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Diversity and characterization of culturable fungi associated with *Gnetum gnemon* Linn. in organic and conventional farming systems and their potential antagonism against pathogenic fungi

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ABSTRACT: This study compared the diversity and abundance of culturable fungi associated with *Gnetum gnemon* Linn. grown in separate organic and conventional fields. A total of 236 fungi were isolated from leaves and soil, of which 124 were from organic fields and 112 were from conventional fields. The colonization rates of endophytic fungi ranged from 80% to 93%, and the mean fungal loads of soil fungi ranged from 4.45×10^3 to 5.65×10^3 CFU/g soil. Based on morphological characteristics and ITS sequence analysis, the isolates were identified to 13 orders and 24 genera. The most abundant endophytes were *Colletotrichum*, *Guignardia*, *Daldinia*, and *Pestalotiopsis*; and the most abundant soil fungi were *Aspergillus*, *Penicillium*, and *Trichoderma*. The fungal communities of *G. gnemon* were diverse and abundant, as indicated by the species richness and diversity indices. Among them, 23 taxa (55%) were common to both organic and conventional fields; however, the dominant endophytic and soil fungi were dissimilar. In antagonistic assays against the plant pathogenic fungi, *Colletotrichum siamense*, *Colletotrichum gloeosporioides* and *Pestalotiopsis mangiferae*, fungal isolates revealed inhibition percentages of 61% to 100%. *Trichoderma hamatum* C212, *Gliocladium* sp. C324, and *Daldinia eschscholtzii* LM12 showed the strongest antagonistic activities. *Aspergillus*, *Colletotrichum*, *Daldinia*, *Talaromyces*, and *Xylaria* showed antimicrobial activities against *Micrococcus luteus*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Vibrio cholerae*, and *Candida albicans*, with inhibition zones ranging from 12 to 22 mm. We demonstrated for the first time that endophytic and soil fungi associated with *G. gnemon* have potential as antagonists and antimicrobial agents for biotechnological applications.

KEYWORDS: *Gnetum gnemon* Linn., fungal diversity, antagonism, antimicrobial activity

INTRODUCTION

Plant-associated fungi are widely distributed in nature. Soil fungi, including root-associated and free-living fungi, make up 10%–30% of the soil rhizosphere, and they are key operators of soil nutrient cycles, acting as main decomposing agents of plant biomass. Most members of soil and root fungal communities are involved in processes that influence plant growth via the saprotrophic decomposition of soil organic residues and the subsequent release of nitrogen, phosphorus, and micronutrients [1, 2]. Similarly, endophytic fungi can be beneficial to their host plants through mutualistic symbiosis; but during certain phases of the growth cycles of hosts, and/or under certain environmental conditions, they can become latent pathogens or saprophytes. Many endophytic fungi can stimulate the production of various compounds and enhance their availability, helping plants to uptake and utilize soil nutrients that have been linked to the promotion of plant growth and development [3]. Endophytic fungi are also capable of improving the resistance of plants to pathogen and insect attacks and their defenses against abiotic stresses [4].

Fungal populations are strongly influenced by the diversity of the topical plant community and soil composition. The fungi in return affects plant growth and soil quality [5]. The interactions between plants and associated fungi can also significantly affect the integrity and sustainability of entire agro-ecosystems [6]. Biodiversity in agricultural landscapes, which usually restrict the application of synthetic chemicals and genetically modified products, is greater and species richness was 30% (on average) higher in organic farming systems than in conventional farming systems [7]. Therefore, organic farming could also enhance fungal diversity. On the other hand, the application of fungicides in conventional farming can reduce fungal abundance and diversity. This may have deleterious effects on plant and soil health, and eventually the sustainability of crop production.

Gnetum gnemon Linn. is a medicinal plant indigenous to Southeast Asia and western Pacific Ocean islands. It has been used in folk medicines for treatments of arthritis, bronchitis and asthma [8]. The fruits and leaves of *G. gnemon* are widely consumed and are a relatively rich source of nutrition. Due to its diverse patterns of bioactive compounds, such as flavonoids, saponins and tannins, *G. gnemon* has a broad range of therapeutic uses [9]. Stilbenoids from the species showed moderate antimicrobial activity and lipase and α-amylase activities [10]. *G. gnemon* var. *tenerum*

Markgr. is the main *Gnetum* variety distributed across southern Thailand. This small evergreen shrub (3 m in height) usually grows in shady areas and is resistant to pests and diseases [11]. In Thailand, this plant has been introduced as a co-plant, intercropped with economic perennials, e.g., rubber (*Hevea brasiliensis*) and durian (*Durio zibethinus* L.), to relieve financial difficulties among monocrop farmers during price crises. However, most plantations in Thailand are still operated conventionally and rely on using chemicals to protect crops from pests and microbial diseases [12].

Extensive use of pesticides and fungicides results in widespread environmental contamination of water, edible plants, and the human food chain. As mentioned, the use of chemicals in conventional farming can reduce fungal abundance and diversity, which subsequently affects plant growth and soil quality. As the leaves of the *tenerum* variety are consumed as a fresh or cooked vegetable in Thailand [12], contamination with agrochemical residues is a real concern. In addition, the overuse or misuse of fungicides can have toxic effects on beneficial organisms, human health, and the environment, and lead to the development of fungicide resistance. For this reason, alternative approaches to crop protection have revealed promising substitutes for chemical fungicides. For example, biocontrol agents are used to increase the diversity of associated microbial communities. Thus, the two interlinked objectives of this study were to investigate the diversity and abundance of culturable fungi associated with *G. gnemon* grown organically and conventionally, and to characterize biological activities of the fungal isolates as an inhibitor of microbial pathogens. *In vitro* activities of fungal isolates against the plant pathogenic fungi were examined via dual cultures, and activities against pathogenic microbes were examined via agar-well diffusion assays. The objectives of this study were to prove our hypothesis that the diversity and abundance of foliar endophytic and soil fungi was greater in the organic farm than in the conventional farm, and to observe the inhibitory potentials against microbial pathogens of selected fungal isolates.

MATERIALS AND METHODS

Sample collection and physicochemical analysis of soil

Representative communities of *G. gnemon* Linn. were sampled from conventional and organic farming systems in Phato District, Chumphon Province, Southern Thailand that have been in operation for more than 10 years. The conventional and organic farming sites were located approximately 500 m away from each other, representing the same climate and geographic conditions in each sampling site. Differences in plants and soil morphology were not observed. Plants were sampled separately during the rainy season in October 2021. Fifteen healthy and mature leaves of five *G. gnemon* plants growing on each site were randomly collected, making a total of 30 leaf specimens. Leaves were kept in sterile plastic bags and stored at 4 °C. Soil samples were collected from both sites at a depth of 5 to 15 cm. At each site, a 1×1-m plot was defined from which the samples were collected. All soil samples from each site were pooled in sterile collection bags and processed in the laboratory within 48 h. During sampling, temperature and soil pH were measured using a portable instrument. The *G. gnemon* plants were intercropped with durian (*D. zibethinus* L.) trees. Physicochemical analysis of soil was performed as described by Wingfield et al [13].

Isolation of soil fungi and endophytic fungi

Soil fungi were isolated using the protocol described by Wingfield et al [13] with minor modification. Briefly, a ten-fold serial dilution of soil suspensions was spread-plated onto potato dextrose agar (PDA) supplemented with 100 μg/ml chloramphenicol. The plates were incubated at 28 °C for 7–21 days. Surface sterilization was carried out to isolate endophytic fungi using a previously described method [14] with minor modification. Briefly, 1.0 cm × 1.0 cm pieces of different parts of leaves (petiole, midrib, vein, and lamina) were immersed in 95% ethanol for 30 s, 5% sodium hypochlorite solution for 5 min, 95% ethanol for 30 s, and sterile distilled water for 3–5 s. The sterilized fragments were placed onto a corn meal agar (CMA) plate containing chloramphenicol (50 μg/ml) and incubated for 7–21 days at 28 °C until the hyphae emerged.

Morphological and molecular identification of fungi

Fungal isolates were initially identified to the genus and species levels based on their macroscopic morphological characteristics. The microscopic appearance of individual fungi was observed using the slide culture technique and the observed mycelial and reproductive structures were used to further identify the fungi. The identification keys described by Wingfield et al [13] were used. Molecular identification of the fungal isolates was performed based on the analysis of DNA sequence of the ITS1-5.8S-ITS2. Genomic DNA was extracted according to a protocol described by Wingfield and Atcharawiriyakul [15]. PCR amplification of fungal ITS regions was carried out using the protocol described by Wingfield et al [13].

Phylogenetic analysis

The closest matched sequences were searched in the National Centre for Biotechnology Information (NCBI) GenBank database using a BLAST search. To confirm the identity of the isolates, phylogenetic and molecular evolutionary analyses were conducted using MEGA version 11 [16]. Multiple sequence alignments were performed using alignments prepared with MUSCLE,

and sequences were manually edited when necessary. The phylogenetic trees were inferred using the maximum-likelihood algorithm. The stability of relationships was evaluated by bootstrap analysis with 1,000 replications. A soil fungus, *Mortierella longigemmata*, was included as an out-group. Sequences were deposited in the GenBank database (OR986518–OR986555).

Diversity and data analysis

The diversity of the fungal isolates was determined by evaluating species richness based on the Menhinick Index (Dmn) [17]. Species diversity was measured by the Shannon (H′) Index [18]. The Shannon index was calculated according to the following formula: $H' = \sum_{i=1}^{k}(P_i \times \ln P_i)$, where k is the total number of species at a site, and P_i is the relative abundance of fungal species at a site. The isolation rate (IR) was used to indicate the fungal richness in each sample. It was calculated as the number of fungal isolates obtained from leaf segments divided by the total number of segments 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 segments infected by fungi divided by the total number of segments 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 7.0 (GraphPad Software, La Jolla, CA).

In vitro antagonism assay

Fungal morphotypes were screened for their antagonistic property using *in vitro* dual culture assays. The fungi grew for the first 5 days on a ¼PDA agar and demonstrated the highest growth radii were selected for antagonism assays with the reference plant pathogenic fungi *Colletotrichum siamense* LV515 (isolated from infected chili, *Capsicum annuum* L. var. *acuminatum* Fingerh), *Pestalotiopsis mangiferae* LM24 (isolated from infected mango leaves, *Mangifera indica* Linn.), and *C. gloeosporioides* (isolated from infected durian leaves, *D. zibethinus* L.). Seven-day-old cultures were used for all antagonism assays. In each assay, a mycelium disc (6 mm in diameter) of a fungal antagonist and a fungal plant pathogen were placed equidistantly (1 cm from the edge) on a PDA plate (9 cm in diameter). Plates inoculated with a fungal pathogen but not with an antagonist were used as negative controls. The assays were performed in triplicate. Observations were carried out at 28 °C for 7 days to determine the antagonistic capability of each fungus. The mycelial radial growth of the test pathogen was measured on a control plate (R) and in the direction of the antagonistic fungus (r), and the percent inhibition (%I) in mycelial growth was calculated according to the formula $\%I = [(R-r)/R] \times 100$. Interactions between fungal antagonists and fungal pathogens were classified according to the following five types of interaction. (1) The two opposing fungi demonstrate similar growth and overlap. (2) The antagonistic fungus outgrows the pathogenic fungus. (3) The pathogenic fungus outgrows the antagonistic fungus. (4) Mutual inhibition of both colonies at a short distance (< 2 mm). (5) Mutual inhibition of both colonies at a longer distance (> 2 mm) (adapted from López et al [19]).

Antimicrobial assays

In vitro antimicrobial activity was tested using the protocol described by Wingfield et al [13] with minor modification. Briefly, fungal isolates were cultivated in a potato dextrose broth (PDB) for 21 days at 28 °C. The culture broths were used for testing antimicrobial activity by agar well diffusion against seven pathogenic bacteria (*Micrococcus luteus* (ATCC9341), *Staphylococcus aureus* (ATCC25923), methicillin-resistant *S. aureus* (MRSA), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi*, and *Vibrio cholerae*) and two pathogenic fungi (*Candida albicans* (ATCC90028) and *Aspergillus fumigatus* Af293). Inhibition zones were measured as the mean diameter of the 8 mm wells plus the clearing zone. Assays were performed in triplicates. Vancomycin and gentamicin were used as standard antibacterial agents, and amphotericin B and miconazole were used as standard antifungal agents.

RESULTS

Plant and soil properties

Representative communities of *G. gnemon* Linn. were sampled from two locations, one a field on a conventional farm, and the other on an organic farm. The genus *Gnetum* presented in both fields belonged to the variety *tenerum* (*G. gnemon* var. *tenerum* Markgr.). This variety is a green shrub of approximately 3–4 m in height. The leaves were oblong-lanceolate, chartaceous, dark green with an entire margin and netted veins, and typically 10–20 cm long and 4–8 cm wide. The *Gnetum* shrubs were planted alongside durian (*D. zibethinus* L.) trees at both locations, and they served as a ground cover crop. At the conventional farm, a herbicide (Glyphosate isopropylammonium, 48% W/V SL) was sprayed four times a month for weed control, and a pesticide (Abamectin, 1.8% W/V EC) once a month for pest control. Details of geographic locations, sampling sites, plant and soil characteristics were shown in Fig. 1. The results of the physicochemical characteristics of soil samples are presented in Table S1. All analyzed parameters of the organic farming soil were much higher than those of the conventional farming soil, except soil pH. Soil samples from

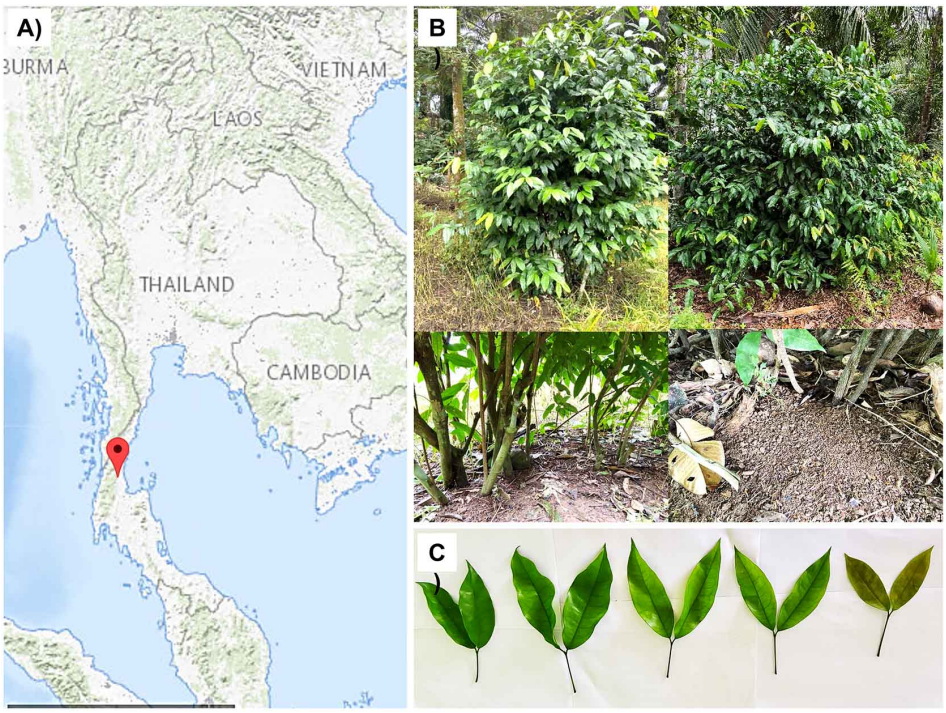

Fig. 1 Plant properties and sampling sites. (A) Geographic location of the sampling sites in Phato District, Chumphon Province, Southern Thailand, and their geographical coordinates were N 10° 27′ 32.4″, E 99° 24′ 10.8″ (USGS National Map Viewer (http://viewer.nationalmap.gov/viewer)). (B) Characteristics of the sampling sites, Thailand. (C) Characteristics of each stage of *G. gnemon* var. *tenerum* Markgr. leaves.

both sites were mildly alkaline. The organic farming soil was naturally rich in organic matter, minerals and water. On the other hand, the conventional farming soil showed a deficiency in soil components, having total nitrogen, total organic carbon, and organic matter levels lower than the optimal levels reported in the literature [20].

Isolation and colonization of soil fungi and endophytic fungi

From 60 leaf segments (petiole, midrib, vein and lamina) of plants growing in the conventional farming system, a total of 79 endophytic fungi were isolated. From 60 leaf segments of plants growing in the organic farming system, a total of 91 endophytic fungi were isolated (Tables S2 and S3). The overall IR of endophytic fungi was higher in the organic farming system (1.52) than the conventional farming system (1.32; Table 1). This result demonstrated that most of the leaf segments tested contained more than one fungal isolate, indicating a high fungal richness in the *G. gnemon* leaves. In the organic system, the greatest number of fungal endophytes was isolated from vein (IR, 1.87) and the lowest from petiole (IR, 1.27). In the conventional system, the greatest number of fungal endophytes was isolated from lamina (IR, 1.53) but the lowest from petiole (IR, 0.93). The total %CR was 91.67 for both sites, but the CRs of different plant tissues were different. The CRs of vein and midrib from the conventional farm (93.33% and 100%, respectively) were significantly higher than those of the vein and midrib from the organic farm (86.67% and 93.33%, respectively). In contrast, the CR of petiole from the conventional farm (80.00%) was significantly lower than the CR of petiole from the organic farm (93.33%). However, the CR of laminas was the same at both sites (93.33%). This result demonstrated a high prevalence of endophytic fungal infection in different tissues of the leaves in both systems. The number of soil fungi isolated from both the conventional and the organic farm soils was the same (33 isolates), giving a total of 66 fungi (Table S2). Mean fungal loads were higher in the soilsoil from the organic farm (Table 1).

Identification of fungi associated with *G. gnemon* Linn.

Based on morphological identification, 236 fungal isolates associated with *G. gnemon* Linn. from the organic and the conventional fields were assigned to 45 representative morphotypes (Fig. 2A). The molecular identification identified 39 fungal species of 24 genera (Table S4 and Fig. 2B) including unidentified

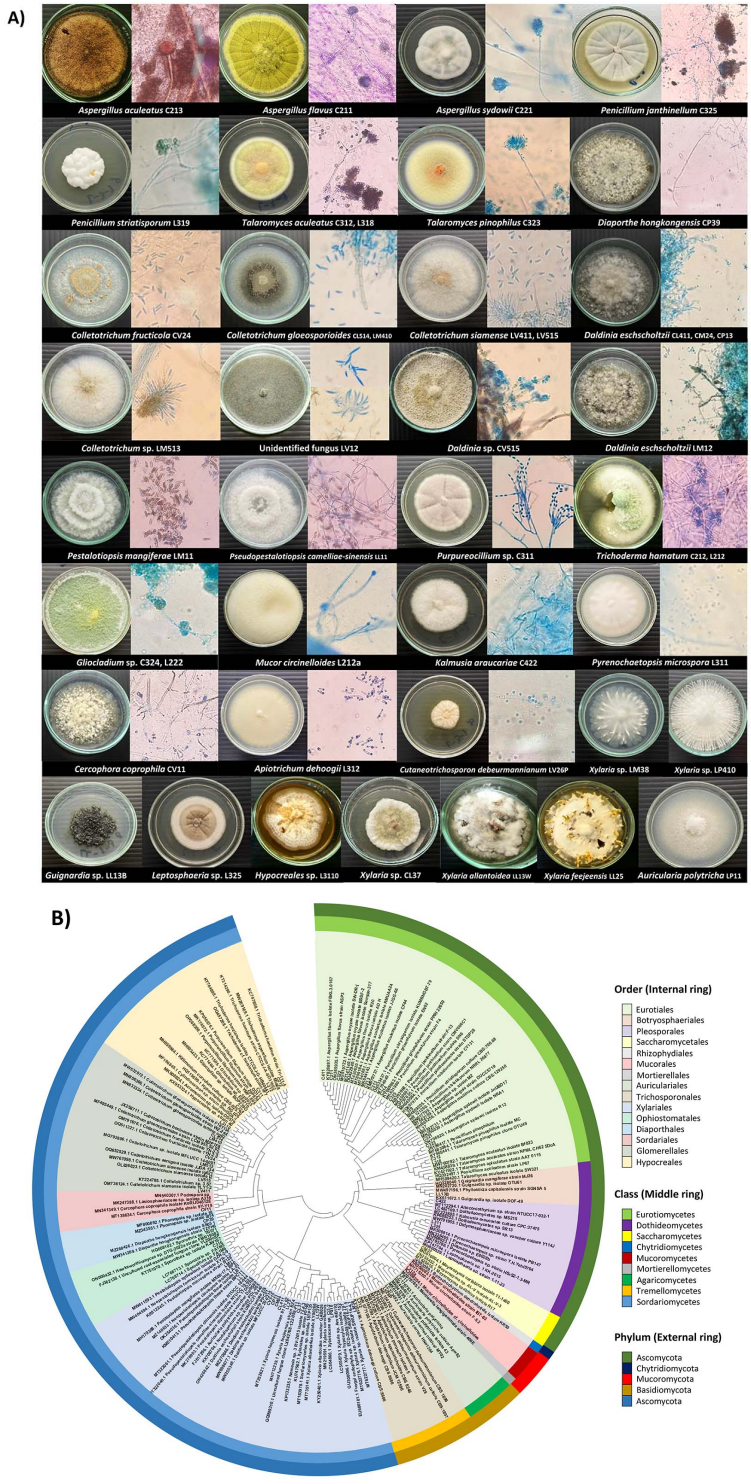

Fig. 2 Morphological and molecular identification of culturable fungi associated with the *G. gnemon* Linn. (A) Representative fungal morphotypes of the isolated fungi. Morphotyping was based on macroscopic and microscopic observations (magnification ×40). All isolates were grown on PDA plates for 7–14 days at 28 °C. (B) The phylogenetic analysis of isolated fungi produced the above tree generated by the Maximum Likelihood method. The circular phylogenetic tree classifies the isolates at the class, order and genus levels. The inner circle presents orders indicated by different colors, and the outer circle presents classes. Percentages of bootstrap sampling derived from 1000 replications are indicated by the numbers on the tree.

Table 1 Isolation rate (IR) and percentage colonization rate (% CR) of endophytic fungi and mean fungal loads of soil fungi associated with *G. gnemon* Linn.

Parameter	Leaf sample										Soil sample
	Lamina		Vein		Midrib		Petiole		Total		
	IR	%CR	IR	% CR	IR	% CR	IR	% CR	IR	% CR	CFU/g soil
Conventional farming	1.53	93.33	1.4	93.33	1.4	100	0.93	80.00	1.32	91.67	5.65×10^3
Organic farming	1.60	93.33	1.87	86.67	1.33	93.33	1.27	93.33	1.52	91.67	4.45×10^3

fungi. The identified isolates belonged to three phyla, seven classes, and thirteen orders. The 24 fungal genera were *Apiotrichum*, *Aspergillus*, *Auricularia*, *Cercophora*, *Colletotrichum*, *Cutaneotrichosporon*, *Daldinia*, *Diaporthe*, *Gliocladium*, *Guignardia*, *Hypocreales*, *Kalmusia*, *Leptosphaeria*, *Meyerozyma*, *Mucor*, *Penicillium*, *Pestalotiopsis*, *Pseudopestalotiopsis*, *Purpureocillium*, *Pyrenochaetopsis*, *Sporothrix*, *Talaromyces*, *Trichoderma*, and *Xylaria*. However, three isolates (C311, CL37 and LL13B representing *Purpureocillium*, *Xylaria* and *Guignardia*, respectively) were potential new taxa because of the low similarities of their ITS sequences. The rDNA ITS sequences of the fungi subjected to molecular identification in this study were deposited in the Genbank (OR986518–OR986555).

Distribution and diversity of fungi associated with *G. gnemon* Linn.

The abundance and the species diversity of fungi in the organic farming were higher than those in the conventional. Of 40 taxa, 32 taxa were obtained from the organic farming system (including 17 from leaves and 14 from soils), whereas 30 taxa were obtained from the conventional farming system (including 13 from leaves and 16 from soils; Table S5). The dominant endophytic fungal genera obtained from the conventional farm were *Colletotrichum* sp., *Daldinia* sp. and *Guignardia* sp., with % RFs of 37.97%, 18.99% and 16.46%, respectively. Similarly, the three endophytic genera were dominant on the organic farm, and their % RFs were 36.27%, 18.68% and 15.38%, respectively (Fig. 3A). The dominant soil fungi from the conventional farm were *Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp., with % RFs of 36.36%, 21.21% and 15.15% respectively; while the dominant soil fungi from the organic farm were *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp. and *Gliocladium* sp., with % RFs of 30.30%, 15.15%, 12.12% and 12.12% respectively.

Among the total fungi associated with *G. gnemon* Linn., the phylum Ascomycota was found to be the most abundant (95.65%) across all the samples analyzed, and the phyla Basidiomycota and Mucoromycota were found to be rare and incidental. Among the endophytic fungi, Xylariales was the most abundant order (RF, 36.48%), followed by Glomerellales (RF, 34.70%) and Diaporthales (RF, 8.82%) (Fig. 3B). Among the soil fungi, Eurotiales was the most abundant order (RF, 54.57%), followed by Hypocreales (RF, 31.83%). Sordariomycetes was the most dominant class among leaf samples, and Eurotiomycetes the most dominant class among soil samples (Fig. 3C). Regardless of the samples analyzed, the numbers of fungal community were equally good on both farms, but fungal isolates from the organic farm were more morphologically diverse. In the analysis of fungal diversity and species richness, the Dmn index of fungi from the organic farming system was slightly higher (2.76) than the index from the conventional farming system (2.74) (Table S5). Shannon's index of species diversity (H′) also showed a similar trend; a slightly higher index from the organic farm (2.49) than the conventional farm (2.47).

The endophytic fungal community among leaves from the two sites was clustered together, as was the fungal community among soil samples from the two sites. Among the isolated fungi, regardless of the samples analyzed, 23 taxa (55%) co-existed in *G. gnemon* Linn. from both farming systems (Fig. S1). However, some endophytic fungi were location-specific. For instance, *Auricularia* sp. and *Cutaneotrichosporon* sp. of the phylum Basidiomycota, *Colletotrichum siamense* and some species of the genus *Xylaria* (*X. allantoidea* and *X. feejeensis*) were only found on the organic farm, while *Cercophora* sp. was found only on the conventional farm. High specificity was found among soil fungi between the two sites. *Mucor* sp., *Leptosphaeria* sp., *Pyrenochaetopsis* sp. and *Hypocreales* sp. were specifically found on the organic farm, while *Kalmusia* sp., *Sporothrix* sp., some species of the genus *Aspergillus* (*Aspergillus aculeatus* and *A. sydowii*), *Penicillium janthinellum* and *Talaromyces pinophilus* were specifically found on the conventional farm.

Antagonism tests against plant pathogenic fungi

Before the antagonism test began, 45 fungal morphotypes were subjected to stressful growth in low nutrient conditions in a 1/3PDA medium. Only fungi that showed to be fast-growing after 5 days (26 isolates) were selected to trial against the plant pathogenic fungi, *C. siamense* LV515, *Pestalotiopsis mangiferae* LM24 and *C. gloeosporioides*. The *Colletotrichum* sp. strain was isolated from infected chili, *Capsicum annuum* L. var. *acuminatum*, presenting anthracnose

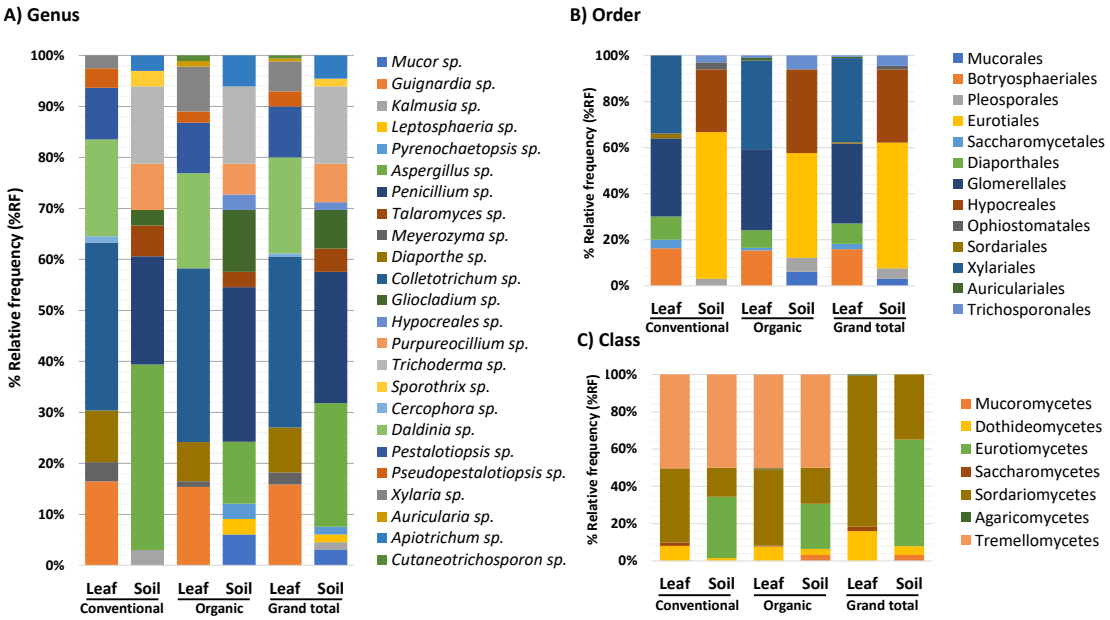

Fig. 3 Percentage relative frequency (%RF) of fungi associated with *G. gnemon* Linn. at the genus (A), order (B) and class (C) levels.

Table 2 *In vitro* antagonism data of selected fungi against plant pathogenic fungi using a dual culture assay.

Isolate name	Plant pathogenic fungi					
	Colletotrichum siamense		*Pestalotiopsis mangiferae*		*Colletotrichum gloeosporioides*	
	Type of interaction	% inhibition growth (%I) ± SD	Type of interaction	% inhibition growth (%I) ± SD	Type of interaction	% inhibition growth (%I) ± SD
Endophytic fungi						
Auricularia polytricha LP11	4	17.32 ± 1.03	4	23.96 ± 6.52	ND	ND
Cercophora coprophila CV11	4	18.99 ± 4.97	4	11.87 ± 5.80	2	54.21 ± 3.67
Colletotrichum sp. LL411	4	21.67 ± 6.40	4	8.99 ± 17.92	ND	ND
Colletotrichum fructicola CV24	4	14.52 ± 7.07	3	12.67 ± 9.45	ND	ND
Colletotrichum gloeosporioides CL514	4	17.20 ± 9.34	4	11.64 ± 13.20	4	19.74 ± 10.01
Colletotrichum gloeosporioides CM19	4	12.98 ± 6.90	4	8.06 ± 10.43	ND	ND
Colletotrichum siamense LV411	4	17.32 ± 13.55	4	17.05 ± 13.03	4	26.73 ± 1.34
Daldinia sp. CV515	4	15.48 ± 14.65	2	30.76 ± 1.14	ND	ND
Daldinia eschscholtzii CM24	4	15.60 ± 4.88	4	17.40 ± 15.80	4	22.05 ± 5.63
Daldinia eschscholtzii LM12	2	30.65 ± 8.00	2	26.96 ± 5.54	4	26.81 ± 1.75
Colletotrichum sp. LM513	4	23.57 ± 6.40	5	21.66 ± 13.03	ND	ND
Diaporthe hongkongensis CP39	2	28.04 ± 12.21	2	33.99 ± 2.44	4	5.63 ± 1.32
Pestalotiopsis mangiferae LM11	4	22.14 ± 12.46	4	20.97 ± 15.32	4	23.23 ± 6.07
Xylaria sp. CL37	4	27.20 ± 11.40	5	49.31 ± 3.26	ND	ND
Soil fungi						
Gliocladium sp. C324	2	100 ± 00	2	64.06 ± 4.56	2	62.12 ± 5.05
Purpureocillium sp. C311	5	61.07 ± 5.05	5	14.14 ± 1.01	5	18.10 ± 8.89
Talaromyces pinophilus C323	3	16.01 ± 2.78	3	8.76 ± 1.63	ND	ND
Talaromyces aculeatus L318	3	13.81 ± 7.41	5	4.38 ± 8.15	ND	ND
Trichoderma sp. L322	2	58.69 ± 8.42	2	32.30 ± 5.21	ND	ND
Trichoderma hamatum C212	2	100 ± 00	2	65.44 ± 6.52	ND	ND

ND refers to not detected.

diseases. The *Pestalotiopsis* sp. strain was isolated from infected mango leaves, *Mangifera indica* Linn., presenting leaf spot disease. The *C. gloeosporioides* was isolated from infected durian leaves, *D. zibethinus* L., presenting anthracnose disease. When the dual culture tests were completed (after 7 days), some fungi presented a similar mycelial growth and mutual inhibition of a Type 4 interaction. These

fungi were *Auricularia polytricha* LP11, *Cercophora coprophila* CV11, *Colletotrichum* sp. LL411, *C. fructicola* CV24, *C. gloeosporioides* CL514 and CM19, and *Daldinia eschscholtzii* CM24 (Table 2 and Fig. S2). Some tested fungi exhibited a Type 5 interaction, where the opposing fungi demonstrated similar growth but maintained a distance of > 2 mm. These fungi were *Colletotrichum* sp. LM513, *Xylaria* sp. CL37, *Purpureocillium* sp. C311 and *Talaromyces aculeatus* L318. Fungi exhibiting a Type 2 interaction were *D. eschscholtzii* LM12, *Diaporthe hongkongensis* CP39, *Gliocladium* sp. C324, *Trichoderma* sp. L322 and *T. hamatum* C212, where the mycelium of the test fungus outgrew that of the pathogen. This was seen to be most true in *Gliocladium* sp. and *Trichoderma* sp. (2++). The inhibition percentage (%I) of each antagonistic fungus against the pathogens revealed different degrees of inhibition (Table 2). *Gliocladium* sp. C324 and *Trichoderma hamatum* C212 recorded the highest %I at 100% against *C. siamense* LV515. Similarly, these two potential antagonists showed the highest %I, up to 65%, against *P. mangiferae* LM24 and *C. gloeosporioides*. *Purpureocillium* sp. C311 showed a good %I of 61.07% against *C. siamense* LV515, whereas *Xylaria* sp. CL37 showed an identical %I but against *P. mangiferae* LM24. Also, over time during the dual culture assay, morphological changes in the test pathogen *P. mangiferae* were observed. The production of either a yellow or black pigmentation was observed on the fungal colony when co-cultured with *Colletotrichum* sp., *Talaromyces* sp. and *Xylaria* sp. antagonists.

Antimicrobial assay of fungi associated with *G. gnemon* Linn.

Using a culture broth filtrate of 80 µl, 22 out of 52 isolates (42.3%) showed antimicrobial activity against at least one pathogen (Table 3 and Fig. S3). The presence of inhibition zones qualitatively indicated positive antimicrobial activity. Endophytic isolates that exhibited antimicrobial activity were from the genera *Colletotrichum* sp., *Daldinia* sp. and *Xylaria* sp., while soil fungi that exhibited antimicrobial activity were from the genera *Aspergillus* sp. and *Talaromyces* sp. In addition, most of the isolates obtained from the conventional farming system showed positive antimicrobial activity against the test pathogens. *M. luteus* was inhibited by most fungal isolates. However, none of the fungi associated with *G. gnemon* could inhibit *E. coli*, *P. aeruginosa*, *S. typhi* and *A. fumigatus*. *V. cholerae* was the only Gram-negative bacterium that was inhibited by two endophytic fungi: *C. siamense* LV515 and *D. eschscholtzii* LM12. The pathogenic yeast *C. albicans* was inhibited by endophyte *Xylaria allantoidea* LL13W. In addition, *D. eschscholtzii* LM12 had positive antimicrobial activity against three bacterial pathogens (*S. aureus*, *M. luteus* and *V. cholerae*).

DISCUSSION

G. gnemon Linn. is a medicinal plant and food in Southeast Asia. In recent years, the biodiversity and pharmacological properties of *G. gnemon* have been the focus of increasing attention [8–11], but very few attempts have been made to evaluate the diversity of fungi associated with this valuable plant. Two studies have reported fungi associated with *Gnetum* spp. root tips, revealing the colonization of ectomycorrhizal fungi of the genus *Scleroderma* and several fungal species from unrelated lineages [21, 22]. These studies thus demonstrated that the roots of *Gnetum* spp. host a narrow range of mycorrhizal symbionts. Here, we investigated the diversity and abundance of culturable fungi associated with *G. gnemon* Linn. collected from two locations: a conventional farm and an organic farm. In addition, biological activities of the isolated fungi were evaluated. As a result, we discovered some fungi with the potential to inhibit microbial pathogens of plants and humans. We investigated the diversity and abundance of culturable foliar endophytic and soil fungi associated with *G. gnemon* var. *tenerum* Markgr. Our study revealed that foliar endophytic and soil fungal communities of *G. gnemon* were more diverse and abundant than the fungal communities associated with the roots of the species. The fungal communities obtained from both farming systems were dominated by Ascomycota. The most abundant order of endophytic fungi obtained from leaves was Xylariales (RF, 36.48%), followed by Glomerellales (RF, 34.70%) and Diaporthales (RF, 8.82%), whereas the most abundant order of soil fungi was Eurotiales (RF, 54.57%), followed by Hypocreales (RF, 31.83%). Regarding fungal genera, *Colletotrichum* sp., *Daldinia* sp., *Guignardia* sp. and *Pestalotiopsis* sp. were dominant in *G. gnemon* leaves, whereas *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp. and *Gliocladium* sp. were dominant in soil. A great number of foliar endophytic fungi have been isolated from leaves of many types of plants (see review by Jia et al [23]. Our findings were similar to those reported in studies of foliar fungal diversity around the world, but different frequencies were observed. This was also true for soil fungi. The dominance of soil fungi by members of the orders Eurotiales and Hypocreales reported by Gaddeyya et al [24] and Rosas-Medina et al [25] was also found in this study. In addition, Tedersoo et al [26] found that Eurotiales and Hypocreales were within the ten most common orders according to data obtained by environmental sequencing.

Differences in soil physicochemical parameters, fungal diversity, and community structure were detected among the sampling sites. Overall, the abundance and species diversity of culturable fungi were slightly higher in the organic farming system than in the conventional farming system. The endophytic fungal community of leaves from both systems was

Table 3 Antimicrobial assays of fungi associated with *G. gnemon* using the agar-well diffusion method.

Isolate	Zone of inhibition (mm)					Total no.
	Bacteria*			Yeast*		
	ML	SA	MRSA	VC	CA	
Endophytic fungi						
Colletotrichum sp. CM410	–	–	12.8 ± 0.5	–	–	1
Colletotrichum sp. CV26	18.6 ± 0.2	–	–	–	–	1
Colletotrichum sp. LL411	18.6 ± 0.5	–	–	–	–	1
Colletotrichum fructicola CM39	20.2 ± 0.1	15.6 ± 1.4	–	–	–	2
Colletotrichum fructicola CV24	–	–	12.4 ± 0.1	–	–	1
Colletotrichum siamense LV515	–	19.5 ± 0.5	–	15.2 ± 0.8	–	2
Daldinia eschscholtzii CL411	20.7 ± 0.0	–	–	–	–	1
Daldinia eschscholtzii CM24	–	–	17.6 ± 0.7	–	–	1
Daldinia eschscholtzii CP13	20.4 ± 1.1	–	–	–	–	1
Daldinia eschscholtzii CP25	–	–	14.4 ± 0.4	–	–	1
Daldinia eschscholtzii CP412	–	–	14.9 ± 0.8	–	–	1
Daldinia eschscholtzii LM12	19.4 ± 1.6	15.5 ± 0.3	–	15.9 ± 1.4	–	3
Meyerozyma caribbica CM37	–	12.5 ± 0.0	–	–	–	1
Xylaria sp. LL12	16.4 ± 0.5	14.4 ± 1.0	–	–	–	2
Xylaria allantoidea LL13W	–	–	–	–	16.6 ± 1.5	1
Xylaria feejeensis LP515	18.0 ± 0.3	–	–	–	–	1
Endophytic fungi						
Aspergillus aculeatus C213	16.1 ± 1.4	–	–	–	–	1
Aspergillus unguis LL37	20.3 ± 0.7	–	17.2 ± 0.1	–	–	2
Talaromyces aculeatus C312	19.0 ± 0.9	–	–	–	–	1
Talaromyces aculeatus L318	18.4 ± 1.3	–	12.1 ± 0.1	–	–	2
Talaromyces aculeatus L513	19.3 ± 1.0	–	12.2 ± 0.5	–	–	2
Talaromyces pinophilus C323	19.0 ± 0.8	–	–	–	–	1
Total no.	14	5	8	2	1	
Positive control	ML	SA	MRSA	VC	CA	
Vancomycin (20 μg/ml)	22.6 ± 1.0	18.0 ± 0.1	16.2 ± 0.7			
Gentamicin (20 μg/ml)				21.3 ± 0.5		
Amphotericin B (20 μg/ml)					15.2 ± 0.3	

* ML: *M. luteus* (ATCC9341), SA: *S. aureus* (ATCC25923), MRSA: methicillin-resistant *S. aureus* (MRSA), VC: *V. cholerae*, and CA: *C. albicans* (ATCC90028). The hyphen – indicates no activity.

clustered together, as was the community of the soil from both systems (Fig. S1). These most common fungal genera have been reported to be pioneer colonizers, and their ability to adapt to various environmental conditions leads to their cosmopolitan distribution [25, 27]. Remarkably, our study demonstrated that fungal habitats (plant vs. soil) were strong determinants of fungal communities. Most endophytic fungi inhabiting the *G. gnemon* leaves were *Colletotrichum* sp. and *Pestalotiopsis* sp., which are considered major pathogens in many crops worldwide. In our study, they existed as fungal endophytes without causing any symptoms to *G. gnemon*. De Silva et al [28] suggested that many species exist as endophytes for part or most of their life cycles in many groups of plants. The differences in lifestyle of these fungal genera might be attributed to their fungal species, the plant species, the physiological maturity of the host and the environmental conditions. Dominant fungi associated with *G. gnemon*, such as the endophytes *Daldinia* sp., *Diaporthe* sp., *Colletotrichum* sp., *Xylaria* sp., and soil fungi *Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp., might be capable of inhibiting other fungal pathogens through different mechanisms. These fungi were reported to produce secondary metabolites that reduce the negative effects from plant pathogenic fungi, including volatile compounds that can suppress pathogen growth [29]. *D. eschscholtzii*, which was the second most frequently found species in *G. gnemon* leaves, has been reported to produce 60 compounds that are effective antifungal agents against plant pathogenic fungi *Colletotrichum gloeosporoides*, *C. nymphaeae* and *C. musae* [30]. Therefore, the diverse fungal communities in *G. gnemon* leaves might provide the plant with a defensive capability against pests and fungal diseases. It was observed by the authors that *G. gnemon* usually requires minimal fertilizing and no pesticides or fungicides during planta-

tion. Furthermore, microbial diseases of *G. gnemon* are rarely reported. This feature of *G. gnemon* could be attributed to their fungal endophytes.

In conventional farming systems, fungicides and pesticides incur great costs and often cause environmental pollution and reduction of biodiversity. Therefore, biocontrol of plant diseases is an attractive strategy. Fungi have been recognized to contain structurally diverse and biologically active compounds; many of which have been reported to produce a variety of novel bioactive metabolites [13, 23, 24]. Alkaloids, aliphatic compounds, peptides, phenylpropanoids, polyketides, and terpenoids have been isolated from fungi and identified. Here, we demonstrated that fungal endophytes *D. eschscholtzii* LM12, *D. hongkongensis* CP39, *Gliocladium* sp. C324, *Trichoderma* sp. L322 and *T. hamatum* C212, successfully inhibited the plant pathogens *C. siamense*, *P. mangiferae* and *C. gloeosporoides* in a dual culture assay. The strong ability of *Gliocladium* sp. and *Trichoderma* sp. to out-grow the pathogenic fungi suggested effective competition for nutrients and space for survival. These fungi are among the most common fungi in nature due to their high level of stress tolerance and rapid growth rate [31]. They are known to secrete certain enzymes that are responsible for breaking down the cell walls of pathogen [32]. In addition, *Gliocladium* sp. C324 and *T. hamatum* C212 showed inhibition rates of 100% against *C. siamense* and restricted the growth of *P. mangiferae*. Variations in fungal interactions observed in this study could be influenced by antibiotics produced by the isolates, which could be fungicidal to certain fungi, but fungistatic to others [33]. During the dual culture assay, we observed morphological changes over time of the test pathogen *P. mangiferae*, which produced either a yellow or black pigmentation in the colony when co-cultured with *Colletotrichum* sp., *Talaromyces* sp. and *Xylaria* sp. These observations were consistent with findings by Hamzah et al [34]. The pigments could include carotenoids, flavins, melanins, quinones, and phenazines [35]; and they might be associated with antibacterial, antifungal, and herbicidal activities [36, 37]

Twenty two out of 52 isolates (42.3%) showed inhibitory activity against at least one human pathogen. Fungal isolates that exhibited antimicrobial activity were from the genera *Aspergillus* sp., *Colletotrichum* sp., *Daldinia* sp., *Talaromyces* sp., and *Xylaria* sp. Among the studied strains, *D. eschscholtzii* LM12 exhibited positive antimicrobial activity against three bacterial pathogens (*S. aureus*, *M. luteus* and *V. cholerae*). *D. eschscholtzii* was previously reported to demonstrate antibacterial activity [24, 38] and antifungal activity [29]. We observed a relatively low level of activity against Gram-negative bacteria from our fungal isolates, which was in accordance with previous studies [13, 14, 39]. Furthermore, most fungal members that showed positive antimicrobial activity against test pathogens, were obtained from the conventional farming system rather than the organic farming system. Renato et al [40] suggested that the chances of discovering new antimicrobial-producing fungal strains were greater if non-mesophilic environments were investigated. Under conditions of nutrient depletion, metal contamination, high salinity or extreme temperatures, fungi must develop survival strategies for growth and reproduction. Besides the exploration of fungal diversity associated with the *G. gnemon* Linn., our significant findings were the discoveries of three potent fungal strains, *D. eschscholtzii* LM12, possessing broad-range antimicrobial activity against several microbial pathogens of plants and humans; and *Trichoderma* and *Gliocladium* strains showing strong antagonistic activity against fungal pathogens. These properties point to potential biotechnological applications of the three strains.

CONCLUSION

We investigated the diversity and abundance of culturable fungi associated with *G. gnemon* Linn. grown in organically and conventionally farmed plantations. Our results demonstrated that both farming systems harbored common native fungal communities in both plant and soil samples. Members of fungi isolated from plants and soil were not clustered to the same group, which suggested that the habitat had more influence on the fungal community than the location. However, some fungal strains were location-specific. Dominant fungi associated with *G. gnemon* were *Colletotrichum* sp., *Daldinia* sp., *Guignardia* sp., *Pestalotiopsis* sp., *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp., and *Gliocladium* sp.; and the fungal communities seemed to be diverse and abundant. The characterization of biological activities of fungal isolates revealed three promising candidates with strong ability to inhibit microbial pathogens; and they could, therefore, be used for biological control. The three candidates were *D. eschscholtzii*, *T. hamatum* and *Gliocladium* strains. Overall, these results suggested that the diversity of fungi associated with *G. gnemon* could be explored as a potential source of bioactive agents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.027.

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Appendix A. Supplementary data

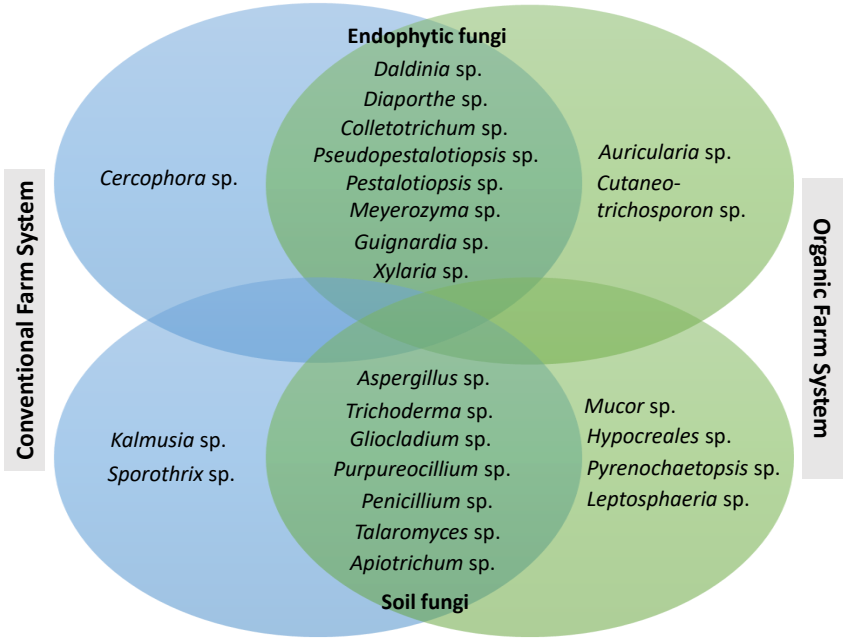

Fig. S1 Venn diagram of the culturable fungi associated with *Gnetum gnemon* Linn. from conventional and organic farming systems.

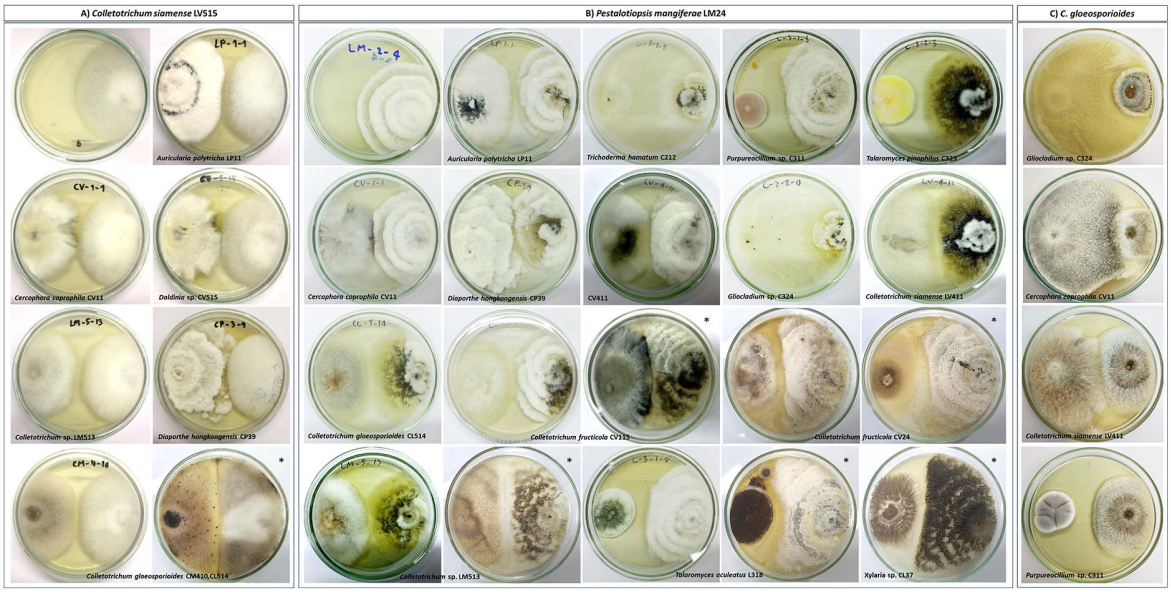

Fig. S2 Dual culture plate assay of test fungi (to the left of plates) against the plant pathogenic fungi (to the right) *Colletotrichum siamense* LV515 (A) and *Pestalotiopsis mangiferae* LM24 (B). The plates were cultivated for 7 days at 27 °C. Radial growths were measured and types of interaction were classified. The asterisks indicate the dual culture after 14 days.

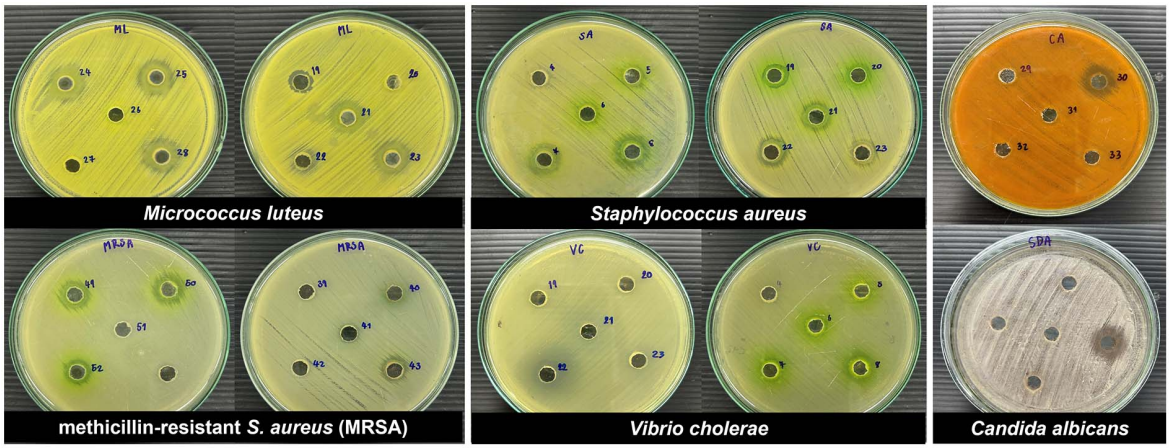

Fig. S3 Antimicrobial activity of fungi associated with *Gnetum gnemon* Linn. determined by the agar well diffusion method.

Table S1 Physicochemical characteristics of soil samples.

Parameter	Test result	
	Conventional farming	Organic farming
pH	7.24[a]	7.16[a]
Moisture content (%)	13.82[a]	17.98[b]
Total nitrogen content (% w/w)	0.10[a]	0.17[a]
Total organic carbon (% w/w)	0.96[a]	1.82[b]
Organic matter (% w/w)	1.66[a]	3.14[b]

The same superscript letter indicates no significant difference ($p > 0.05$) between individual parameter from conventional and organic farming.

Table S2 Numbers of soil fungi and endophytic fungi associated with *Gnetum gnemon* Linn.

Parameter	Leaf sample					Soil sample	
	No. of fungal isolates (No. of leaf samples)					No. of isolates	Colony forming units/ gram soil (CFU/g soil)
	Lamina	Vein	Midrib	Petiole	Total		
Conventional farming	23 (15)	21 (15)	21 (15)	14 (15)	79 (60)	33	5.65×10^3
Organic farming	24 (15)	28 (15)	20 (15)	19 (15)	91 (60)	33	4.45×10^3

Table S3 Numbers of infected leaf segments of the *Gnetum gnemon* Linn.

Parameter	No. of infected leaf segments (Total no. of leaf segments)				
	Lamina	Vein	Midrib	Petiole	Total
Conventional farming	14 (15)	14 (15)	15 (15)	12 (15)	55 (60)
Organic farming	14 (15)	13 (15)	14 (15)	14 (15)	55 (60)

Table S4 BLAST analysis results of the representative fungal morphotypes associated with *Gnetum gnemon* Linn. and their closest relatives.

Taxa	Code	Closest relative (BLAST)	Accession no.	Identity (%)
Mucoromycota				
Mucoromycetes				
Mucorales	L212a	*Mucor circinelloides*	KJ588204.1	99.07
Ascomycota				
Dothideomycetes				
Botryosphaeriales	LL13B	*Guignardia mangiferae* strain MJ26	HQ328040.1	94.64
Pleosporales	C422	*Kalmusia araucariae* culture CPC:37475	MT223805.1	99.81
	L325	*Leptosphaeria* sp. strain 1-11-23	MH800329.1	98.65
	L311	*Pyrenochaetopsis microspora* isolate PB147	MK508814.1	98.77
Eurotiomycetes				
Eurotiales	C213	*Aspergillus aculeatus* isolate JJGG-66	MK644143.1	98.95
	C211	*Aspergillus flavus* strain ASP3	ON365935.1	99.83
	C411	*Aspergillus flavus* isolate FBKL3.0167	KY828897.1	99.49
	C322	*Aspergillus oryzae* isolate SW-DR-j	MG519722.1	99.83
	C221	*Aspergillus sydowii* isolate JnUBD17	MH392731.1	99.81
	LL37	*Aspergillus unguis* strain DUCC5719	MT582759.1	96.35
	C313	*Penicillium chrysogenum* isolate KUMBNGBT-79	ON533437.1	97.93
	C314	*Penicillium griseofulvum* strain PPRI22659	KY069863.1	99.24
	C325	*Penicillium janthinellum* strain CMV006C1	MK450697.1	98.63
	L319	*Penicillium striatisporum* NRRL 26877	NR 121260.1	98.60
	C312	*Talaromyces aculeatus* isolate Bf023	MW760782.1	99.46
	L318	*Talaromyces aculeatus* isolate Bf023	MW760782.1	98.43
	C323	*Talaromyces pinophilus* clone OTU49	KY965441.1	97.83
Saccharomycetes				
Saccharomycetales	CM37	*Meyerozyma caribbica* isolate 11-1400	MG016004.1	98.64
Sordariomycetes				
Diaporthales	CP39	*Diaporthe hongkongensis* strain TZFH6	MW341269.1	98.61
Glomerellales	CV24	*Colletotrichum fructicola* isolate 1YN-2-2	OQ511327.1	99.65
	CL514	*Colletotrichum gloeosporioides* isolate F16T1IA	MW532972.1	99.29
	LM410	*Colletotrichum gloeosporioides* strain LCM 955.S2.02	MN833334.1	99.82
	LV411	*Colletotrichum siamense* isolate lg621	MW767098.1	99.10
	LV515	*Colletotrichum siamense* isolate YLY1-2	OM736124.1	99.29
	LM513	*Colletotrichum* sp. isolate MFLUCC14-0091	MG792806.1	99.80
Hypocreales	C324	*Gliocladium* sp. isolate BOP239O	MH003433.1	98.20
	L3110	*Hypocreales* sp. strain 40	KX953324.1	99.41
	C311	*Purpureocillium lilacinum* strain YMF1.665	OP268300.1	93.38
	C212	*Trichoderma hamatum* strain Tri-111-5	KC747808.1	100.00
	L212	*Trichoderma harzianum* isolate CTCCSJ-G-HB40441	KY764898.1	99.67
Ophiostomatales	C316	*Sporothrix* sp. FA1M6	LC768714.1	98.40
Sordariales	CV11	*Cercophora coprophila* isolate KoRLI046128	MN341349.1	97.72
Xylariales	CL411	*Daldinia eschscholtzii* strain KUMCC21-0440	ON426842.1	99.28
	CM24	*Daldinia eschscholtzii* strain KUMCC21-0440	ON426842.1	99.08
	CP13	*Daldinia eschscholtzii* strain KUMCC21-0440	ON426842.1	98.93
	CV515	*Daldinia* sp. isolate MFLUCC 20-0215	MW255346.1	98.19
	LM11	*Pestalotiopsis mangiferae* isolate MX06-17-LBPE	MH179308.1	98.90
	LL11	*Pseudopestalotiopsis camelliae-sinensis* NTUCC18-024	MT322040.1	98.91
	CL37	*Xylaria* sp. strain F0146	KU747562.1	90.32
	LM38	*Xylaria* sp. isolate SWUF16-11.4	MT622776.1	99.02
	LP410	*Xylaria* sp. Am-NB192	LC505079.1	98.97
	LL13W	*Xylaria allantoidea* isolate XY00266	MT735141.1	98.87
	LL25	*Xylaria feejeensis* strain BZ4	MH712239.1	99.12
	LV12	Unidentified fungus		
Basidiomycota				
Agaricomycetes				
Auriculariales	LP11	*Auricularia polytricha*	LC176783.1	98.79
Tremellomycetes				
Trichosporonales	L312	*Apiotrichum dehoogii* culture CBS:8686	KY101656.1	99.03
	LV26P	*Cutaneotrichosporon debeurmannianum* CBS 1896	NR 154752.1	99.05

Table S5 Relative frequency (%RF) and diversity indices of fungi associated with *Gnetum gnemon* Linn.

Taxa	% Relative frequency (%RF)								
	Conventional farming			Organic farming			Grand total		
	Leaf	Soil	Total	Leaf	Soil	Total	Leaf	Soil	Total
Mucoromycota									
Mucoromycetes									
Mucorales									
Mucor circinelloides L212a	0	0 7 0	0	6.06	1.61	0	3.03	0.85	
Ascomycota									
Dothideomycetes									
Botryosphaeriales									
Guignardia sp. LL13B	16.46	0	11.61	15.38	0	11.29	15.88	0	11.44
Pleosporales									
Kalmusia araucariae C422	0	3.03	0.89	0	0	0	0	1.52	0.42
Leptosphaeria sp. L325	0	0	0	0	3.03	0.81	0	1.52	0.42
Pyrenochaetopsis microspora L311	0	0	0	0	3.03	0.81	0	1.52	0.42
Eurotiomycetes									
Eurotiales									
Aspergillus aculeatus C213	0	9.09	2.68	0	0	0	0	4.55	1.27
Aspergillus flavus C211, C411	0	15.15	4.46	0	6.06	1.61	0	10.50	2.94
Aspergillus oryzae C322	0	6.06	1.79	0	3.03	0.81	0	4.65	1.30
Aspergillus sydowii C221	0	3.03	0.89	0	0	0	0	1.52	0.42
Aspergillus unguis LL37	0	3.03	0.89	0	3.03	0.81	0	3.03	0.85
Penicillium chrysogenum C313	0	9.09	2.68	0	18.18	4.84	0	13.64	3.81
Penicillium griseofulvum C314	0	6.06	1.79	0	9.09	2.42	0	7.58	2.12
Penicillium janthinellum C325	0	3.03	0.89	0	0	0	0	1.52	0.42
Penicillium striatisporum L319	0	3.03	0.89	0	3.03	0.81	0	3.03	0.85
Talaromyces aculeatus C312, L318	0	3.03	0.89	0	3.03	0.81	0	3.03	0.85
Talaromyces pinophilus C323	0	3.03	0.89	0	0	0	0	1.52	0.42
Saccharomycetes									
Saccharomycetales									
Meyerozyma caribbica CM37	3.80	0	2.68	1.10	0	0.81	2.35	0	1.69
Sordariomycetes									
Diaporthales									
Diaporthe hongkongensis CP39	5.06	0	3.57	5.49	0	5.65	5.29	0	4.66
Glomerellales									
Colletotrichum fructicola CV24	10.13	0	7.14	9.89	0	7.26	10.00	0	7.20
Colletotrichum gloeosporioides LM410, CM19	22.78	0	16.07	20.88	0	15.32	21.76	0	15.68
Colletotrichum siamense LV411, LV515	0	0	0	3.30	0	2.42	1.76	0	1.27
Colletotrichum sp. LM513	5.06	0	3.57	2.20	0	1.61	3.58	0	2.54
Hypocreales									
Gliocladium sp. C324, L222	0	3.03	0.89	0	12.12	3.23	0	7.58	2.12
Hypocreales sp. L3110	0	0	0	0	3.03	0.81	0	1.52	0.42
Purpureocillium sp. C311	0	9.09	2.68	0	6.06	1.61	0	7.58	2.12
Trichoderma hamatum C212	0	9.09	2.68	0	6.06	1.61	0	7.66	2.14
Trichoderma harzianum L212	0	6.06	1.78	0	9.09	2.42	0	7.49	2.10
Ophiostomatales									
Sporothrix sp. C316	0	3.03	0.89	0	0	0	0	1.52	0.42
Sordariales									
Cercophora coprophila CV11	2.17	0	0.89	0	0	0	0.59	0	0.42

Table S5 Continued …

Taxa	% Relative frequency (%RF)								
	Conventional farming			Organic farming			Grand total		
	Leaf	Soil	Total	Leaf	Soil	Total	Leaf	Soil	Total
Xylariales									
Daldinia sp. CV515	5.06	0	3.57	3.30	0	2.42	4.12	0	2.97
Daldinia eschscholtzii CL411, CM24, CP13, LM12	13.92	0	9.82	15.38	0	11.29	14.71	0	10.59
Pestalotiopsis mangiferae LM11	10.13	0	7.14	9.89	0	7.26	10.00	0	7.20
Pseudopestalotiopsis camelliae-sinensis LL11	3.80	0	2.68	2.20	0	1.61	2.94	0	2.12
Xylaria sp. CL37	2.53	0	1.79	3.30	0	2.42	2.94	0	2.12
Xylaria sp. LM38	0	0	0	1.10	0	0.81	0.59	0	0.42
Xylaria sp. LP410	0	0	0	1.10	0	0.81	0.59	0	0.42
Xylaria allantoidea LL13W	0	0	0	1.10	0	0.81	0.59	0	0.42
Xylaria feejeensis LL25	0	0	0	2.20	0	1.61	1.18	0	0.85
Unidentified fungus LV12	1.27	0	0.89	1.10	0	0.81	1.18	0	0.85
Basidiomycota									
Agaricomycetes									
Auriculariales									
Auricularia polytricha LP11	0	0	0	1.10	0	0.81	0.59	0	0.42
Tremellomycetes									
Trichosporonales									
Apiotrichum dehoogii L312	0	3.03	0.89	0	6.06	1.61	0	4.55	1.27
Cutaneotrichosporon debeurmannianum LV26P	0	0	0	1.10	0	0.81	0.59	0	0.42
Total %RF	100	100	100	100	100	100	100	100	100
Number of detected species	13	18	31	17	16	33	20	22	42
Menhinick Index (Dmn)	1.46	2.79	2.74[a]	1.78	2.44	2.76[a]	1.53	2.46	2.59
Shannon (H′) Index	1.77	1.79	2.47[b]	1.89	2.09	2.49[b]	1.92	2.07	2.56

The same superscript letter indicates no significant difference ($p > 0.05$) between individual parameter from conventional and organic farming.