates produced by *Streptomyces* spp. against *B. cinerea* on tomato leaves

The culture filtrates of *S. mycarofaciens* SS-2-243 and *S. philanthi* RM-1-138 and RL-1-178 were prepared in a 250 ml flask containing 100 ml GYM medium (pH adjusted at 7.0 before autoclaving) and incubated in the light (photoperiod L:D 14:10 h) growth chamber at 28°C. After 3 days of incubation, 5 ml aliquots of this culture was transferred into 100 ml fresh GYM medium and incubated for 10 days under the same condition. The culture broth was centrifuged (8880*g* for 20 min), then the supernatant was filtered through a 0.45 mm Millipore membrane to get the culture filtrate. The efficacy of culture filtrates produced by the three strains of *Streptomyces* spp. were evaluated for their biocontrol capacities against *B. cinerea* on tomato leaves using detached leaf assay [9]. These culture filtrates of each *Streptomyces* spp. were sprayed on tomato leaf (10 ml/leaf). When the leaves were dried (1 h), they were removed, and three leaflets were placed in a transparent plastic box with humid absorbent paper to maintain high relative humidity (close to 90%–95%). Mycelial plugs (5-mm in diameter) of *B. cinerea* excised from the growing margin of 3-day-old PDA cultures were deposited onto the center of each tomato leaflet. Leaves with GYM medium as a control. After 2 days of growth with 12 h of light and 12 h of darkness alternately (photoperiod L:D 12:12 h) at 28°C, the lesion areas were measured [34]. Each

treatment included three replicates and each replicate consisted of three leaflets (three leaflets/plastic boxes). The strain of *Streptomyces* spp. possessing the highest in vivo antagonistic activity was selected for the next study.

2.5 | Identification of antifungal compounds produced by the selected strain of *Streptomyces* spp. grown in different light/dark conditions and temperatures by GC-MS

A 5 ml aliquots of the selected strain of *Streptomyces* spp. was transferred into a 200 ml GYM medium, incubated in the growth chamber under either light or dark (24 h) condition at 21°C and 28°C. After 10 days of incubation, the culture broth was centrifuged (8880*g* for 20 min), then the supernatant was filtered through a 0.45 mm Millipore membrane to get the culture filtrate. The chemical composition of the antifungal compounds in all culture filtrates was determined. Each culture filtrate was acetylated [35], then analyzed by a gas chromatograph-mass spectrometer (GC-MS) (7890 B GC-7000 MS; Agilent) equipped with a VF-WAXms column (30 m film thickness 0.25 μm, I.D. 0.25 mm). Mass spectra were obtained using the scan modus (total ion count, 35–500 *m*/*z*). The retention time and fragmentation pattern in the mass spectra were used to identify each compound by comparing them to those available standards from the Library of the National Institute of Standards and Technology (NIST).

2.6 | Statistical analysis

The data were analyzed by analysis of variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS) program version 26 (IBM SPSS Statistics for Windows, Version 26.0. IBM Corp). A *p*-value of less than 0.05 was considered as the significant level.

3 | RESULTS

3.1 | The influence of culture media, temperature, and light conditions on the growth of *Streptomyces* spp.

The growth of *S. mycarofaciens* SS-2-243 and *S. philanthi* RM-1-138 and RL-1-178 depended on the nutrient composition of the media and incubation temperature (Table 1A). The two strains of *S. philanthi* (RM-1-138 and RL-1-178) showed higher growth on GYM medium and incubation temperature at 28°C than on PDA medium incubated at 21°C. Unlike *S. philanthi*, culture media had no influence on the growth of *S. mycarofaciens* but the strain was more affected by incubation temperature. The strain grew better at 28°C than at 21°C. Therefore, the optimum temperature for growth of all three strains of *Streptomyces* spp. was 28°C. While the two strains of *S. philanthi* (RM-1-138 and RL-1-178) preferred GYM to PDA, the medium composition did not affect *S. mycarofaciens* SS-2-243.

TABLE 1 Influences of culture media and temperature (A) and light conditions (B) on growth of *Streptomyces mycarofaciens* SS-2-243 and *Streptomyces philanthi* RM-1-138 and RL-1-178 after 14 days incubation at 21°C or 28°C

A	21°C		28°C	
***Streptomyces* species**	**PDA**	**GYM**	**PDA**	**GYM**
S. mycarofaciens SS-2-243	++	++	+++	+++
S. philanthi RM-1-138	+	+	++	+++
S. philanthi RL-1-178	+	+	++	+++

B		
Streptomyces species	**Light**	**Dark**
S. mycarofaciens SS-2-243	++	+++
S. philanthi RM-1-138	+	+
S. philanthi RL-1-178	+	+

Abbreviations: +++, good growth and produced black spore; ++, moderate growth and produced white spore; +, weak growth and no produced spore; GYM, glucose yeast-malt extract medium; PDA, potato dextrose medium.

Light/dark conditions influenced the growth of *S. mycarofaciens* SS-2-243 but did not affect the two strains of *S. philanthi* (RM-1-138 and RL-1-178) (Table 1B). *S. mycarofaciens* SS-2-243 grew better under dark condition than the light condition.

In the case of *B. cinerea*, the culture media (PDA and GYM) and temperatures (21°C and 28°C) had a profound effect on the mycelial growth (colony diameter) after 3 days of incubation (Figure 1). The PDA medium and temperature at 21°C showed a significantly ($p < 0.05$) higher mycelial growth of *B. cinerea* with an average colony diameter of 8.41 cm as compared with the GYM (6.86 cm).

3.2 | The influence of culture media and temperature on antifungal activity of *Streptomyces* spp. against *B. cinerea*

Influence of culture media and temperature on antifungal activity of *S. mycarofaciens* SS-2-243 and the two strains of *S. philanthi* (RM-1-138 and RL-1-178) against

B. cinerea were investigated. Figure 2 shows that the three strains of *Streptomyces* spp. exhibited 83%–100% inhibition on the mycelial growth of the pathogenic fungi on PDA or GYM medium after 3 days incubation at 21°C or 28°C, compared with the control treatment without *Streptomyces* spp. Significant differences in antifungal activity of *S. mycarofaciens* SS-2-243 were observed between medium and temperature (the same as the result of growth). Notably, the strains RM-1-138 and RL-1-178 of *S. philanthi* showed the strongest antagonistic activity (100% inhibition) against the mycelial growth of *B. cinerea* tested on both PDA and GYM medium after incubation at 21°C or 28°C.

3.3 | In vivo antagonistic activity of culture filtrate produced by *Streptomyces* spp. against *B. cinerea* on tomato leaves

Because control efficacy in vitro is not always consistent with the results in vivo, the disease controls of the efficacy of culture filtrate produced by the three strains of *Streptomyces* spp. against gray mold were evaluated on tomato leaves as a screening procedure for predicting the actual control efficacy. The development and expansion of disease symptoms induced by *B. cinerea* were inhibited effectively by the culture filtrate produced by *S. mycarofaciens* SS-2-243 and *S. philanthi* RM-1-138 and RL-1-178 and in in vivo tomato leaf test. As shown in Figure 3a, the lesion areas on tomato leaves inoculated by *B. cinerea* were significantly reduced ($p < 0.05$) after being treated with culture filtrate produced by *Streptomyces* spp. using detached leaf assay. For leaves in the control group with no treatment of culture filtrate, the lesion areas extended to 3.12 cm^2 after 2 days incubation at 28°C. For the leaves treated with the culture filtrate produced by *S. mycarofaciens* SS-2-243 and the strains RL-1-178 and RM-1-138 of *S. philanthi*, the lesion areas were limited to 1.78, 0.98, and 0.33 cm^2, respectively (Figure 3b). The cultural filtrates of *S. philanthi* RM-1-138 showed strong efficacy protection on tomato leaf caused by *B. cinerea* (82.89%) (Figure 3c). Therefore, the culture filtrates of this strain RM-1-138 were selected to identify the chemical composition.

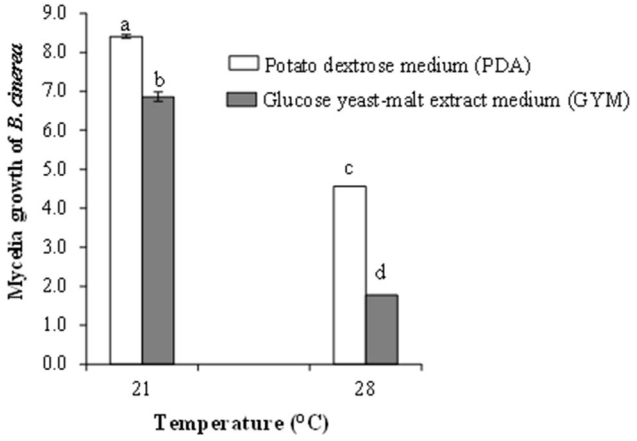

FIGURE 1 Influences of culture media and temperature on mycelial growth of pathogenic *Botrytis cinerea* after 3 days incubation. Values are the meaning of three replications (±SD)

3.4 | Identification of antifungal compounds produced by *S. philanthi* RM-1138 grown at different light/dark conditions and temperatures by GC-MS

The metabolic compounds produced by *S. philanthi* RM-1-138 greatly varied under the light and dark (24 h) conditions at the two temperatures (21°C and 28°C) (Table 2). The chemical compounds mainly fell into several categories including acids, ketones, alcohol, amines, amides, and others. Among them, there were only three components: acetic acid, 2-propanone, and 2-furanmethanol that were detected in all conditions tested. In the light condition, 11 and 19 compounds were identified after incubation at 21°C and

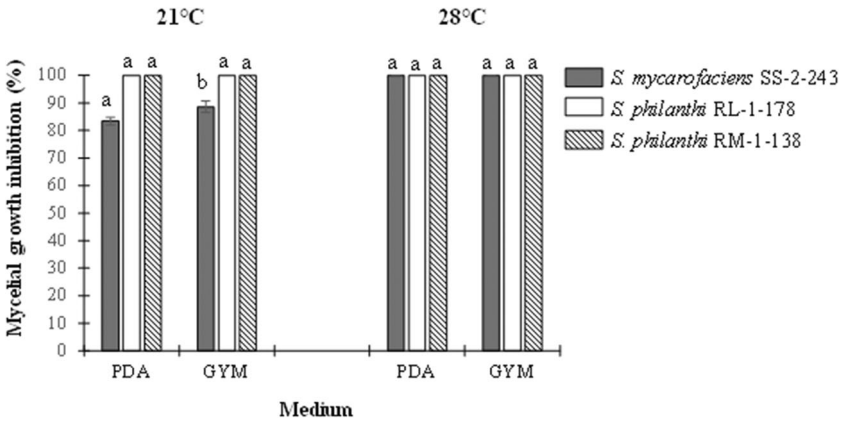

FIGURE 2 Mycelial growth inhibition of *Botrytis cinerea* caused by *Streptomyces mycarofaciens* SS-2-243 and Streptomyces *philanthi* RM-1-138 and RL-1-178 using dual culture technique on potato dextrose agar (PDA) or glucose yeast-malt (GYM) agar after incubated at 21°C or 28°C

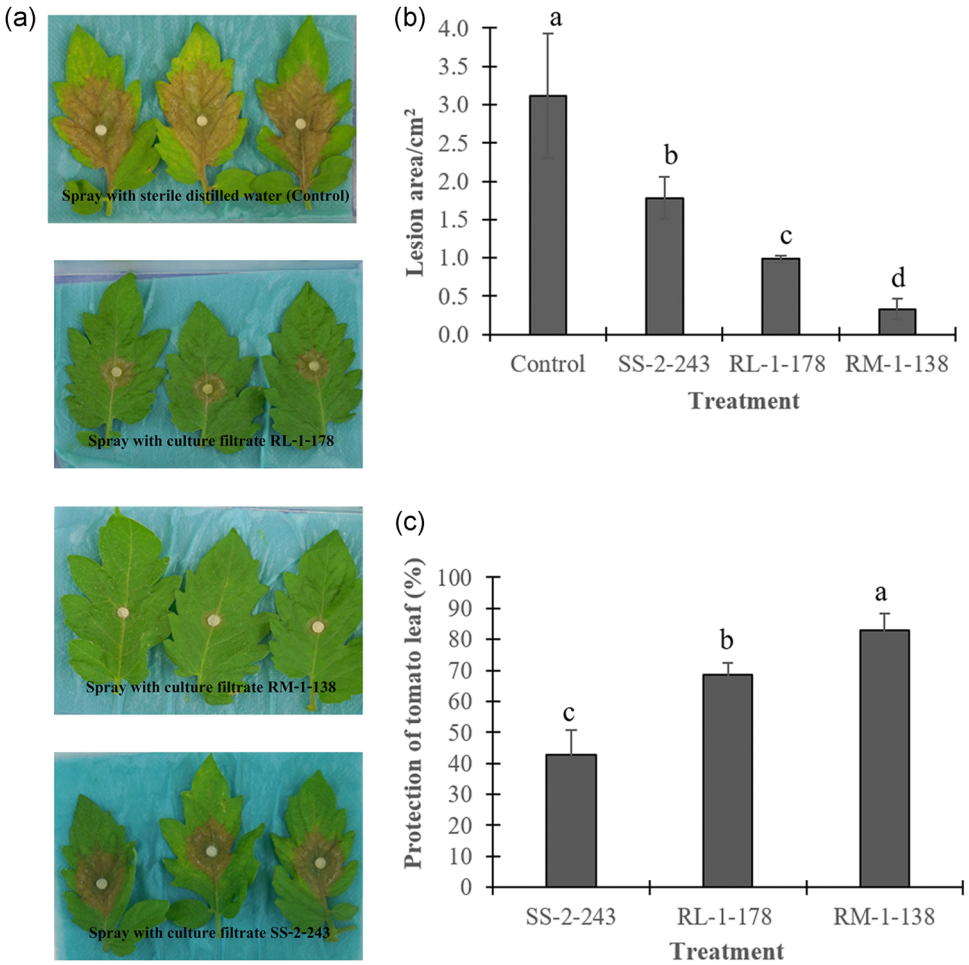

FIGURE 3 Antagonistic effects of culture filtrate produced by *Streptomyces mycarofacien* SS-2-243 and *Streptomyces philanthi* RM-1-138 and RL-1-178 on the development of blight symptoms in tomato leaf (a), lesion areas of tomato leaves with or without treatment by the culture filtrates (b), and protection of tomato leaf (in percentage) against *Botrytis cinerea* with the culture filtrate produced by the three strains of *Streptomyces* spp. (c). The data presented are the mean ± SD (n = 6). The same letter on the bars for each column indicates no significant difference according to a Tukey test at $p < 0.05$

28°C, respectively. At 21°C, the dominant component was acetic acid (67.41%) (Figure 4a), while at 28°C the dominant component was benzeneacetamide (43.58%) followed by propanamide (20.39%) (Figure 4b). In the dark condition, *S. philanthi* RM-1-138 produced 11 and 32 antifungal compounds after cultivation at 21°C and 28°C, respectively. Surprisingly, acetic acid (68.77%) (Figure 4c) was produced abundantly when incubated at 21°C while at 28°C the dominant components were propanamide (20.68%) followed by benzeneacetamide (10.34%) (Figure 4d).

4 | DISCUSSION

This paper provides insightful information about the influence of culture media, temperatures, and light/dark conditions on growth and antifungal activity by the antagonistic strains of *S. mycarofaciens* SS-2-243 and *S. philanthi* RL-1-178 and RM-1-138 against *B. cinerea* in vitro as well as in vivo on tomato leaves. Besides, the antifungal compounds produced by the promising strain were identified by GC-MS. The results revealed that culture media, temperatures, and light/dark condition had a profound effect on the growth of the two antagonistic strains of *S. philanthi* (RM-1-138 and RL-1-178) and fungal pathogenic strain. Moreover, temperatures and light conditions influenced the number of metabolic compounds produced by the selected strain of *S. philanthi* RM-1-138. The culture media and environmental factors were found to be the most influential factors on the growth and production of secondary metabolites of microorganisms [36,37]. Kathiresan et al. [38] reported that glucose and soyabean meal were the best carbon and nitrogen sources for the production of antimicrobial agents under different culture conditions. In our study, the growth medium, temperature, and light/dark

TABLE 2 Comparative chemical composition analyzed by GC-MS of the culture filtrate from *S. philanthi* RM-1-138 grown in a GYM medium under the light and dark condition, and incubated at 21°C and 28°C for 10 days

	% relative concentration			
	Light condition		Dark condition	
Chemical compositions	**21°C**	**28°C**	**21°C**	**28°C**
Acid				
Acetic acid ($C_2H_4O_2$)	67.41	4.91	68.77	4.27
Propanoic acid ($C_3H_6O_2$)	2.66	-	3.08	-
Propanoic acid, 2-methyl- ($C_4H_8O_2$)	1.22	-	2.00	-
Butanoic acid, 2-methyl- ($C_5H_{10}O_2$)	1.85	-	2.24	-
Lactic anhydride ($C_6H_{10}O_5$)	-	1.49	-	3.09
2-Propenoic acid, 2-hydroxyethyl ester ($C_5H_8O_3$)	-	3.13	-	-
Pentanoic acid, 2-hydroxy-, ethyl ester ($C_7H_{14}O_3$)	-	-	-	7.61
Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester) ($C_9H_{11}NO_4$)	-	-	-	0.24
Ketones				
2-Propanone, 1-hydroxy ($C_3H_6O_2$)	1.68	3.55	2.15	7.15
2(3H)-Furanone, dihydro-4-hydroxy ($C_4H_6O_3$)	1.03	1.96	0.79	-
2(3H)-Furanone, dihydro ($C_4H_6O_2$)	-	-	-	0.39
2,5-Pyrrolidinedione, 1-methyl ($C_5H_7NO_2$)	-	0.70	-	0.34
2-Butanone, 3-hydroxy ($C_4H_8O_2$)	-	-	-	0.52
2(5H)-furanone ($C_4H_4O_2$)	-	-	-	1.19
Alcohol				
2-Furanmethanol ($C_5H_6O_2$)	0.75	1.49	0.74	3.15
3,4-Furandimethanol ($C_6H_8O_3$)	-	2.46	1.69	-
1,2-Propanediol ($C_3H_8O_2$)	-	1.30	-	-
Benzenemethanol, alpha.-(1-aminoethyl), [S-(R*, R*)] ($C_9H_{13}NO$)	-	-	-	5.01
1,3-Butanediol, (S)-($C_4H_{10}O_2$)	-	-	-	1.31
Cyclobutanal-DO (C_4H_7DO)	-	-	-	1.28
Carveol ($C_{10}H_{16}O$)	-	-	-	0.95
9-Hydroxy-linalool ($C_{10}H_{18}O_2$)	-	-	-	0.22
p-Mentha-1,8-dien-7-ol ($C_{10}H_{16}O$)	-	-	-	1.79
3,4-Furandimethanol ($C_6H_8O_3$)	-	-	-	5.09
(2-[1-(3,3-Dimethoxy-propyl)-vinyl]-5-methylcyclopentyl)-methanol ($C_{14}H_{26}O_3$)	-	-	-	0.23
Amines				
Benzeneethanamine, 2,5-difluoro-beta,3,4-trihydroxy-*N*-methyl ($C_9H_{11}F_2NO_3$)	-	2.44	1.54	-
Amides				
Propanamide (C_4H_9NO)	-	20.39	-	20.68
Heptanamide ($C_7H_{15}NO$)	-	0.85	-	-

(Continues)

TABLE 2 (Continued)

Chemical compositions	% relative concentration			
	Light condition		Dark condition	
	21°C	28°C	21°C	28°C
Butanamide, 3-methyl ($C_5H_{11}NO$)	-	2.15	-	-
Benzeneacetamide (C_8H_9NO)	-	43.58	-	10.34
Hexanamide ($C_6H_{13}NO$)	-	-	-	0.36
Others				
Benzeneacetaldehyde (C_8H_8O)	10.01	-	-	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl ($C_6H_8O_4$)	6.80	-	8.52	-
1,4:3,6-Dianhydro-.alpha.-D-glucopyranose ($C_6H_8O_4$)	4.46	-	-	-
2-Furancarboxaldehyde, 5-methyl ($C_6H_6O_2$)	2.14	-	-	-
Butyl aldoxime, 2-methyl-, syn ($C_5H_{11}NO$)	-	1.34	-	3.76
Diethyl alpha-acetylglutarate ($C_{11}H_{18}O_5$)	-	0.58	-	-
Nonanal ($C_9H_{18}O$)	-	1.41	-	-
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl) ($C_{10}H_{16}O$)	-	2.14	-	-
1,4:3,6-Dianhydro-.alpha.-D-glucopyranose ($C_6H_8O_4$)	-	2.47	2.97	-
3-Carene ($C_{10}H_{16}$)	-	-	-	0.31
4,4-Ethylenedioxy-pentanenitrile ($C_7H_{11}NO_2$)	-	-	-	0.24
(Z)-4-(Methylamino)-3-penten-2-one ($C_6H_{11}NO$)	-	-	-	0.25
5-Methyl-2-hexanone oxime ($C_7H_{15}NO$)	-	-	-	0.42
1,3,5-Triazine-2,4,6-triamine, 1,3,5-trioxide	-	-	-	0.43
Arginine ($C_6H_{14}N_4O_2$)	-	-	-	1.51
2,7-Anhydro-L-galacto-heptulofuranose	-	-	-	0.23
2-*N*-propyl-5-oxohexanal	-	-	-	0.37
1,4:3,6-Dianhydro-.alpha-D-glucopyranose ($C_6H_8O_4$)	-	-	-	9.15
2-hydroxy-butanedial ($C_4H_6O_3$)	-	-	-	5.36

Abbreviation: GC-MS, gas chromatography–mass spectrometry; GYM, glucose yeast-malt.

conditions showed a significant effect on the mycelia growth of the three strains of *Streptomyces* spp. The two strains of *S. philanthi* (RM-1-138 and RL-1-178) grew and produced a spore mass on GYM medium at 28°C higher than on PDA medium at 21°C. GYM medium containing glucose and yeast-malt extract was more enriched with nutrients for microbial growth than PDA containing only potato and dextrose. Yeast extract and soybean meal as nitrogen source were reported to have a profound influence on the growth and production of secondary metabolites by *S. albidoflavus* [39]. The study indicated that the nutrient source was an important factor on bacterial and fungal growth.

The light/dark cycle influenced the growth of the three antagonistic strains of *Streptomyces* spp. There was no significant difference in the growth of *S. philanthi* RL-1-178 and RM-1-138 between light and dark conditions. On the other hand, *S. mycarofaciens* SS-2-243 had a significantly higher growth rate in the dark condition than in the light condition. This was due to the fact that light remarkably inhibited the spore germination as also found in *S. viridosporus* and *S. coelicolor* [40]. The influence of light on the growth of various *Streptomyces* spp. was varied. *S. phaeopurpureus* 5125, *S. salmonicida* 5472, and *S. fulvissimus* could grow well in light

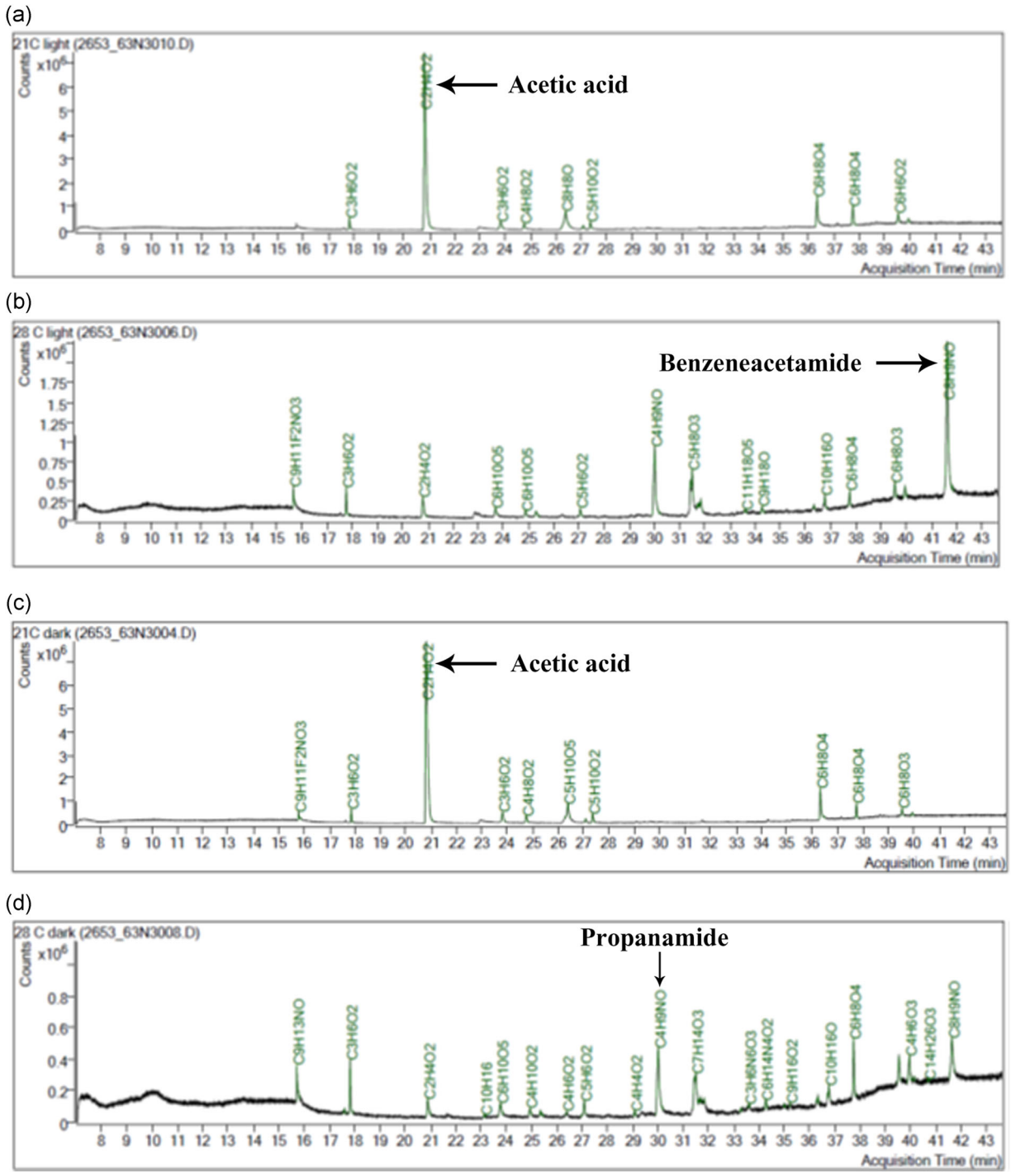

FIGURE 4 GC chromatograms of the culture filtrate from *Streptomyces philanthi* RM-1-138 grew in a glucose yeast-malt (GYM) medium under the light and dark condition, and incubated at 21°C and 28°C for 10 days: Light condition and incubated at 21°C (a) and 28°C (b) and dark condition and incubated at 21°C (c) and 28°C (d), highlighting one major compound of each condition

conditions [27]. On the other hand, *S. viridosporus*, *S. lividans*, and *S. coelicolor* were sensitive to light, while *S. ambofaciens* and *S. pilosus* remained unchanged and unaffected [40]. It has been demonstrated that light conditions influenced the growth of *Streptomyces* spp. The intracellular superoxide dismutase levels were found to be remarkably enhanced by the light, indicating that light and oxygen together might produce high amounts of reactive oxygen species.

B. cinerea is used a wide range of strategies to complete its infection cycle. The induction of these strategies is mainly related to environmental conditions. Culture media

(PDA and GYM) and temperature (21°C and 28°C) influenced the mycelial growth of *B. cinerea*. Many researchers reported that the PDA and incubation temperature, ranging from 18°C to 22°C were the best conditions for the growth of *B. cinerea* [24–26]. However, the growth conditions on GYM medium and incubation temperature at 28°C have never been reported. The PDA medium and temperature at 21°C enhanced a rapid mycelial growth of *B. cinerea* while GYM medium showed no significantly difference after incubation at 21°C or 28°C. The finding is consistent with the study by Ciliberti et al. [41], which reported that the highest mycelial growth and conidia production of *B. cinerea* was observed on PDA and temperature at 20°C. Several other research also stated that PDA was the best media for mycelial growth [42,43].

The PDA medium, with a temperature of 21°C not only had a profound positive effect on growth but also on the antifungal activity of *S. mycarofaciens* SS-2-243 against gray mold *B. cinerea*. The two strains of *S. philanthi* (RM-1-138 and RL-1-178) showed the strongest antagonistic activity (100% inhibition) against the mycelial growth of *B. cinerea* tested on both media at an incubation temperature of 21°C and 28°C in the in vitro test. The three antagonistic strains of *Streptomyces* spp. produced antifungal substances and secreted them into the agar medium in dual culture plates. In addition to the in vitro trials, the three antagonistic strains of *Streptomyces* spp. also exhibited positive results against *B. cinerea* during the in vivo trials on tomato leaves with remarkable protection efficiency after 2 days of cultivation at 28°C. Blight symptoms and lesion diameter caused by the fungal pathogen in tomato leaves were all significantly ($p < 0.05$). reduced by the strains of *S. mycarofaciens* SS-2-243 as well as *S. philanthi* RM-1-138 and RL-1-178 when compared with the control. Among the three strains tested, the antifungal compounds in the culture filtrates produced by *S. philanthi* RM-1-138 showed the highest inhibitory effect (82.89%) on the protection of tomato leaf caused by *B. cinerea* when compared with *S. philanthi* RL-1-178 (68.45%) and *S. mycarofaciens* SS-2-243 (42.78%), respectively. These results confirmed the potential of an antifungal compound in the secondary metabolites produced by antagonistic microorganisms such as *Streptomyces* spp. [15,44,45], *Bacillus* sp. [46,47], and *Pseudomonas* sp. [48,49] to control gray mold *B. cinerea* in this study or other plant pathogenic fungi.

The antimicrobial substances produced by *Streptomyces* were variable and diversified depending on multiple factors. Therefore, the influence of temperatures (21°C and 28°C) and light/dark condition on antifungal production of the selected strain *S. philanthi* RM-1-138 was studied. Interestingly, the metabolic compounds produced by this particular strain were greatly altered by relative concentration components under the conditions tested. In light/dark conditions, the anti-*B. cinerea* of *S. philanthi* RM-1-138 was established at a higher level in several metabolic compounds in the dark condition (11 and 32 antifungal compounds after incubation at 21°C and 28°C, respectively) than in the light condition (11 and 19 antifungal compounds after incubation at 21°C and 28°C, respectively). Moreover, it was found that the dominant antifungal compounds showed significant differences between dark and light conditions. The incubation temperature plays a seminal role in the number of antifungal and dominant compounds of *S. philanthi* RM-1-138. Incubation at 21°C, the dominant component of the antifungal compound was acetic acid (68.77% and 67.41% in the dark and light condition, respectively). On the contrary, at 28°C the dominant components in the dark and light condition were propanamide (20.68%) and benzeneacetamide (43.58%), respectively. Besides, it should be noted that acetic acid (67.41% light and 68.77% dark condition at 21°C; 4.91% light and 4.27% dark condition at 28°C), 2-propanone (1.68% light and 2.15% dark condition at 21°C; 3.55% light and 7.15% dark condition at 28°C), and 2-furanmethanol (0.75% light and 0.74% dark condition at 21°C; 1.49% light and 3.15% dark condition at 28°C) were detected in all conditions tested. Moreover, some of the antifungal compounds such as lactic anhydride, 2,5-pyrrolidinedione, 1-methyl, propanamide, benzeneacetamide, butyl aldoxime, 2-methyl-, syn- were found only under incubation at 28°C both under light and dark condition. According to this study, different environmental factors may release similar and different concentrations of antifungal substances, with a variety of effects such as acetic acid, 2-propanone, and 2-furanmethanol. Many researchers proposed that *Streptomyces* spp. was commonly reported for its broad spectrum of antimicrobial activity [50,51]. Our results clearly demonstrated that the light/dark condition and incubation temperature were the most influential factor on the production of secondary metabolites of *Streptomyces* spp.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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