RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.027

ScienceAsia 51 (2): 2025: ID 2025027: **1–11**

Distribution and diversity of cultured fungi from *Rhodomyrtus tomentosa* (Aiton) Hassk. leaves in southern Thailand and their antimicrobial activities

Alisara Ma[a], Nurida Chealee[a], Sara Bumrungsri[a], Supayang Piyawan Voravuthikunchai[b,c], Lakkhana Kanhayuwa Wingfield[a,*]

[a] Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110 Thailand
[b] Center of Antimicrobial Biomaterial Innovation-Southeast Asia, Faculty of Science, Prince of Songkla University, Songkhla 90110 Thailand
[c] Natural Product Research Center of Excellence, Faculty of Science, Prince of Songkla University, Songkhla 90110 Thailand

*Corresponding author, e-mail: Lakkhana.k@psu.ac.th

Received 1 Feb 2024, Accepted 5 Nov 2024
Available online 22 Apr 2025

ABSTRACT: *Rhodomyrtus tomentosa* (Aiton) Hassk. is a medicinal plant known for its therapeutic potential on bacterial infections. We first investigated the distribution, diversity, and antimicrobial activities of cultured endophytic fungi isolated from *R. tomentosa* across the southern region of Thailand (13 provinces). The endophytes were isolated and characterized based on their morphological and molecular characteristics. A total of 29 representative morphotypes were phylogenetically classified into 12 genera. The most frequently isolated genera were *Neopestalotiopsis* (RF, 29.46%), *Endomelanconiopsis* (RF, 17.24%), *Colletotrichum* (RF, 13.72%), and *Phyllosticta* (RF, 12.43%). The Margalef and Menhinick species richness indices were 3.74 and 0.32, respectively; and the Shannon and Simpson species diversity indices were 1.98 and 0.83, respectively. These values indicated that *R. tomentosa* leaves harbored a low diversity of fungal endophytes. The study revealed native endophytic fungal communities that were common across the southern region; but in some provinces, variations in fungal communities were observed. The variations were possibly influenced by different climatic, geographical, and geological characteristics. *Colletotrichum* and *Neopestalotiopsis* were the genera most commonly found in all provinces, while *Pseudopestalotiopsis* and *Gnomoniopsis* were area specific. Four out of 13 fungal morphotypes exhibited inhibitory activities against at least one bacterial pathogen. *Chaetomium cupreum* strain KBSK-V1-T8 exhibited antimicrobial activities against both Gram-positive and Gram-negative bacteria. Our results suggested that fungal endophytes from *R. tomentosa* could be exploited as a potential source of bioactive agents.

KEYWORDS: endophytic fungi, fungal diversity, *Rhodomyrtus tomentosa* (Aiton) Hassk., antimicrobial activity

INTRODUCTION

Rhodomyrtus tomentosa (Aiton) Hassk., a flowering plant in the family Myrtaceae, is native to Southeast Asia. The plant was identified by the Agrofolio scientific project as one of 240 neglected and underutilized crop species of Cambodia, China, Thailand, and Vietnam (www.Agrofolio.eu/db). *R. tomentosa* commonly grows in wet forests up to 2,400 m in elevation on sandy soils, although acid soils are preferred [1]. In Thailand, *R. tomentosa* is most frequently distributed in coastal sandy soils on both the western and the eastern coasts of the Thai Peninsular [2]. It has long been used in Asian traditional medicine, and the known biological activities of *R. tomentosa* are antibacterial, anticancer, anti-inflammatory, antidiarrhea, antidysentery, antioxidant, and antipyretic [3–7].

Endophytic fungi are considered new sources of bioactive secondary metabolites that play an important role in medical, industrial and agricultural fields. They are ubiquitous symbionts that live within plant tissues. Their resistance mechanisms produce secondary metabolites that protect their host plants from abiotic or biotic stresses [8]. It has been shown that some endophytic fungi can produce bioactive compounds that are equivalent to those of their host plants [9]. Secondary metabolites produced by endophytic fungi include alkaloids, terpenoids, phenylpropanoids, steroids, quinones, phenols, isocoumarins, lignans, and lactones [10, 11]. Since drug-resistant microorganisms have become a worldwide serious public health concern, recent research has investigated endophytic fungi that exhibit inhibitory activity toward microbial pathogens. However, to date, there has been no comprehensive report on the diversity and antimicrobial activities of fungal endophytes isolated from *R. tomentosa*.

This study focused on the distribution and characteristics of endophytic fungi associated with *R. tomentosa* (Aiton) Hassk. leaves from different geographical locations and environments across the southern region of Thailand. Fungal isolates were characterized based on morphological observation and molecular identification. Taking into account variations in host plant characteristics in different climatic, geographical, and geological contexts, a comparative analysis and characterization of fungal endophytes may shed light on endophytic interactions in plant growth, as well as

antibiotic and secondary metabolite production [12]. To our knowledge, this was the first comparative study to demonstrate culturable fungal biota interacting with *R. tomentosa* (Aiton) Hassk. in southern Thailand. We also discovered fungal endophytes that could present a novel source of bioactive compounds.

MATERIALS AND METHODS

Sampling sites and plant materials

Representative communities of *R. tomentosa* (Aiton) Hassk. native to the southern region of Thailand were sampled from 13 provinces (46 sampling sites) located on the western and the eastern coasts along the Thai Peninsula. Details of geographical locations and climatic characteristics of sampling sites were presented in Table S1. Southern Thailand occupies the top part of the long, narrow Malay Peninsula. Situated between the Andaman Sea and the Gulf of Thailand, the southern region is divided by mountains into western and eastern sides. The western side (submerged shoreline) has steep coasts featuring bays and islands, fringed with mangrove forests interspersed with sandy beaches from Ranong in the north to Satun in the south. The eastern side (emerged shoreline) is dominated by river plains, generating a flat coastline with narrow plains from Chumphon in the north down to Narathiwat on the border with Malaysia. The tropical climate on both sides of the region is influenced by the sea, causing heavy rainfalls for most of the year. The average annual rainfall on the western coast is 2,467.7 mm, and 2,044 mm on the eastern coast.

Healthy mature leaves of *R. tomentosa* from 13 provinces across the Thai Peninsula (10°57′38.2″ N 99°29′21.8″ E to 6°32′23.82″ N 101°16′52.61″ E) were collected during dry season (November 2021 to January 2022) (Fig. 1 and Table S2). Leaves with physical damage or showing signs of pathogenic infection were excluded from the study. Mature leaves were carefully selected to control the leaf age. An adult leaf of *R. tomentosa* is opposite the eighth node on a branch, showing large leaf area and leathery green [13]. Three leaves of three independent *R. tomentosa* plants per district site were randomly collected and used as one biological replication. Then, three biological replications were performed (making 9 leaves per site and 414 leaves in total). Numbers of sampling sites in each province varied as *R. tomentosa* was sparsely populated. The leaf samples were kept in sterile plastic bags, stored at 4 °C, and transported to the laboratory. During the sampling, temperature, soil pH, and relative humidity (RH) were measured using portable instruments (Table S3). The temperature within the Peninsula ranged from 25 °C to 32 °C. Relative humidity (RH) was measured using Portable Thermo-Hygrometer (KEPLER, China), and the RH level ranged from 85% to 90% across the peninsula during the sampling period. The soil pH range was 4.49–5.95. Total Nitrogen (N), total P_2O_5, total K_2O, and organic matter were measured as described by Wingfield et al [14]. The electrical conductivity (EC) of soil was measured by the electrode method using EC Soil Meter (Hanna HI98331, Romania).

Isolation of endophytic fungi

Different segments of leaves; petiole, midrib, vein, and lamina; were used to investigate effects of host tissues on the colonization, diversity, and composition of endophytic fungi. Leaf samples were washed several times to remove soil and then air-dried. Surface sterilization was carried out using a previously described method [15] with minor modification, as follows. Sterile surgical blades were used to cut 1.0×1.0 cm fragments of different segments of leaves, and sample fragments were then immersed in 95% ethanol for 30 s, 5% sodium hypochlorite solution (NaOCl) (Sigma-Aldrich®, USA) for 5 min, 95% ethanol for 30 s, and sterile distilled water for 3–5 s. Four sterilized fragments were then placed onto a potato dextrose agar (PDA) (HiMedia®, India) plate containing chloramphenicol (Sigma-Aldrich®) (50 µg/ml) and incubated for 7 to 21 days at 28 °C until the fungal hyphae emerged. Hyphal tips were isolated and transferred to new PDA plates without antibiotics. Fungal colonies were observed periodically for morphological characterization. The endophytic fungal isolates were stored at the Mycology Laboratory, Department of Microbiology, Prince of Songkla University.

Morphological identification of endophytic fungi

Endophytic fungal isolates were identified to the genus and species levels based on their macroscopic morphological characteristics such as colony topography and growth pattern. The microscopic appearances of individual endophytic fungi were observed using the slide culture technique. Then, the observed mycelial and reproductive structures were investigated to further identify the fungi, using the identification keys of Samson et al [16], Dugan [17] and Ellis et al [18].

Molecular identification of endophytic fungi

Molecular identification of the endophytic fungal isolates was performed based on the analysis of the DNA sequence of the ITS1-5.8S-ITS2, ITS regions of their rRNA gene. Genomic DNA was extracted according to a protocol described by Wingfield and Atcharawiriyakul [19], using the DNeasy® Plant Mini Kit (QIAGEN, UK) with a mini protocol provided by the manufacturer. PCR amplification of fungal ITS regions was carried out using an ITS primer set; ITS5/ITS4N, which amplified a 600–800 bp section of the ITS and had the following sequences: ITS5, (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4N, (5′-TCCTCCGCTTATTGATATGC-3′). Each 50 µl reaction mixture contained 5 µl of 10x *Taq* buffer, 5µl of dNTP

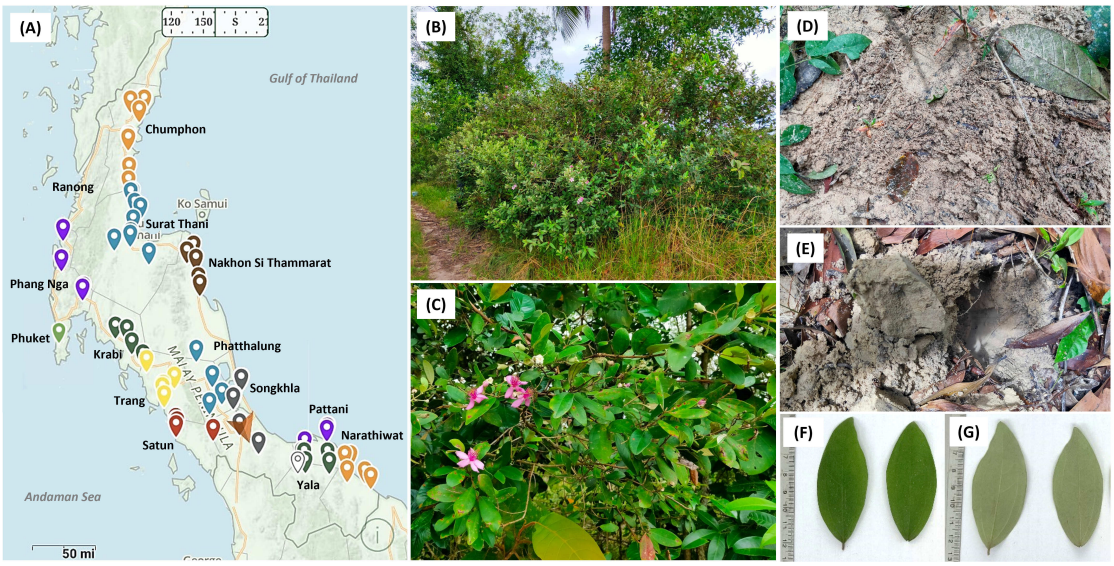

Fig. 1 Locations of sampling sites and habitats of the *Rhodomyrtus tomentosa* plants: (A), map of the locations of sample plots; (B), *R. tomentosa* community; (C), individual characteristics; (D, E), physical characteristics of the bulk soil; (F), characteristics of the front of mature leaves; and (G), characteristics of the back of mature leaves.

mix (2 mM each), 2 µl of each primer at 1.0 µM, 0.5 µl of *Taq* DNA polymerase (1.25U) (New England Biolabs, USA), and 1 µg of genomic DNA. The PCR reaction was performed using a DNA Engine DYAD ALD 1244 thermocycler (MJ Research Inc., USA) with the following cycles: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 10 min. The PCR products were visualized by electrophoresis on a 1% agarose gel in 1XTAE buffer at 100 V for 30 min, purified using the MinElute® Gel Extraction kit (QIAGEN), and then sent for sequencing.

Phylogenetic analysis

A search for closest matched sequences in the National Centre for Biotechnology Information (NCBI) GenBank database was done using a BLAST search tool. To confirm the identity of the isolates, phylogenetic and molecular evolutionary analyses were conducted using MEGA version 11 [20]. Multiple sequence alignments were performed using alignments prepared with ClustalW, and sequences were manually edited when necessary to maximize the alignment. The phylogenetic tree was inferred using the maximum-likelihood algorithm. The stability of relationships was evaluated by bootstrap analysis with 1,000 replications.

Diversity and data analysis

The diversity of the fungal isolates from *R. tomentosa* was determined by evaluating species richness based on the Menhinick (Dmn) [21] and Margalef (Dmg) indices [22]. Species diversity was measured by the Shannon (H′) and Simpson (D) indices [23]. The isolation rate (IR) [24] was used to indicate the fungal richness in a given leaf sample. It was calculated as the number of fungal isolates obtained from leaf fragments divided by the total number of fragments tested. The degree of infection by endophytic fungi was evaluated for different leaf tissues by comparing colonization rates (%CR), which were calculated as the total number of leaf fragments infected by fungi divided by the total number of fragments tested. The representation of fungal genera was expressed as relative frequency (%RF) calculated as the frequency of a specific genus divided by the total number of fungal isolates. The statistical analysis was conducted using Graph Pad Prism, version 6.0.

Antimicrobial activities

Fungal endophytes were cultivated in a potato dextrose broth (PDB) (HiMedia®) for 21 days at 28 °C under shaking condition at 150 rpm. Culture broths were used for screening antimicrobial activity by the agar well diffusion method [25] against six pathogenic bacteria (*Kocuria rhizophila* ATCC 9341, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Vibrio cholerae*), and a pathogenic yeast (*Candida albicans* (ATCC90028)). The bacteria were grown on Mueller-Hinton Agar (MHA) (HiMedia®) at 35 °C for 18 h, and the yeast was grown on Sabouraud dextrose agar (SDA) (HiMedia®) at 28 °C for 24–48 h. After incu-

bation, inhibition zones were measured in triplicates as the mean diameter of the wells (8 mm) plus the clearing zone. The values obtained were presented as means ± standard deviation ($n = 3$). Crude ethyl acetate extracts of the culture broths and mycelia were also tested for antimicrobial activities at concentrations from 1 to 100 mg/ml (dissolved in 0.1% DMSO) (Merck, Germany), using the agar well diffusion method. Initially, fungal broths and mycelia were separated using filter papers (Whatman, No. 1). The filtered broth was then extracted thrice with an equal volume of ethyl acetate (Sigma-Aldrich®) (1:1; v/v), and only the organic phase was collected. Meanwhile, the mycelia (10 g) were mixed with 100 ml of ethyl acetate with shaking at room temperature for 24 h. Both ethyl acetate extracts were concentrated using a rotary evaporator, left to air dry in a fume hood, weighed, and stored at −15 °C until further use. DMSO was used as a vehicle control. Vancomycin and gentamicin (as standard antibacterial agents) and amphotericin B (as standard antifungal agents) were from Sigma-Aldrich®.

RESULTS

Isolation and colonization of endophytic fungi from *R. tomentosa* leaves

A total of 1,623 endophytic fungi were isolated from 1,656 leaf fragments obtained from petiole, midrib, vein and lamina (Table S4), giving an overall IR of 0.98 (Table 1). This result demonstrated that most of the leaf segments tested contained at least one fungal isolate, indicating a moderate fungal richness in the leaf samples. Meanwhile, out of the 1,656 leaf fragments. a total of 1,454 fragments were infected (Table S5), giving an overall %CR of 88 (Table 1). This result demonstrated a moderate prevalence of endophytic fungal infection in different tissues of the leaves.

Table 1 shows the IR values of endophytic fungi in vein, petiole, midrib, and lamina segments with the highest value of 1 in the vein. The greatest number of fungal endophytes was isolated from samples collected in Songkhla (IR, 1.17), followed by samples from Phatthalung (IR, 1.10) and Trang (IR, 1.08). Leaf samples from Narathiwat produced the lowest IR (IR, 0.69). The %CR was higher in the midrib segment than the vein, petiole and lamina segments. The highest %CR of 97 was obtained from samples collected in Songkhla and Pattani, followed by samples from Nakhon Si Thammarat, Phatthalung, Krabi and Satun with %CR of 92. The lowest %CR was obtained from samples collected in Narathiwat (%CR, 66). The IR was higher among specimens collected on the eastern coast (excluding Narathiwat and Yala) than the western coast (IR, 1.03 and 0.99, respectively), but the %CR was not different between coasts (%CR, 92).

Identification of endophytic fungi from *R. tomentosa* (Aiton) Hassk. leaves

On the basis of morphological identification, 1,080 fungal isolates were assigned to 13 representative morphotypes, and these morphotypes were selected for molecular identification (Fig. 2). Fungal isolates were categorized at the genus level based on a sequence similarity threshold of 97–100% (Table 2). Phylogenetic analyses using maximum likelihood (Fig. 3) identified 12 fungal genera representing the single phylum, Ascomycota; the two classes, *Dothideomycetes* and *Sordariomycetes*; and the seven orders, *Botryosphaeriales*, *Diaporthales*, *Glomerellales*, *Hypocreales*, *Pleosporales*, *Sordariales*, and *Xylariales*. The 12 endophytic fungal genera identified in this study were *Chaetomium*, *Colletotrichum*, *Daldinia*, *Endomelanconiopsis*, *Fusarium*, *Gnomoniopsis*, *Lasiodiplodia*, *Neopestalotiopsis*, *Nigrospora*, *Phyllosticta*, *Preussia*, and *Pseudopestalotiopsis*. However, two isolates (KBHN-M1 and KBNK-V1) were potential new taxa because of the low similarities of their ITS sequences. The ITS sequences of the studied endophytic fungi were deposited in the Genbank (accession Nos. OP890913 to OP890924) (Table S6).

Distribution and diversity of endophytic fungi from the *R. tomentosa* (Aiton) Hassk. leaves

In the present study, *Xylariales* was the most abundant order (RF, 51.67%), followed by *Botryosphaeriales* (RF, 30.75%) (Fig. 4A). The genera *Neopestalotiopsis* (RF, 29.46%), *Endomelanconiopsis* (RF, 17.24%), *Colletotrichum* (RF, 13.72%), and *Phyllosticta* (RF, 12.43%) were most frequently isolated (Fig. 4B).

The results demonstrated that the distribution of endophytic fungi varied in different leaf tissues (Tables S7 and S8). *Chaetomium*, *Daldinia*, *Colletotrichum*, *Neopestalotiopsis*, *Endomelanconiopsis*, *Nigrospora*, and *Phyllosticta* were found in all segments of the leaves, with the majority in the midrib. On the other hand, *Pseudopestalotiopsis* was only found in leaf veins. Different fungal communities were observed in different areas of the southern region (Fig. 4C) but certain native endophytic fungal communities were common across the region. *Neopestalotiopsis* was found on samples collected from all provinces but was most common on samples from Chumphon, Nakhon Si Thammarat and Trang. *Colletotrichum* was found on specimens from every province. *Nigrospora* and *Fusarium* were more dominant on the western coast. *Gnomoniopsis* and *Preussia* were only found on samples from the eastern coast. *Daldinia* was dominant in Phatthalung and Songkhla. *Endomelanconiopsis* was also dominant in Phatthalung, while *Nigrospora* was dominant in Surat Thani. In addition, *Pseudopestalotiopsis* and *Gnomoniopsis* were only found on samples from Yala.

Our findings also revealed that the %RF of en-

Table 1 Isolation (IR) and colonization (%CR) rates of endophytic fungi from *R. tomentosa* leaves.

Side	Sampling province	Leaf segment								Total	
		Vein		Midrib		Lamina		Petiole			
		IR	%CR	IR	%CR	IR	%CR	IR	%CR	IR	%CR
Western coast	Phang Nga	0.89	89	0.78	100	0.78	78	0.89	78	0.94	86
	Phuket	1.00	100	1.00	100	1.00	56	1.00	67	1.00	81
	Krabi	1.00	92	0.92	100	0.92	83	1.08	92	1.00	92
	Trang	1.14	78	1.00	100	1.00	94	1.08	83	1.08	89
	Satun	0.89	89	0.89	100	0.89	89	1.00	89	0.94	92
Eastern coast	Chumphon	1.09	93	0.73	100	0.73	67	0.84	80	0.95	85
	Surat Thani	0.93	96	0.93	93	0.93	84	0.93	80	0.93	88
	Nakhon Si Thammarat	1.00	100	1.00	100	1.00	78	1.00	89	1.00	92
	Phatthalung	1.17	100	1.17	92	1.17	92	1.00	83	1.10	92
	Songkhla	1.25	100	1.17	100	1.17	97	1.08	92	1.17	97
	Pattani	1.04	100	0.96	100	0.96	89	1.11	100	1.03	97
	Narathiwat	0.67	61	0.50	67	0.50	53	0.92	83	0.69	66
LL[†]	Yala	0.93	89	1.07	78	1.07	89	0.89	78	0.92	83
	Total	1.00	91	0.92	94	0.92	82	0.98	85	0.98	88

[†] LL refers to a landlocked province in the south.

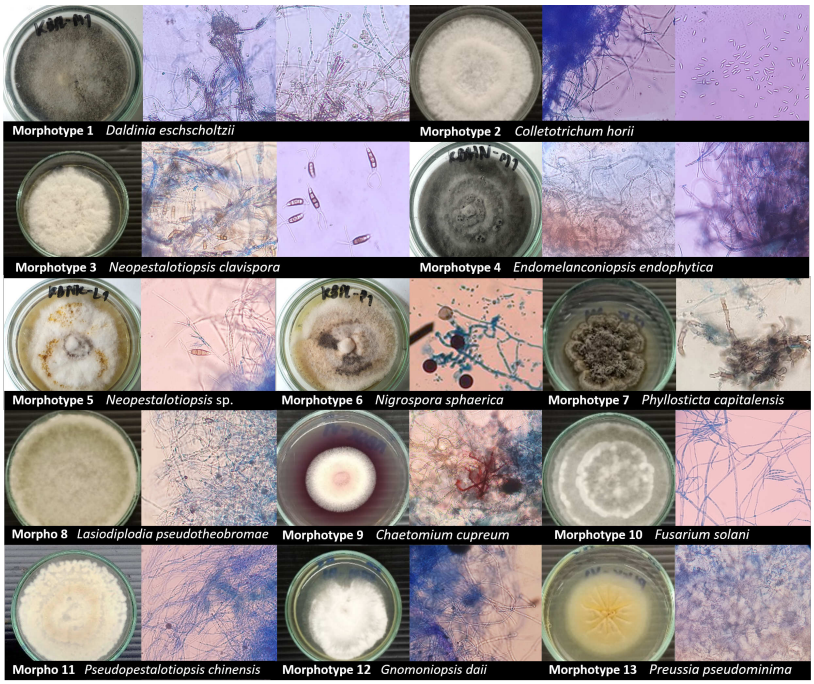

Fig. 2 Thirteen fungal morphotypes of the isolated endophytic fungi based on their macroscopic and microscopic morphology (magnification × 40). All isolates were grown on PDA plates for 7–14 days at 28 °C.

dophytic fungal morphotypes was higher among isolates from the eastern coast (excluding Narathiwat and Yala) than among those from the western coast (%RF, 54.69 and 33.44 respectively) (Table S8). The numbers of fungal morphotypes shared between the two coasts were equally good, but fungal isolates from the western coast were more morphologically diverse. Narathiwat and Yala, which exhibited low %RF values, showed the highest fungal diversity (11 morphotypes). In contrast, Chumphon, Surat Thani, Phatthalung, and Songkhla exhibited distinctly high %RF values of 10% or above but presented only 6 or 7 morphotypes.

In the analysis of fungal diversity and species richness (Table 3), the Dmg index describes the number of different fungal genera represented in an ecological community. The Dmg index was higher on the western coast (2.928) than the eastern coast (2.375, excluding Narathiwat and Yala). With regard to dif-

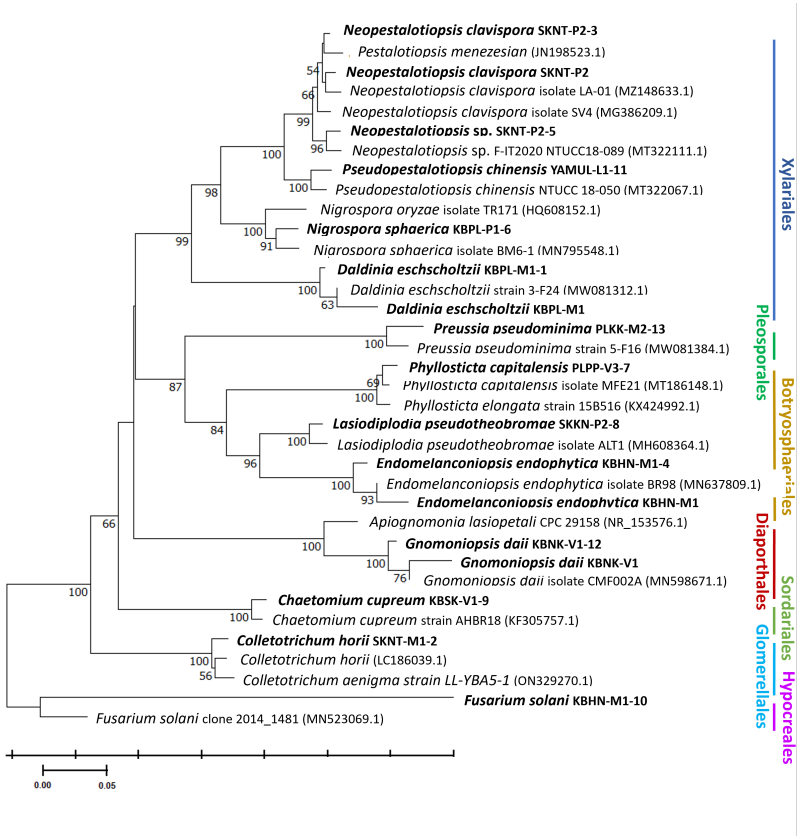

Fig. 3 Unrooted phylogenetic tree generated using maximum likelihood method based on a comparison of the ITS ribosomal DNA sequences of fungal isolates and their closest phylogenetic relatives. Percentages of bootstrap sampling derived from 1000 replications are indicated by the numbers on the tree.

Table 2 BLAST analysis results of the representative fungal isolates from *R. tomentosa* (Aiton) Hassk. leaves and their closest relatives.

Morphotype	Code	Closest relative (BLAST)	Order	Accession no.	Identity (%)
1	KBPL-M1	*Daldinia eschscholtzii* strain 3-F24	*Xylariales*	MW081312.1	98.76
2	SKNT-M1	*Colletotrichum horii*	*Glomerellales*	LC186039.1	98.40
3	SKNT-P2	*Neopestalotiopsis clavispora* isolate SV4	*Xylariales*	MG386209.1	99.81
4	KBSK-P1	*Endomelanconiopsis endophytica* isolate BR9	*Botryosphaeriales*	MN637809.1	99.46
5	KBNK-L1	*Neopestalotiopsis* sp. F.IT-2020 NTUCC 18-089	*Xylariales*	MT322111.1	100.00
6	KBPL-P1	*Nigrospora sphaerica* isolate BM6-1	*Xylariales*	MN795548.1	97.44
7	PLPP-V3	*Phyllosticta capitalensis* strain 15B516	*Botryosphaeriales*	MT186148.1	99.52
8	SKKN-P2	*Lasiodiplodia pseudotheobromae* isolate ALT1	*Botryosphaeriales*	MH608364.1	99.25
9	KBSK-V1	*Chaetomium cupreum* strain AHBR18	*Sordariales*	KF305757.1	99.00
10	KBHN-M1	*Fusarium solani* clone 2014_1481	*Hypocreales*	MN523069.1	74.78
11	YAMUL-L1	*Pseudopestalotiopsis chinensis* NTUCC 18-050	*Xylariales*	MT322067.1	99.45
12	KBNK-V1	*Gnomoniopsis daii* CPC 29158	*Diaporthales*	MN598671.1	94.66
13	PLKK-M2	*Preussia pseudominima* strain 5-F16	*Pleosporales*	MW081384.1	98.69

ferent plant tissues, the Dmg index was highest in vein tissue (4.203) but was not significantly different among midrib, lamina and petiole tissues. Shannon's index of species diversity (H′) showed no difference between samples from the western and the eastern coasts. Species diversity in different leaf segments varied as follows: vein (2.033) > midrib (1.932) > lamina (1.869) > petiole (1.833). Simpson's index (D) showed the same declining order as the diversity.

Antimicrobial assay

In primary screening using culture broth filtrates, four out of 13 isolates (31%) showed antimicrobial activities against at least one pathogen (Table 4). *C. cupreum* isolate KBSK-V1-T8 had positive antimicrobial activities against both Gram-positive (*K. rhizophila*) and

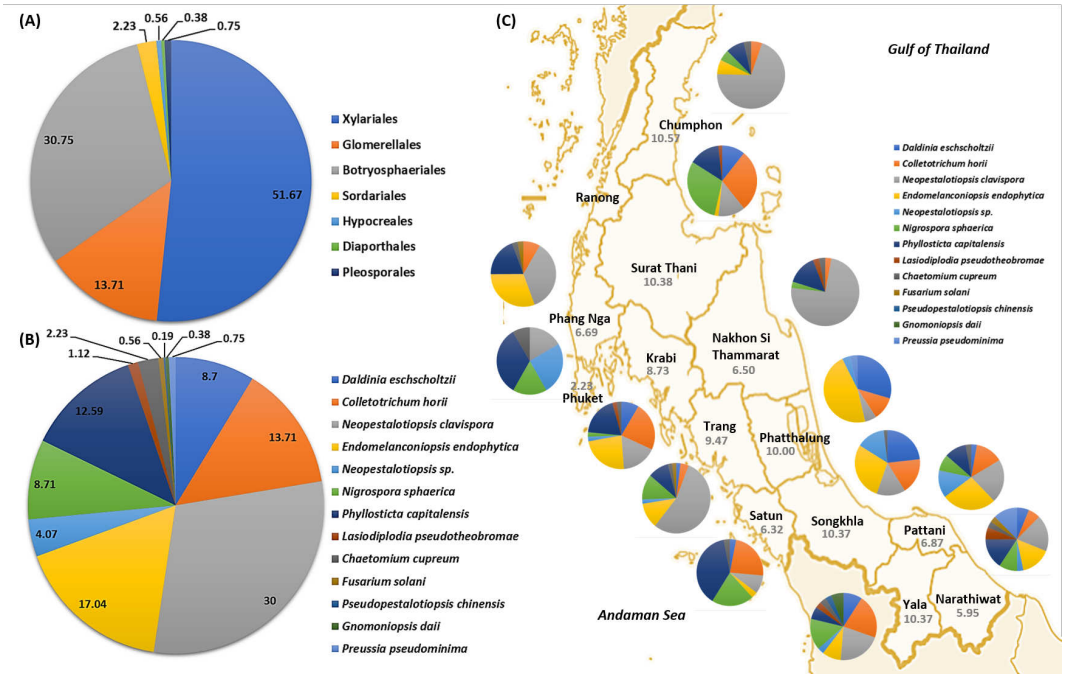

Fig. 4 Relative frequency (%RF) of endophytic fungi at the order (A) and genus (B) levels. Geographical distribution of endophytic fungi from *R. tomentosa* (Aiton) Hassk. leaves across the southern region of Thailand (C). Numbers indicate relative frequency (%RF) of fungal communities.

Table 3 Fungal diversity analysis of *R. tomentosa* leaves assessed from different leaf segments and different sampling provinces.

Diversity analysis		S	Dmg	Dmn	H′	D
(A) Leaf segment						
	Vein	12	4.203	0.590	2.033	0.840
	Midrib	10	3.439	0.491	1.932	0.828
	Lamina	10	3.473	0.506	1.869	0.816
	Petiole	10	3.456	0.499	1.833	0.787
	Total	13	3.739	0.323	1.983	0.829
(B) Sampling province						
Western coast	Phang Nga	6	2.459	0.577	1.475	0.742
	Phuket	5	2.570	0.833	1.517	0.786
	Krabi	9	3.722	0.758	1.835	0.822
	Trang	9	3.662	0.728	1.496	0.663
	Satun	7	2.987	0.693	1.559	0.753
Eastern coast	Chumphon	6	2.239	0.459	1.076	0.491
	Surat Thani	7	2.696	0.540	1.641	0.783
	Nakhon Si Thammarat	6	2.474	0.586	0.902	0.428
	Phatthalung	6	2.263	0.471	1.366	0.684
	Songkhla	6	2.247	0.463	1.618	0.794
	Pattani	8	3.422	0.759	1.865	0.831
	Narathiwat	11	5.045	1.123	2.200	0.883
LL[†]	Yala	11	5.011	1.106	2.143	0.868
	Total	13	3.739	0.323	1.998	0.832

[†] LL refers to a landlocked province in the south. S, number of genera; Dmg, Margalef's richness; Dmn, Menhinick's index; H′, Shannon's index and D, Simpson's index.

Table 4 Antimicrobial activity screening of endophytic fungi isolated from *R. tomentosa* (Aiton) Hassk. leaves using agar well diffusion method.

Test organisms	Zone of inhibition (mm)						
	Fungal isolates				Antibiotics**		
	C. cupreum-T8 (morpho9)	*Neopestalotiopsis* sp.T9 (morpho5)	*E. endophytica*-T13 (morpho4)	*G. daii*-T14 (morpho12)	Vancomycin	Gentamicin	Amphotericin B
G. (+ve) bacteria							
K. rhizophila	21.8 ± 0.8^{a}	–	17.2 ± 0.3^{c}	14.7 ± 0.5^{d}	18.7 ± 0.3^{b}	–	–
S. aureus	–	–	–	–	15.1 ± 0.5	–	–
MRSA*	–	–	–	–	13.2 ± 0.1	–	–
G. (-ve) bacteria							
E. coli	–	–	–	–	–	19.3 ± 0.7	–
P. aeruginosa	–	–	–	–	–	14.1 ± 0.9	–
V. cholerae	22.2 ± 0.4^{a}	20.8 ± 0.8^{b}	–	–	–	20.5 ± 0.2^{b}	–
Yeast							
C. albicans	–	–	–	–	–	–	20.3 ± 0.9

* MRSA: methicillin-resistant *S. aureus*; ** Concentration of antibiotics used in this study was 20 µg/ml; Different superscript letters (a–d) indicate significant differences ($p < 0.05$) between zone of inhibition values from different endophytic fungi against each test organism (same row); – indicates no activity.

Gram-negative (*V. cholerae*) bacteria. *E. endophytica* isolate KBHN-M1-T13 and *G. daii* isolate KBNK-V1-T14 had positive antimicrobial activities against *K. rhizophila*, while *Neopestalotiopsis* sp. isolate KBNK-L1-T9 had positive antimicrobial activity against *V. cholerae*. None of the fungal isolates had antifungal activity against *C. albicans*. The four active fungal isolates were tested for antimicrobial activities at different concentrations (1–100 mg/ml) of ethyl acetate extracts of mycelia and culture broths. The results showed that extracts of the culture broths showed greater inhibitory activities against the test pathogens than the mycelial extracts (Table S9). At 1 mg/ml concentration, none of the extracts inhibited any test organism. Overall, strong antimicrobial activities against most of the test organisms of the culture broth and the mycelial extracts were observed at the concentrations of 10 mg/ml and 100 mg/ml, respectively. This study indicated that endophytic fungi isolated from *R. tomentosa* leaves could be a good source of natural antimicrobial products.

DISCUSSION

Rhodomyrtus tomentosa has been used in Asian traditional medicine for a long time. Moreover, the biodiversity and pharmacological properties of *R. tomentosa* have been the focus of increasing attention [5–7], and few studies attempted to evaluate the diversity of endophytic fungi associated with this valuable plant [26]. In this study, we investigated the diversity of cultured fungal endophytes isolated from *R. tomentosa* (Aiton) Hassk. leaves collected in the dry season from different locations across the southern region of Thailand. Interestingly, some of the fungal endophytes had the potential to inhibit microbial pathogens.

Previous studies of endophytic fungi associated with Myrtaceae plants reported *Diaporthales*, *Botryosphaeriales*, *Glomerellales*, *Xylariales*, and *Hypocreales* to be dominant orders [26–28]. In our study, the most abundant order was *Xylariales* (RF, 51.67%), followed by *Botryosphaeriales* (RF, 30.75%) and *Glomerellales* (RF, 13.71%). Regarding fungal genera, *Colletotrichum*, *Diaporthe*, *Phomopsis*, *Guignardia*, *Pestalotiopsis*, and *Xylaria* were reported to dominate [26, 27]. The present study produced similar findings (*Neopestalotiopsis* (RF, 33.56%) and *Colletotrichum* (RF, 13.72%)), and fungi representing the genera *Daldinia*, *Endomelanconiopsis*, *Fusarium*, *Gnomoniopsis*, *Lasiodiplodia*, *Nigrospora*, *Phyllosticta*, *Preussia*, and *Pseudopestalotiopsis* were also observed. Lina et al [27] described the endophytic fungal compositions of leaves and twigs of *Blepharocalyx salicifolius*, *Myrceugenia glaucescens*, and *Acca sellowiana* (Myrtaceae-Myrtoideae) in the South-Central region of Uruguay. Their results revealed %CRs ranging from 27% to 78%, which was a narrower range than what observed in this study (65% to 98%). Other studies on fungal endophytes associated with Myrtaceae were reported from Argentina and Brazil, where the authors found *Sordariomycetes* to be the dominant class, and *Xylariales* the dominant order [28, 29]. In addition, we discovered two fungal isolates (KBHN-M1 and KBNK-V1) that might represent new species since the similarity of their sequences compared with the sequences in the NCBI GenBank database was low.

The low diversity of endophytic fungi harbored by *R. tomentosa* leaves in this study was consistent with the findings in other studies of Myrtaceae plants [26, 27]. It was notable that only seven morphotypes (*Daldinia*, *Colletotrichum*, *Neopestalotiopsis*, *Endomelanconiopsis*, *Nigrospora*, and *Phyllosticta*) were cul-

tured from all leaf tissues. The ubiquity of these fungi suggested that they had no preference for types of leaf tissue. However, most of the fungal endophytes isolated from *R. tomentosa* leaves exhibited a preference to colonize the midrib and the vein tissues, while some fungal morphotypes were only found in one specific leaf tissue. The results suggested that different leaf tissues harbored different endophytic fungal morphotypes at different levels of frequency. Our results were conformable to other findings obtained from several plant hosts [30–33]. Notably, the unique characteristics of the vein, the petiole, and the midrib tissues might promote endophytic fungal species richness in these leaf segments, compared with the lamina. Toofanee and Dulymamode [32] proposed that the physical properties of leaves could affect spore retention and spore deposition. The effects included the behavior of water reaching the leaves and the pattern of runoff and evaporation, all of which favored the petiole and the midrib. Furthermore, the petiole and the midrib tended to have more vascular bundles than the lamina; therefore, they could support nutrient accumulations for the endophytic fungi.

Our findings also revealed native endophytic fungal communities that are common across the southern region of Thailand, especially *Neopestalotiopsis* and *Colletotrichum*. A common distribution of fungal genera at all sites could indicate close interactions with host plants. However, the distribution of some fungal communities varied at a regional scale, perhaps as a result of geographic distance. For instance, *Phyllosticta* was dominant on the western coast, while *Preussia* was dominant on the eastern coast. At a local scale, environmental factors such as soil pH, soil quality, and rainfall might influence the distribution of some endophytic fungi. For instance, *Daldinia* was dominant in Phatthalung and Songkhla on the eastern coast while *Gnomoniopsis* was only found in the landlocked Yala. These findings suggested that some endophytic fungi of *R. tomentosa* were not randomly distributed, and they could be influenced either by topography or environmental factors. Furthermore, these factors might play a role in fungal species richness in certain locations.

The fungal morphotypes were shared fairly evenly between the western and the eastern coasts. However, fungal isolates from the western coast were more morphologically diverse. The growth preferences of *R. tomentosa* are for coastal sandy soil and wet forest [1, 2], and these preferences might have contributed to the aforementioned geographic difference in diversity. Notably, isolates from Narathiwat exhibited the lowest total %RF, but the highest diversity; even though it is located on the eastern coast. As evidenced by Wei et al [1], acid soils are preferred by *R. tomentosa*; hence, the strong acidity of the soil in Narathiwat might promote the distribution of *R. tomentosa* and consequently the diversity of the fungal community in this host. In addition, the genetic diversity within and among *R. tomentosa* populations could have an effect on fungal diversity and distribution. However, the level of genetic diversity of *R. tomentosa* across the southern region of Thailand has not yet been documented.

Endophytic fungi have been recognized to contain structurally diverse and biologically active metabolites [10, 11, 38]. In this study, 31% (four out of 13) ethyl acetate extracts of the endophytic showed inhibitory activities against pathogenic bacteria. These four isolates represented fungal genera previously reported to produce antimicrobial compounds [34, 35]. We observed a relatively low level of activity against Gram-negative bacteria from the fungal endophytes, which was in accordance with previous studies [15, 35, 36, 38]. Among the studied strains, *C. cupreum* strain KBSK-V1-T8 and *Neopestalotiopsis* sp. strain KBNK-L1-T9 exhibited strong antimicrobial activities against *V. cholerae* with inhibition zones of 22.2 ± 0.4 and 20.8 ± 0.8 mm, respectively. Only *C. cupreum* strain KBSK-V1-T8 exhibited antimicrobial activities against both Gram-positive and Gram-negative bacteria. Antimicrobial compounds produced by the strain KBSK-V1-T8 are being investigated. Endophytes of the genus *Chaetomium* was recently reported to exert wide spectrum antimicrobial activities against *S. aureus*, MRSA, *E. coli*, and *Klebsiella pneumoniae* [35, 37]; and, therefore, antimicrobial compounds produced by the strain KBSK-V1-T8 should be further investigated. We observed that crude extracts of culture broths showed greater inhibitory activity than those of the mycelia. A high antibacterial activity of the fungal culture broths was expected due to the ability of endophytic fungi to produce antibacterial components needed to compete and survive in natural habitats. Our study, therefore, indicated that *R. tomentosa* could be exploited as a potential source of fungal endophytes with antimicrobial activities.

CONCLUSION

In the present study, the diversity, distribution, and antimicrobial activities of fungal endophytes from *Rhodomyrtus tomentosa* (Aiton) Hassk. leaves were investigated. Our results demonstrate that *R. tomentosa* harbored common native endophytic fungal communities across the southern region of Thailand, representing a diversity of taxonomic affiliations, including potentially new species. The results showed that four out of 13 fungal morphotypes exhibited inhibitory activities against at least one of the tested pathogenic bacteria. Specifically, the *C. cupreum* strain KBSK-V1-T8 exhibited antimicrobial activities against both Gram-positive and Gram-negative bacteria. Overall, these results suggested that fungal endophytes from *R. tomentosa* could be exploited as a potential source of bioactive agents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.027.

Acknowledgements: We thank Hattaya Jaroensap for plant collection. This research was supported by the National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University (Grant No. SCI6505182M).

REFERENCES

1. Wei MS, Chen ZH, Ren H, Yin ZY (2009) Reproductive ecology of *Rhodomyrtus tomentosa* (Myrtaceae). *Nord J Bot* **3**, 154–160.
2. Winotai A, Wright T, Goolsby JA (2005) Herbivores in Thailand on *Rhodomyrtus* sp. (Myrtaceae), an invasive weed in Florida. *Florida Entomol* **88**, 104–105.
3. Chuakul W (2005) Medicinal plants in the Khok Pho District, Pattani Province (Thailand). *Thai J Phytopharm* **12**, 23–45.
4. Limsuwan S, Trip EN, Kouwen TR, Piersma S, Hiranrat A, Mahabusarakam W, Voravuthikunchai SP, van Dijl JM, et al (2009) Rhodomyrtone: A new candidate as natural antibacterial drug from *Rhodomyrtus tomentosa*. *Phytomedicine* **16**, 645–651.
5. Do T (2011) *Medicine Plants and Remedies of Vietnam*, Thoi Dai Publisher, Hanoi, Vietnam.
6. Hamid HA, Mutazah SSZR, Yusoff MM (2017) *Rhodomyrtus tomentosa*: A phytochemical and pharmacological review. *Asian J Pharm Clin Res* **10**, 10–16.
7. Vo TS, Ngo DH (2019) The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. *Biomolecules* **9**, 76.
8. Kuldau G, Bacon C (2008) Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biol Control* **46**, 57–51.
9. Kusari P, Kusari S, Spiteller M, Kayser O (2013) Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. *Fungal Divers* **60**, 137–151.
10. Deshmukh SK, Verekar SA, Bhave SV (2015) Endophytic fungi: A reservoir of antibacterials. *Front Microbiol* **5**, 715.
11. Meena H, Hnamte S, Siddhardha B (2019) Secondary metabolites from endophytic fungi: chemical diversity and application. In: Singh B (ed) *Advances in Endophytic Fungal Research*, Fungal Biology, Springer, Cham.
12. Baron NC, Rigobelo EC (2022) Endophytic fungi: A tool for plant growth promotion and sustainable agriculture. *Mycology* **13**, 39–55.
13. He SM, Wang X, Yang SC, Dong Y, Zhao QM, Yang JL, Cong K, Zhang JJ, et al (2018) *De novo* transcriptome characterization of *Rhodomyrtus tomentosa* leaves and identification of genes involved in α/β-pinene and β-caryophyllene biosynthesis. *Front Plant Sci* **9**, 1231.
14. Wingfield LK, Jitprasitporn N, Che-alee N (2023) Isolation and characterization of halophilic and halotolerant fungi from man-made solar salterns in Pattani Province, Thailand. *PLoS One* **18**, e0281623.
15. Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. *FEMS Immunol Med Microbiol* **48**, 367–372.
16. Samson RA, Hoekstra ES, Frisvad JC (2004) *Introduction to Food and Airborne Fungi*, 7th edn, Centraalbureauvoor Schimme Cultures, Utrecht.
17. Dugan FM (2015) *The Identification of Fungi – An Illustrated Introduction with Keys, Glossary, and Guide to Literature*, The American Phytopathological Society Press, St. Paul, USA.
18. Ellis D, Davis S, Alexiou H, Handke R, Bartley R (2016) *Description of Medical Fungi*, 3rd edn, South Australia Nexus Print Solutions.
19. Wingfield LK, Atcharawiriyakul J (2021) Evaluation of simple and rapid DNA extraction methods for molecular identification of fungi using the ITS regions. *Asia Pac J Sci Technol* **26**, APST-26.
20. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. *Mol Biol Evol* **38**, 3022–3027.
21. Whittaker RH (1972) Evolution of species diversity in land communities. *Evol Biol* **10**, 1–67.
22. Margalef DR (1958) Information theory in ecology. *Gen Syst* **3**, 36–71.
23. Lambshead PJ, Platt HM, Shaw KM (1983) The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *J Nat Hist* **17**, 859–874.
24. Gong LJ, Guo SX (2009) Endophytic fungi from *Dracaena cambodiana* and *Aquilaria sinensis* and their antimicrobial activity. *Afr J Biotechnol* **8**, 731–736.
25. Lorian V (1996) *Antibiotics in Laboratory Medicine*, 3rd edn, Williams and Wilkins, Baltimore.
26. Jeenkeawpieam J, Phongpaichit S, Rukachaisirikul V, Sakayaroj J (2012) Antifungal activity and molecular identification of endophytic fungi from the angiosperm *Rhodomyrtus tomentosa*. *Afr J Biotechnol* **11**, 14007–14016.
27. Lina B, Sofía S, Raquel A, Sandra L (2008) Endophytic fungi of twigs and leaves of three native species of Myrtaceae in Uruguay. *Sydowia* **56**, 8.
28. Vaz AB, Fontenla S, Rocha FS, Brandão LR, Vieira MLA, de Garcia V, Góes-Neto A, Rosa CA (2014) Fungal endophyte β-diversity associated with Myrtaceae species in an Andean Patagonian Forest (Argentina) and an Atlantic Forest (Brazil). *Fungal Ecol* **8**, 28–36.
29. Vaz AB, da Costa AG, Raad LV, Góes-Neto A (2014b) Fungal endophytes associated with three South American Myrtae (Myrtaceae) exhibit preferences in the colonization at leaf level. *Fungal Biol* **118**, 277–286.
30. Brown KB, Hyde KD, Guest DI (1998) Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. *Fungal Divers* **1**, 26–50.
31. Rodrigues KF, Samuels GJ (1999) Fungal endophytes of *Spondias mombin* leaves in Brazil. *J Basic Microbiol* **39**, 131–135.
32. Toofanee SB, Dulymamode R (2002) Fungal endophytes associated with *Cordemoya integrifolia*. *Fungal Divers* **11**, 169–175.
33. Kannan KP, Muthumary J (2012) Comparative analysis of endophytic mycobiota in different tissues of medicinal plants. *Afr J Microbiol Res* **6**, 4219–4225.

34. Tanapichatsakul C, Khruengsai S, Monggoot S, Pripdeevech P (2019) Production of eugenol from fungal endophytes *Neopestalotiopsis* sp. and *Diaporthe* sp. isolated from *Cinnamomum loureiroi* leaves. *PeerJ* **7**, e6427.
35. Kaur N, Arora DS (2020) Prospecting the antimicrobial and antibiofilm potential of *Chaetomium globosum* an endophytic fungus from *Moringa oleifera*. *AMB Express* **10**, 206.
36. Santos IP, Silva LCN, Silva MV, Araújo JM, Cavalcanti MS, Lima VLM (2015) Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). *Front Microbiol* **6**, 350.
37. Gao W, He Y, Li F, Chai C, Zhang J, Guo J, Chen C, Wang J, et al (2019) Antibacterial activity against drug-resistant microbial pathogens of cytochalasan alkaloids from the arthropod-associated fungus *Chaetomium globosum* TW1-1. *Bioorg Chem* **83**, 98–104.
38. Wingfield LK, Yungsatit Y, Jitprasitporn N, Nikornsaen S (2024) Diversity and characterization of culturable fungi associated with *Gnetum gnemon* Linn. in organic and conventional farming systems and their potential antagonism against pathogenic fungi. *ScienceAsia* **50**, 2024027.

Appendix A. Supplementary data

Table S1 Geographical locations and climatic characteristics of sampling sites.

Side	Sampling area		GPS information			Geographical position	Climate	Temp.	RH
	Province	District	Latitude	Longitude	Elevation			(°C)	(%)
Western coast	Phang Nga	Takua Pa Khura Buri Thap Put	8.8267 9.1796 8.4868	98.3425 98.3626 98.5911	43 ft 48 ft −57 ft	Complex mountain stretching in long line from north to south; flat area slopes down east to west.	Tropical monsoon climate and is influenced by southwestern and northeast monsoon winds; 2 seasons, summer and rainy.	25–33	80–85
	Phuket	Thalang	8.0358	98.2976	40 ft	The archipelago landscape consists of mountains, seas, and beaches.	Tropical monsoon climate and is influenced by southwestern and northeast monsoon winds; 2 seasons, summer and rainy.	25–33	80–85
	Krabi	Sai Khao Pe Lah Huay Nam Khao Nuea Khlong	7.7221 8.0163 7.8620 8.0449	99.3003 99.1157 99.1690 98.9778	65 ft 118 ft 121 ft 41 ft	Long mountain ranges extending in line from north to south; unique terrain mountains scattered undulating area in the south.	Tropical monsoon climate and is influenced by southwestern and northeast monsoon winds; 2 seasons, summer and rainy.	25–33	80–85
	Trang	Yan Ta Khao Hat Samran Kantang Sikao	7.4629 7.2433 7.3682 7.6557	99.6711 99.5503 99.5377 99.3430	62 ft 14 ft 90 ft 71 ft	High-low hill interspersed with large and small mountains scattered in the east; long mountain ranges from north to south.	Hot, humid climate influenced by southwest and northeast monsoon winds; 2 seasons, summer and rainy.	27–30	80–85
	Satun	La-ngu Thung Wa Khuan Kalong	6.9461 6.9804 6.8530	99.6956 99.6852 100.1257	18 ft 37 ft 174 ft	Hills and mountains in the north and east; slopes down to the sea from west to south, with narrow plains running parallel to seacoast.	Influenced by the northeast monsoon that blows over the Gulf of Thailand and the southwest monsoon from the Indian Ocean; 2 seasons, summer and rainy.	25–32	80–85
Eastern coast	Chumphon	Lamae Sawi Mueang Tha Sae Lang Suan	9.7420 10.2594 10.5522 10.6641 9.8971	99.1303 99.1317 99.2580 99.1544 99.1378	36 ft 48 ft 83 ft 72 ft 71 ft	Mountain ranges with high and low peaks; to the east is a plain by the sea.	Northwest and southeast monsoon zones resulting in a climate under the influence of the monsoon (summer and rainy).	25–30	85–90
	Surat Thani	Tha Chana Chaiya Tha Chang Khiri Rat Nikhom Phunphin	9.6089 9.4303 9.2891 9.0664 9.1116	99.1785 99.2711 99.1855 98.9630 99.1520	45 ft 23 ft 21 ft 102 ft 24 ft	Lowland area in the north; to the east is a plain by the sea. South and west are mountains and plateaus.	The Southwest monsoon every year causes cloudy and abundant rain; 2 seasons, summer and rainy.	25–30	85–90
	Nakhon Si Thammarat	Mueang Tha Sala Sichon	8.5395 8.6193 8.9196	99.9683 99.9519 99.8175	41 ft 22 ft 94 ft	Mountain ranges with high and low peaks; to the east is a plain by the sea.	Northwest and southeast monsoon zones resulting in a climate under the influence of the monsoon (summer and rainy).	25–30	85–90
	Phatthalung	Pa Bon Pak Phayun Khao Chaison Khuan Khanun	7.1628 7.2791 7.4796 7.7740	100.0828 100.2276 100.1162 99.9279	425 ft 44 ft 45 ft 106 ft	Mountain ranges with high and low peaks; to the east is a plain by the sea.	Northwest and southeast monsoon zones resulting in a climate under the influence of the monsoon (summer and rainy).	27–29	62–93
	Songkhla	Khuan Niang Na Thawi Khlong Hoi Khong Sathing Phra	7. 6.7003 6.9176 7.4368	100.3716 100.6595 100.4361 100.4558	32 ft 142 ft 49 ft 27 ft	Lowland area in the north; to the east is a plain by the sea. South and west are mountains and plateaus.	The Southwest monsoon every year causes cloudy and abundant rain; 2 seasons, summer and rainy.	27–28	85–95
	Pattani	Mae Lan Yaring Panare	6.7079 6.8432 6.8695	101.2016 101.4621 101.4663	48 ft 28 ft 25 ft	Coastal plain with mountainous areas in the south and east.	Tropical climate around the equator causing tropical monsoon; 2 seasons, summer and rainy.	25–33	85–93
	Narathiwat	Bacho Yi-ngo Mueang Tak Bai	6.5447 6.3707 6.5454 6.2916	101.6735 101.7002 101.7373 101.9781	30 ft 74 ft 32 ft 15 ft	Slope area from west to east. Most of the plains are adjacent to the Gulf of Thailand and the river plains.	Tropical monsoon; southwest monsoon; 2 seasons, summer and rainy.	25–33	85–93
LL[†]	Yala	Mueang Raman Lam Mai	6.4757 6.5781 6.5796	101.2098 101.4690 101.1944	152 ft 63 ft 119 ft	Land-locked area with mountains and hill valleys from middle to south; plains in the north covered with rainforest and rubber plantations.	Northeast monsoon and southwest monsoon; 2 seasons, summer and rainy.	28–30	85–90

[†] LL refers to a landlocked province in the south.

Table S2 Geographical location of the sampling sites based on provinces, soil analysis, temperature, relative humidity, and number of the *R. tomentosa* leaf samples.

Side	Province	Coordinate	Soil pH	Total N (%w/w)	Total P_2O_5 (%w/w)	Total K_2O (%w/w)	Organic matter (%w/w)	EC	Average temp. (°C)	Average RH (%)	Sample (*n*)
Western coast	Phang Nga	8°49′36.1″ N 98°20′33.0″ E	5.05	0.069	0.002	0.014	1.510	0.034	26.4	81.70	27
	Phuket	8°02′09.1″ N 98°17′51.5″ E	5.68	0.051	0.002	0.001	1.260	0.034	27.3	75.40	9
	Krabi	7°51′43.4″ N 99°10′08.4″ E	4.99	0.066	0.002	0.004	1.875	0.017	27.0	80.10	36
	Trang	7°14′35.9″ N 99°33′01.4″ E	4.90	0.084	0.003	0.010	2.193	0.019	26.7	79.90	36
	Satun	6°56′46.2″ N 99°41′45.5″ E	5.17	0.080	0.005	0.010	2.463	0.041	27.0	78.00	27
Eastern coast	Chumphon	9°53′49.8″ N 99°08′16.2″ E	5.12	0.060	0.010	0.010	1.820	0.020	26.6	80.90	45
	Surat Thani	9°25′49.2″ N 99°16′16.2″ E	5.54	0.054	0.005	0.003	1.824	0.025	26.5	82.90	45
	Nakhon Si Thammarat	8°37′09.7″ N 99°57′07.2″ E	5.47	0.060	0.010	0.005	1.557	0.008	26.9	81.40	27
	Phatthalung	7°09′46.3″ N 100°04′58.4″ E	4.82	0.079	0.006	0.013	2.620	0.023	26.7	81.70	36
	Songkhla	7°13′45.0″ N 100°22′21.5″ E	5.06	0.091	0.007	0.008	2.435	0.021	27.1	76.80	36
	Pattani	6°52′10.4″ N 101°27′58.8″ E	5.99	0.069	0.006	0.008	2.057	0.019	27.0	83.70	27
	Narathiwat	6°17′30.0″ N 101°58′41.3″ E	4.62	0.075	0.004	0.004	1.653	0.016	27.1	77.50	36
LL[†]	Yala	6°32′23.82″ N 101°16′52.61″ E	5.41	0.076	0.006	0.013	1.740	0.032	27.1	81.00	27

[†] LL refers to a landlocked province in the south.

Table S3 Soil characteristics of the sampling sites.

Side	Sampling area		Soil	Total N	Total P_2O_5	Total K_2O	Organic	Electrical
	Province	District	pH	(%w/w)	(%w/w)	(%w/w)	matter (%w/w)	conductivity
Western coast	Phang Nga	Takua Pa	5.24	0.074	0.002	0.008	1.83	0.014
		Khura Buri	5.39	0.038	0.001	0.001	0.72	0.055
		Thap Put	4.52	0.094	0.002	0.034	1.98	0.034
	Phuket	Thalang	5.68	0.051	0.002	0.001	1.26	0.034
	Krabi	Sai Khao	5.40	0.031	0.001	0.002	1.01	0.008
		Pe Lah	4.80	0.110	0.004	0.003	3.02	0.019
		Huay Nam Khao	4.66	0.068	0.001	0.005	1.85	0.022
		Nuea Khlong	5.10	0.053	0.003	0.004	1.62	0.018
	Trang	Yan Ta Khao	5.04	0.076	0.004	0.004	1.89	0.013
		Hat Samran	4.82	0.089	0.003	0.003	2.71	0.016
		Kantang	4.31	0.097	0.003	0.033	1.89	0.021
		Sikao	5.36	0.091	0.002	0.007	2.60	0.030
	Satun	La-ngu	5.32	0.152	0.002	0.007	2.42	0.071
		Thung Wa	4.99	0.013	0.009	0.014	2.96	0.020
		Khuan Kalong	5.21	0.076	0.004	0.009	2.01	0.032
Eastern coast	Chumphon	Lamae	4.93	0.066	0.004	0.004	2.24	0.013
		Sawi	5.20	0.081	0.005	0.028	1.80	0.019
		Mueang	5.14	0.082	0.010	0.008	2.10	0.035
		Tha Sae	5.12	0.055	0.005	0.005	1.53	0.012
		Lang Suan	5.23	0.036	0.001	0.001	1.42	0.017
	Surat Thani	Tha Chana	5.54	0.050	0.005	0.003	1.59	0.014
		Chaiya	5.61	0.056	0.003	0.001	1.53	0.021
		Tha Chang	5.50	0.064	0.005	0.003	2.10	0.034
		Khiri Rat Nikhom	5.43	0.048	0.006	0.004	1.92	0.029
		Phunphin	5.60	0.050	0.004	0.004	1.98	0.025
	Nakhon Si Thammarat	Mueang	5.15	0.058	0.011	0.004	1.12	0.008
		Tha Sala	5.31	0.078	0.011	0.007	2.01	0.007
		Sichon	5.95	0.043	0.007	0.004	1.54	0.010
	Phatthalung	Pa Bon	5.07	0.038	0.003	0.023	2.36	0.018
		Pak Phayun	4.60	0.091	0.008	0.012	2.63	0.029
		Khao Chaison	4.83	0.077	0.006	0.003	2.09	0.019
		Khuan Khanun	4.77	0.109	0.006	0.012	3.40	0.027
	Songkhla	Khuan Niang	5.02	0.080	0.014	0.002	1.95	0.029
		Na Thawi	4.99	0.094	0.004	0.003	2.99	0.018
		Khlong Hoi Khong	5.08	0.146	0.002	0.021	3.23	0.027
		Sathing Phra	5.13	0.044	0.007	0.007	1.57	0.011
	Pattani	Mae Lan	5.67	0.101	0.005	0.005	2.72	0.024
		Yaring	4.65	0.049	0.006	0.016	1.61	0.010
		Panare	5.99	0.057	0.006	0.003	1.84	0.022
	Narathiwat	Bacho	4.67	0.106	0.005	0.002	2.11	0.020
		Yi-ngo	4.49	0.056	0.007	0.009	2.23	0.022
		Mueang	4.68	0.043	0.003	0.002	1.28	0.012
		Tak Bai	4.62	0.094	0.002	0.002	0.99	0.010
LL[†]	Yala	Mueang	5.28	0.050	0.003	0.010	1.27	0.009
		Raman	5.44	0.055	0.006	0.003	1.37	0.031
		Lam Mai	5.52	0.122	0.010	0.027	2.58	0.056

[†] LL refers to a landlocked province in the south.

Table S4 Numbers of endophytic fungi isolated from the *R. tomentosa* leaves.

Side	Province	No. of fungal isolates (No. of fragments)				
		Vein	Midrib	Lamina	Petiole	Total
Western coast	Phang Nga	24 (27)	33 (27)	21 (27)	24 (27)	102 (108)
	Phuket	9 (9)	9 (9)	9 (9)	9 (9)	36 (36)
	Krabi	36 (36)	36 (36)	33 (36)	39 (36)	144 (144)
	Trang	41 (36)	40 (36)	36 (36)	39 (36)	156 (144)
	Satun	24 (27)	27 (27)	24 (27)	27 (27)	102 (108)
Eastern coast	Chumphon	49 (45)	51 (45)	33 (45)	38 (45)	171 (180)
	Surat Thani	42 (45)	42 (45)	42 (45)	42 (45)	168 (180)
	Nakhon Si Thammarat	27 (27)	27 (27)	27 (27)	27 (27)	108 (108)
	Phatthalung	42 (36)	39 (36)	42 (36)	36 (36)	159 (144)
	Songkhla	45 (36)	42 (36)	42 (36)	39 (36)	168 (144)
	Pattani	28 (27)	27 (27)	26 (27)	30 (27)	111 (108)
	Narathiwat	24 (36)	24 (36)	18 (36)	33 (36)	99 (144)
LL[†]	Yala	25 (27)	21 (27)	29 (27)	24 (27)	99 (108)
	Total	416 (414)	418 (414)	382 (414)	407 (414)	1,623 (1,656)

[†] LL refers to a landlocked province in the south.

Table S5 Numbers of infected leaf segments of the *R. tomentosa*.

Side	Province	No. of infected leaf segments (Total no. of leaf segments)				
		Vein	Midrib	Lamina	Petiole	Total
Western coast	Phang Nga	24 (27)	27 (27)	21 (27)	21 (27)	93 (108)
	Phuket	9 (9)	9 (9)	5 (9)	6 (9)	29 (36)
	Krabi	33 (36)	36 (36)	30 (36)	33 (36)	132 (144)
	Trang	28 (36)	36 (36)	34 (36)	30 (36)	128 (144)
	Satun	24 (27)	27 (27)	24 (27)	24 (27)	99 (108)
Eastern coast	Chumphon	42 (45)	45 (45)	30 (45)	36 (45)	153 (180)
	Surat Thani	43 (45)	42 (45)	38 (45)	36 (45)	159 (180)
	Nakhon Si Thammarat	27 (27)	27 (27)	21 (27)	24 (27)	99 (108)
	Phatthalung	36 (36)	33 (36)	33 (36)	30 (36)	132 (144)
	Songkhla	36 (36)	36 (36)	35 (36)	33 (36)	140 (144)
	Pattani	27 (27)	27 (27)	24 (27)	27 (27)	105 (108)
	Narathiwat	22 (36)	24 (36)	19 (36)	30 (36)	95 (144)
LL[†]	Yala	24 (27)	21 (27)	24 (27)	21 (27)	90 (108)
	Total	375 (414)	390 (414)	338 (414)	351 (414)	1,454 (1,656)

[†] LL refers to a landlocked province in the south.

Table S6 ITS sequences of endophytic fungi deposited in the Genbank (accession Nos. OP890913 to OP890924).

Morphotype	Fungal code	Accession number
1	KBPL-M1	OP890913
2	SKNT-M1	OP890914
3	SKNT-P2	OP890915
4	KBHN-M1	OP890916
5	KBNK-L1	OP890917
6	KBPL-P1	OP890918
7	PLPP-V3	OP890919
8	SKKN-P2	OP890920
9	KBSK-V1	OP890921
10	KBHN-M1	OP890916
11	YAMUL-L1	OP890922
12	KBNK-V1	OP890923
13	PLKK-M2	OP890924

Table S7 Numbers of endophytic fungi grouped by morphotypes and categorized by leaf segment and sampling areas (provinces).

Parameter		Fungal morphotype													Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	
(A) Leaf segment															
	Vein	36	53	105	93	18	54	27	9	6	0	3	3	9	416
	Midrib	39	46	126	63	18	60	51	3	9	3	0	0	0	418
	Lamina	42	45	99	104	12	39	27	0	9	0	0	3	2	382
	Petiole	25	84	150	19	18	51	36	6	12	6	0	0	0	407
	Total	142	228	480	279	66	204	141	18	36	9	3	6	11	1623
(B) Sampling province															
Western coast	Phang Nga	1	9	39	28	0	18	0	0	4	3	0	0	0	102
	Phuket	1	2	6	1	9	11	6	0	0	0	0	0	0	36
	Krabi	12	33	23	33	3	31	3	3	3	0	0	0	0	144
	Trang	3	7	84	18	3	17	18	0	3	3	0	0	0	156
	Satun	3	24	9	3	0	39	21	0	3	0	0	0	0	102
Eastern coast	Chumphon	0	9	115	17	0	15	9	0	6	0	0	0	0	171
	Surat Thani	18	48	21	6	0	24	48	3	0	0	0	0	0	168
	Nakhon Si	0	5	78	0	0	15	3	4	3	0	0	0	0	108
	Phatthalung	47	18	15	71	6	0	0	0	0	0	0	0	2	159
	Songkhla	39	31	24	48	24	0	0	0	2	0	0	0	0	168
	Pattani	3	15	24	30	15	12	9	0	3	0	0	0	0	111
	Narathiwat	6	6	21	15	3	16	9	5	6	3	0	0	9	99
LL[†]	Yala	9	21	21	9	3	6	15	3	3	0	3	6	0	99
	Total	142	228	480	279	66	204	141	18	36	9	3	6	11	1623

[†] LL refers to a landlocked province in the south.

Table S8 Relative frequency (%RF) of endophytic fungal morphotypes isolated from different segments of *R. tomentosa* leaves collected from sampling sites in different provinces of southern Thailand.

Parameter		Fungal morphotype (%RF)													Total	Number of
		1	2	3	4	5	6	7	8	9	10	11	12	13	(%RF)	morpho-type
(A) Leaf segment																
	Vein	2.22	3.27	6.47	5.73	1.11	3.33	1.66	0.55	0.37	0.00	0.18	0.18	0.55	25.63	12
	Midrib	2.40	2.83	7.76	3.88	1.11	3.70	3.14	0.18	0.55	0.18	0.00	0.00	0.00	25.75	10
	Lamina	2.59	2.77	6.10	6.41	0.74	2.40	1.66	0.00	0.55	0.00	0.00	0.18	0.12	23.54	10
	Petiole	1.54	5.18	9.24	1.17	1.11	3.14	2.22	0.37	0.74	0.37	0.00	0.00	0.00	25.08	10
	Total	8.75	14.05	29.57	17.19	4.07	12.57	8.69	1.11	2.22	0.55	0.18	0.37	0.68	100	13
(B) Sampling province																
Western coast	Phang Nga	0.06	0.55	2.40	1.73	0.00	1.11	0.00	0.00	0.25	0.18	0.00	0.00	0.00	6.28	7
	Phuket	0.06	0.12	0.37	0.06	0.55	0.68	0.37	0.00	0.00	0.00	0.00	0.00	0.00	2.22	7
	Krabi	0.74	2.03	1.42	2.03	0.18	1.91	0.18	0.18	0.18	0.00	0.00	0.00	0.00	8.87	9
	Trang	0.18	0.43	5.18	1.11	0.18	1.05	1.11	0.00	0.18	0.18	0.00	0.00	0.00	9.61	9
	Satun	0.18	1.48	0.55	0.18	0.00	2.40	1.29	0.00	0.18	0.00	0.00	0.00	0.00	6.28	7
Eastern coast	Chumphon	0.00	0.55	7.09	1.05	0.00	0.92	0.55	0.00	0.37	0.00	0.00	0.00	0.00	10.54	6
	Surat Thani	1.11	2.96	1.29	0.37	0.00	1.48	2.96	0.18	0.00	0.00	0.00	0.00	0.00	10.35	7
	Nakhon Si Thammarat	0.00	0.31	4.81	0.00	0.00	0.92	0.18	0.25	0.18	0.00	0.00	0.00	0.00	6.65	6
	Phatthalung	2.90	1.11	0.92	4.37	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	9.80	6
	Songkhla	2.40	1.91	1.48	2.96	1.48	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	10.35	6
	Pattani	0.18	0.92	1.48	1.85	0.92	0.74	0.55	0.00	0.18	0.00	0.00	0.00	0.00	6.84	8
	Narathiwat	0.37	0.37	1.29	0.92	0.18	0.99	0.55	0.31	0.37	0.18	0.00	0.00	0.55	6.10	11
LL[†]	Yala	0.55	1.29	1.29	0.55	0.18	0.37	0.92	0.18	0.18	0.00	0.18	0.37	0.00	6.10	11
	Total	8.75	14.05	29.57	17.19	4.07	12.57	8.69	1.11	2.22	0.55	0.18	0.37	0.68	100	13
	No. of provinces	11	13	13	12	8	11	10	6	10	3	2	2	3		

[†] LL refers to a landlocked province in the south.

Table S9 Antimicrobial activities of endophytic fungi isolated from *R. tomentosa* leaves and antimicrobial activities of ethyl acetate extracts of the culture broths and mycelia of four active isolates using agar well diffusion method.

Test organism	Concentration of the extract (mg/ml)												(+)ve[†] control
	C. cupreum-T8			*Neopestalotiopsis* sp.-T9			*E. endophytica*-T13			*G. daii*-T14			
	1	10	100	1	10	100	1	10	100	1	10	100	(20 µg/ml)
Ethanolic extracts of the fungal culture broths													
K. rhizophila	–	17.9 ± 1.2	22.3 ± 1.1	–	–	–	–	8.8 ± 0.6	19.3 ± 0.5	–	–	16.1 ± 0.3	18.6 ± 0.9
V. cholerae	–	16.9 ± 0.5	23.9 ± 1.3	–	23.4 ± 0.3	25.1 ± 0.1	–	–	–	–	–	–	21.4 ± 0.2
Ethanolic extracts of the fungal mycelia													
K. rhizophila	–	8.3 ± 0.2	15.7 ± 0.7	–	–	–	–	–	13.0 ± 0.3	–	–	12.0 ± 0.5	18.6 ± 0.9
V. cholerae	–	9.1 ± 1.1	20.9 ± 0.4	–	12.9 ± 0.4	14.2 ± 1.1	–	–	–	–	–	–	21.4 ± 0.2

[†] Concentration of antibiotics used in this study was 20 µg/ml; DMSO was used as a vehicle control; – indicates no activity.