



OPEN Entomopathogenic fungi as potential biological control agents against basal stem rot in oil palm plantations of Sabah, Malaysia

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Basal stem rot (BSR) is a serious oil palm disease caused by *Ganoderma* fungi resulting in severe yield losses in Southeast Asia. Conventional management strategies including chemical treatments, sanitation and cultural practices remain largely ineffective, necessitating the exploration of sustainable biological alternatives. This study aimed to evaluate the antagonistic potential of selected entomopathogenic fungi (EPF) against *G. boninense* isolates from oil palm plantations in Sabah, Malaysia. Six *G. boninense* isolates were obtained from infected oil palm trees, while five EPF — *Cordyceps jakajanicola*, *Cordyceps tenuipes*, *Blackwellomyces calandulinus*, *Purpureocillium lilacinum*, and *Simplicillium formicae* — were newly isolated from Danum Valley Conservation Area, INFAPRO, Kinabalu Park, and Tawau Hills Park and identified using morpho-molecular approaches. Antagonistic activity was evaluated using a dual culture assay, with nystatin (200 and 300 $\mu\text{L/mL}$) included as a positive control. Among EPF, *C. jakajanicola* and *P. lilacinum* exhibited strong antagonistic activity against *G. boninense*, with inhibition ranging from 38.6 to 70.37%, and 32.76–70.18%, respectively. Moderate inhibition was observed for *C. tenuipes* (23.81–64.41%) and *B. calandulinus* (13.64–45.54%), while *S. formicae* showed the lowest inhibitory effect (13.73–39.34%). For comparison, the positive control nystatin showed the highest inhibition, ranging from 76.02 to 79.76% at 300 $\mu\text{L/mL}$ and 67.86–75.57% at 200 $\mu\text{L/mL}$. All observed inhibitory effects were statistically significant ($p \leq 0.05$). These findings illustrate that selected EPF, particularly, *C. jakajanicola*, and *P. lilacinum*, possess promising antagonistic potential against *G. boninense* and may serve as eco-friendly biocontrol agents for the management of basal stem rot in oil palm.

Keywords Antagonistic activity, Dual culture, Growth inhibition, Pathogen and sustainable agriculture

Oil palm (*Elaeis guineensis*) is one of the most important cash crops globally, contributing approximately 40% of the world's total vegetable oil production, with Malaysia and Indonesia accounting for over 85% of global supply^{1–3}. Despite its substantial economic significance, oil palm productivity is severely constrained by basal stem rot (BSR), a destructive disease caused by *Ganoderma* species. BSR leads to extensive yield losses due to palm mortality and reduced fruit production, resulting in annual economic losses exceeding USD 0.5 billion in Malaysia alone^{4,5}. The pathogenicity of *Ganoderma* spp. is associated with the secretion of lignocellulolytic enzymes, including ligninases, cellulases, proteases, laccases, as well as bioactive metabolites such as phytotoxins and ganoderic acids, which collectively disrupt host tissue integrity and physiological functions^{6,7}. Disease progression is further influenced by environmental factors such as temperature, humidity, and soil characteristics, which enhance pathogen survival and infection efficiency⁸. Although various management approaches, including sanitation, pathogen-free planting materials, crop rotation, and chemical fungicides, have been implemented, effective and sustainable control of BSR remains elusive^{9–11}.

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This challenge is particularly critical for major producing countries, where compliance with sustainability framework such as the Roundtable on Sustainable Palm Oil (RSPO) and Malaysian Sustainable Palm Oil (MSPO) necessitates environmentally compatible disease management strategies^{12–14}.

Biological control has emerged as a promising eco-friendly alternative to synthetic fungicides for the management of plant diseases, particularly where chemical interventions have shown limited effectiveness and sustainability concerns. Among biological agents, Entomopathogenic fungi (EPF) have attracted increasing attention due to their ability to penetrate host cuticle, adapt to diverse environmental conditions, and exhibit broad-spectrum antagonistic activity against viruses, bacteria, and fungal pathogens¹⁵. In contrast to conventional fungicides, EPF-based formulations offer advantages such as target specificity, environmental compatibility, and reduced risk of resistance development, making them suitable candidates for sustainable disease management in oil palm plantations^{16,17}. Previous reports described the antifungal activity of EPF against phytopathogens. Different EPF including *Beauveria*, *Metarhizium*, *Lecanicillium*, and *Clonostachys rosea*, have reported with their antifungal activities¹⁸. Similarly, *Akanthomyces muscarius* possess inhibition rates from 39.61% to 52.94%¹⁹. In the context of basal stem rot, fungal biocontrol agent *Trichoderma* spp. has been widely evaluated against *G. boninense* using dual culture assay²⁰.

Despite their well-documented biological active properties, the antagonistic potential of EPF against *Ganoderma boninense* remains insufficiently explored, particularly under Malaysian oil palm plantations conditions.

Therefore, the present study aimed to investigate the antagonistic activity of selected entomopathogenic fungi against *G. boninense* isolates and to evaluate their potential as natural biocontrol agents for the sustainable management of basal stem rot disease in oil palm.

Methodology

Collection and isolation of *Ganoderma* spp

The fruiting bodies of *Ganoderma* fungi were collected from privately managed commercial oil palm plantations, specifically from infected oil palm trees exhibiting clear symptoms of basal stem rot (BSR) disease, obtained from the lower trunk region. All sampling was conducted with permission from the respective estate managements and restricted to palms exhibiting typical basal stem rot (BSR) symptoms. A total of six samples was collected between 2023 and 2024 from different oil palm plantations in Sabah, Malaysia. Two samples (BORH4448, BORH4449) were collected from Kota Marudu (6.542936, 116.717606), one sample (BORH4453) from Beluran (5.790436, 117.596970), one sample (BORH4452) from Sandakan (5.821093, 117.839278), one sample (BORH4451) from Kinabatangan (5.589462, 118.065875), and one sample (BORH4450) from Lahad datu (5.232570, 118.434440) of Sabah, Malaysia. All *Ganoderma* fruiting bodies samples were molecularly identified as *Ganoderma boninense* and labelled as *Ganoderma boninense* 1 - *Ganoderma boninense* 6 (Fig. 1) throughout this study. A map was created using QGIS 3.44.3 software (<https://qgis.org/download/>) to point the different areas of oil palm plantations in Sabah, Malaysia (Fig. 1). The infected plant material was carefully washed to remove soil particles and surface sterilized using 10% bleach (sodium hypochlorite (NaClO) and ethanol before further processing²¹. The fungus was isolated by culturing on *Ganoderma* Selective Medium (GSM). Mycelial plugs from infected tissues were placed on these media and incubated in the dark at 27 °C for optimal growth²².

Collection and isolation of Entomopathogenic Fungi (Ef)

Entomopathogenic fungi (EPF) samples including *Cordyceps jakajanicola* (BORH(F)03653), *Cordyceps tenuipes* (BORH(F)03654), *Blackwellomyces calendulinus* (BORH(F)03655), *Purpureocillium lilacinum* (BORH(F)03656) and *Simplicillium formicae* (BORH(F)03640), that will be used for biocontrol agents were collected from Danum Valley Conservation Area (N° 0457.783 E° 11748.354), INFAPRO (N° 4.97816, E° 117.86502), Kinabalu park (N° 61229.9 E° 1163827.0), and Tawau Hills Park (N° 4.3992481 E° 117.8892033). After collections, the samples were deposited in Borneensis Herbarium Collection, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia. The protocol followed by Vivekanandhan et al.²³ was used to isolate *Cordyceps jakajanicola* (BORH(F)03653), *Cordyceps tenuipes* (BORH(F)03654), *Blackwellomyces calendulinus* (BORH(F)03655), *Purpureocillium lilacinum* (BORH(F)03656) and *Simplicillium formicae* (BORH(F)03640), from infected insects. Initially, samples were surface sterilized three times using 70% ethanol for 3 min followed by distilled water. The sterilized samples were individually cut into fine pieces and placed onto potato dextrose agar (PDA) plates supplemented with streptomycin as an antibacterial agent. The Petri dishes were incubated at 25 °C for 7–14 days. The newly emerged fungal colony was sub-cultured into PDA to obtain the axenic culture.

Morpho-molecular identification

Morphological identifications were carried out using 14 days old culture. The morphological characters such as colony colour, growth rate, and colony diameter were recorded. A small piece of fungal tissue was mounted on a glass slide with 5% KOH, and microscopic characteristics such as phialide number, size, arrangement, and conidial size were examined under a light microscope²⁴.

DNA extraction, PCR amplification and DNA sequencing

DNA extraction was performed using E.Z.N.A Fungal DNA Mini Kit (Omega Bio-Tek, USA) following the protocol outlined by Garibaldi et al.²⁵. The extracted DNA was preserved at –20 °C. Amplification of the internal transcribed spacer (ITS) region and translation elongation factor 1-alpha (TEF1) were conducted via PCR following the conditions described by Garibaldi et al.²⁵. The resulting PCR products were purified and sequenced at Apical Scientific Sdn. Bhd., Selangor, Malaysia.

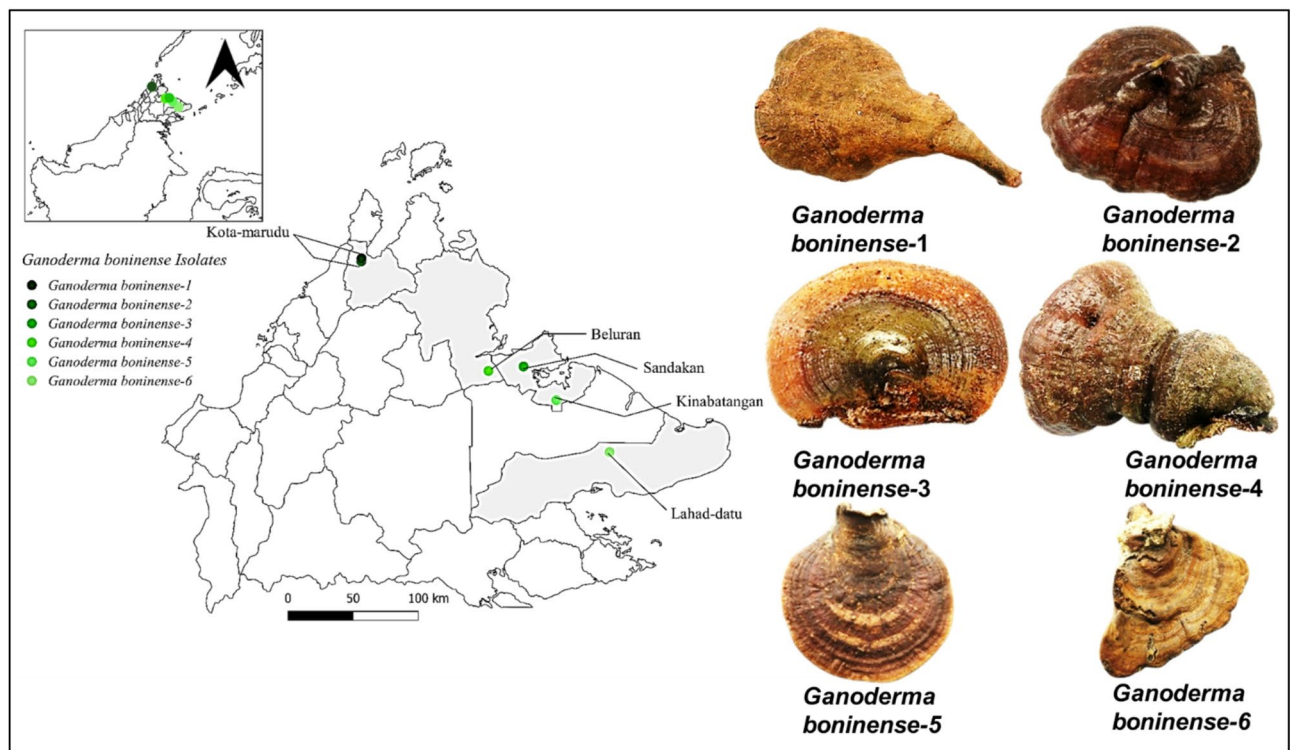


Fig. 1. *Ganoderma* fruiting bodies collected from different areas of oil palm plantations in Sabah, Malaysia. Kota Marudu = *Ganoderma boninense*-1 and *Ganoderma boninense*-2; Beluran = *Ganoderma boninense*-3; Sandakan = *Ganoderma boninense*-4; Kinabatangan = *Ganoderma boninense*-5; Lahad Datu = *Ganoderma boninense*-6. A map was created using QGIS 3.44.3 software (<https://qgis.org/download/>).

Phylogenetic tree construction

The ITS and TEF1 generated sequences for all fungal isolates were visually screened first and then subjected to BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for quality control. BioEdit (Sequence Alignment Editor, file version 7.2.5.0) was used to create consensus sequences. Further editing was performed using the Aliview software (version 3.0 (GPLv3)²⁶. Reference sequences from GenBank (S1Tables 1 and 2) were used to construct phylogenetic tree based on parameters as proposed by Bich et al.²⁷, Lu et al.²⁸ and Zhang et al.²⁹. Using RAXML-HP2 on ACCESSION v. 8.2.12, 1000 nonparametric bootstrap iterations were used to conduct Maximum Likelihood (ML) analysis³⁰. For machine learning analysis, a bootstrap value of 70% or more is deemed to provide considerable support. TrNef + G was then identified as the best-fit models of nucleotide substitution for the dataset using JModelTest2 on XSEDE v. 2.1.6 with the following parameters: lsetnst = 6; rates - gamma; prset revmatpr fixed 1.0000, 1.7639, 1.0000, 1.0000, 3.1308, 1.000; shapepr = fixed 0.725³¹. Finally, XSEDE v. 3.2.7a was used to conduct Bayesian Inference (BI) analysis using MrBayes³². Five million generations of four Markov chains were executed, with a tree sample taken every 1000 generations. Bootstrap values (BS) of $\geq 70\%$ and posterior probabilities (PP) of $\geq 90\%$ were considered to indicate significant support. Phylogenetic trees were generated using FigTree v1.4.4 and edited with Adobe Illustrator v27.6.1³³.

Antagonistic activity assay

A dual culture assay was conducted by inoculating 8-mm mycelial plugs of five entomopathogenic fungi *Cordyceps jakajanicola* (BORH(F)03653), *Cordyceps tenuipes* (BORH(F)03654), *Blackwellomyces calendulinus* (BORH(F)03655), *Purpureocillium lilacinum* (BORH(F)03656) and *Simplicillium formicae* (BORH(F)03640) individually and separately with *Ganoderma boninense* at a distance of 20 mm from one another on potato dextrose agar (PDA) plates. The control contained an 8-mm mycelial plugs of *G. boninense* without any EPF. The plates were incubated at 28 °C in dark for 7 days³⁴. Nystatin oral suspension (100,000 units/mL; T.O. Chemicals, Thailand) was used as the positive control. The tested concentrations of 200 $\mu\text{L/mL}$ (equivalent to 20,000 units/mL) and 300 $\mu\text{L/mL}$ (equivalent to 30,000 units/mL) refer to the volume of Nystatin suspension added per millilitre of culture medium. The suspensions were prepared under controlled conditions using sterilized double-distilled water³⁵. To observe the antagonistic effects of treatments against *G. boninense*, individual culture samples were prepared for observation under the scanning electron microscope (SEM). Samples were individually fixed using glutaraldehyde and osmium tetroxide. Then, series of graded ethanol was applied to remove excess water and followed by application of Hexamethyldisilazane (HMDS) to further dry the samples. Finally, the specimen was mounted on stubs, then spotter coated with gold as a conductive material and observed under scanning electron microscopy^{36,37}.

Evaluation of antagonistic activity

Radial growth of *G. boninense* toward the antagonist (R2) and in controls (R1) was measured to calculate percentage inhibition of radial growth (PIRG) as

$$\text{Percentage of Growth Inhibition (PGI)} = \frac{R1 - R2}{R1} \times 100$$

The efficacy classified as very weak (1–20%), weak (21–40%), moderate (41–60%), strong (61–80%), or very strong (81–100%)^{38,39}.

Statistical analysis

The experiment was performed in triplicates, and the data were expressed as Mean ± standard error. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's test to determine significant differences between treatments at $p < 0.05$ ⁴⁰.

Results

Morphological description of *Ganoderma boninense*

In current study, morphological observations of six fruiting bodies collected from an oil palm plantation in Sabah clearly show characteristics of *Ganoderma* fungi. The size of sporophores is within the range of 5–15 cm, which are either stalked or sessile, indicating traits of *Ganoderma* fungi. Structurally, *Ganoderma* were reported to be either flat, bracket-shaped, or lobed as observed in these fruiting body samples. The dorsal surface of all samples was observed to be glossy, blackish-brown in color with concentric markings, evident for *Ganoderma* species but varied in contrast and color's depth which could be influenced by the environment, chemicals application in the plantation, or animal. The edge was white when fresh which similarly observed on under-surface sporophores. The incubation of the fruiting body's fraction on GSM at 25 °C for one-week reveal white, fluffy mycelium development (Fig. 2B) which indicate strong presence of basidiomycetes. Proceeding to subculturing on PDA, the fungus front colony was observed to be denser, cottony, white mycelium, often radiating from the inoculation point while a light brown to yellowish pigmentation was observed in the reverse colony, strong indication for *Ganoderma* fungal growth on enriched medium. The *Ganoderma* culture colony attained a diameter of 15 mm within 6 days of incubation on PDA and fully grown on the plate within 9 days of incubation (Fig. 2C & D). Basidia are usually clavate (club-shaped) measuring 45–65 × 8–12 µm (Fig. 2E). The septate hyphae, measuring 3–5 µm in diameter, possessed clamp connection, measuring 3–4 µm in diameter (2 F&G). Basidiospores appeared as ellipsoid to oblong, double-walled, and brownish in colour with 8–12 × 4–6 µm diameter (Fig. 2H&I) which previously described for *Ganoderma* fungi.

Morphological description of entomopathogenic fungi

The entomopathogenic fungi (EPF) used in current research work were collected from naturally infected arthropods hosts and were identified based on association with their hosts, features of culture colony on PDA, and microscopic study. Colony colour, texture, pigmentation, and growth rate on potato dextrose agar (PDA) at 25 °C were measured for macroscopic assessments while microscopic observations include arrangement of phialides and conidia (Fig. 3).

Cordyceps jakajanicola was obtained from naturally infected cicada that exhibited stromata emerging from host body (Fig. 3A). The culture front colony appeared as dense, cottony, white with a concentric ring while cream to pale-yellow pigmentation was observed in reverse colony (Fig. 3A1 & A2). Phialides were slender, tapering and conidia were cylindrical to ellipsoidal (Fig. 3A3).

Cordyceps tenuipes was isolated from lepidopteran pupa with fibrous fungal growth (Fig. 3B). On PDA, white fluffy mycelium was observed in both front and reverse colony (Fig. 3B1 & B2). Phialides were arranged vertically, and oval to fusiform conidia with slimy heads were noted (Fig. 3B3).

Blackwellomyces calendulinus was obtained from naturally infected cocoon with a stromata bright orange in colour, emerging from host (Fig. 3C). *B. calendulinus* produced front colony as white to light yellow velvety while pal-yellow pigmentation was observed on reverse colony (Fig. 3C1 & C2). Under microscope, smooth-walled, septate hyphae were observed. Conidia were cylindrical to ellipsoid and elongated phialides were observed (Fig. 3C3).

Purpureocillium lilacinum was obtained from beetles surrounded with purple fungal growth (Fig. 3D). The front colony was circular with powdery texture and pale to brown reverse colony on PDA. Elongated and flask shaped phialides were observed. Conidia were elliptical to oblong with long chains (Fig. 3D3).

Simplicillium formicae was parasitised on *Ophiocordyceps loidii* (Fig. 3E). *S. formicae* forms white, floccose with yellow pigmentation on PDA. The front and reverse colony is circular (Fig. 3E1 & E2). The phialides are simple, unbranched, and tapering structures while conidia are elliptical, single-celled, formed in slimy heads (Fig. 3E3).

Phylogenetic analysis of *Ganoderma boninense*

The ITS phylogenetic tree of *G. boninense* alignment has 72 sequences with 358 columns, 72 distinct patterns, 64 parsimony-informative, 10 singleton sites, 284 constant sites. Akaike information criterion TNe+G4, corrected Akaike information criterion TNe+G4, Bayesian Information Criterion (BIC) K2P+G4 and best-fit model was K2P+G4 chosen according to BIC. Optimal log-likelihood –1179.785, Rate parameters A-C: 1.00000 A-G: 4.78790 A-T: 1.00000 C-G: 1.00000 C-T: 4.78790 G-T: 1.00000 Base frequencies: A: 0.250 C: 0.250 G: 0.250 T: 0.250, and Gamma shape alpha: 0.246. Performs final model parameters optimization, estimate model parameters (epsilon = 0.010), Initial log-likelihood: -1178.791, Optimal log-likelihood: -1178.791, Rate parameters: A-C:

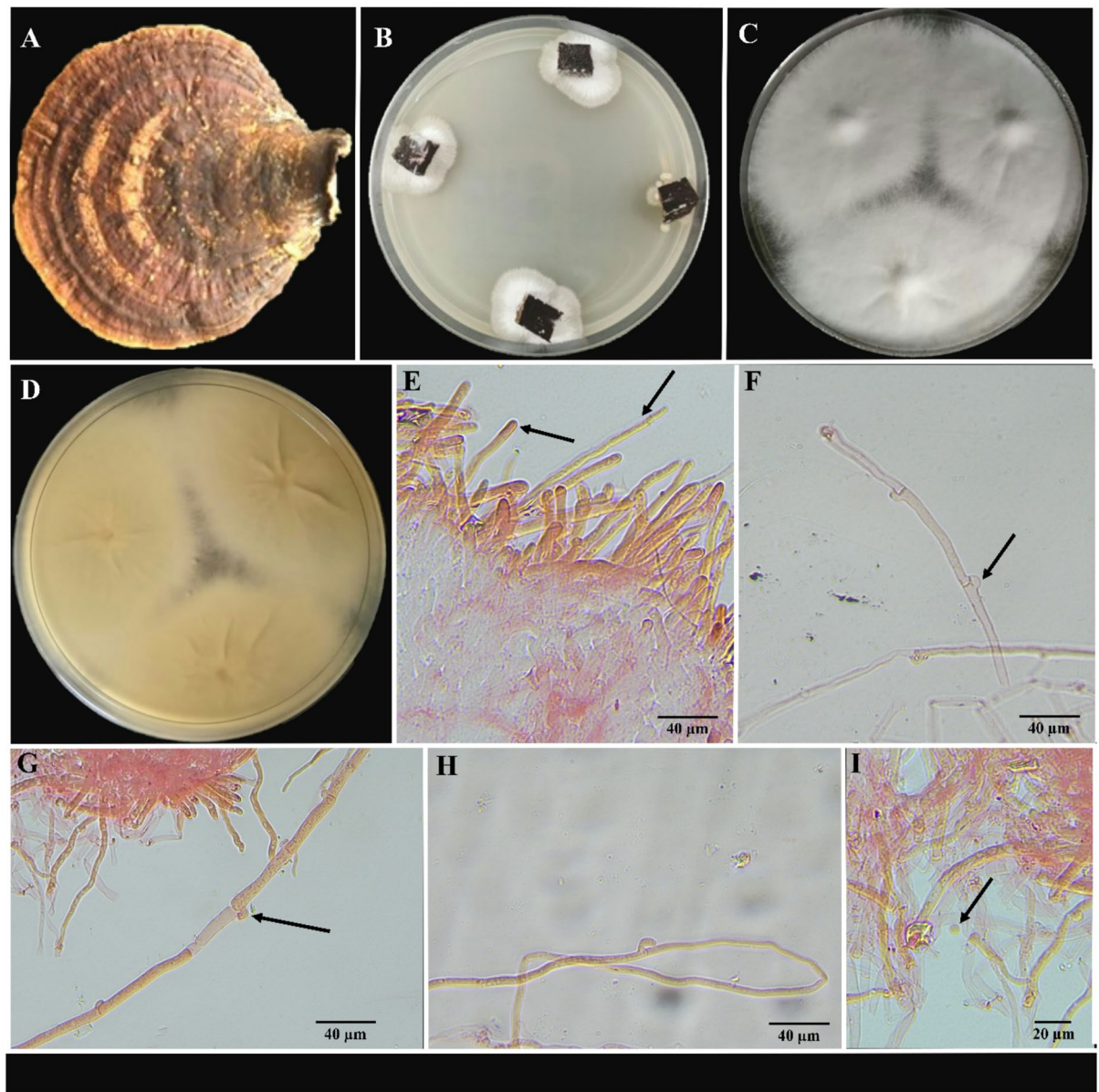


Fig. 2. Morphological identification of *Ganoderma boninense*. (A) Fresh basidiocarp of *Ganoderma boninense*, (B) Isolation, (C) Front Colony, (D) Reverse colony (Cells of crust hymeniderm and hyphae, (F–H) Hyphae showing clamp connection and (I) Basidiospores.

1.00000 A–G: 4.81322 A–T: 1.00000 C–G: 1.00000 C–T: 4.81322 G–T: 1.00000, Base frequencies: A: 0.250 C: 0.250 G: 0.250 T: 0.250, Gamma shape alpha: 0.238, Parameters optimization took 1 round (0.010 s) (Fig. 4).

Phylogenetic analysis of Entomopathogenic Fungi (EPF)

Two-gene data (ITS+TEF) set used for phylogenetic analysis for the genus *Cordyceps*, *Simplicillium*, *Purpureocillium*, and *Blackwellomyces* that consists of 77 sequences with 1259 columns, 612 distinct patterns, 458 parsimony-informative, 174 singleton sites, 627 constant sites. Optimal log-likelihood: -10112.909, rate parameters: A–C: 0.71482 A–G: 1.58007, A–T: 1.00000, C–G: 0.71482, C–T: 3.58177, G–T: 1.00000, base frequencies: A: 0.225, C: 0.323, G: 0.248, T: 0.204, and gamma shape alpha: 0.320. *Aspergillus iizukae* was selected for outgroup. Both ML and BI analyses shows high bootstrap value for the terminal clades at the species level. In this study, *C. jakajanicola* (BORH(F)03653) was identical to *C. jakajanicola* (NTUCC 17036) already reported from China with maximum likelihood analysis, with bootstrap support values (MLB) 96% and Bayesian posterior probabilities (BPP) 0.99 while *Cordyceps tenuipes* (BORH(F)03654) in this study aligned well with *C. tenuipes* (NTUCC 18137) from Taiwan and formed an identical clade with maximum likelihood analysis value 100% and Bayesian posterior probabilities (BPP) 1. Similarly, *Blackwellomyces calendulinus* (BORH(F)03655) is associated to *B. calendulinus* (BCC 68500, 685002) from Thailand with supportive bootstrap value maximum likelihood analysis value 82% and Bayesian posterior probabilities (BPP) 0.99. Both *Purpureocillium lilacinum*

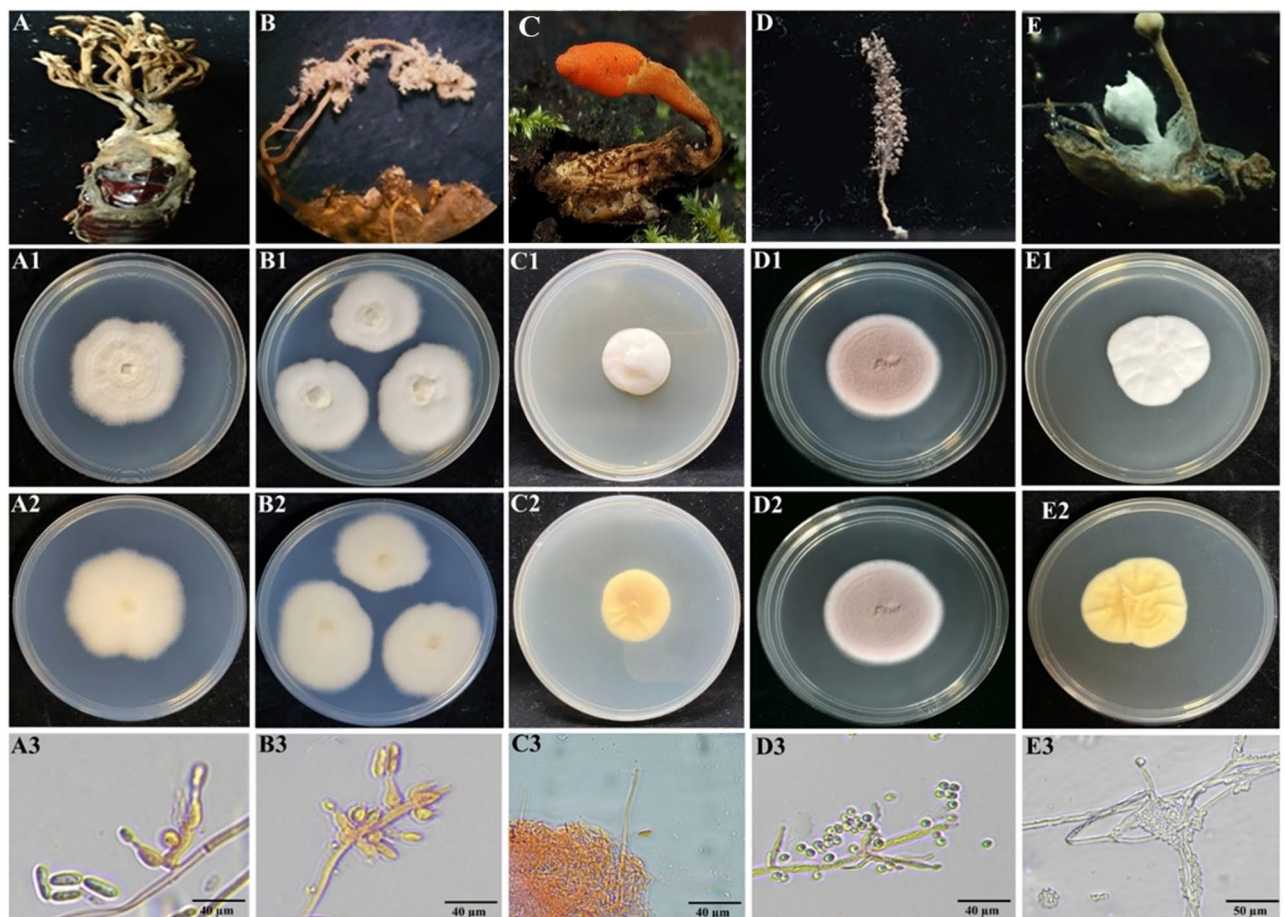


Fig. 3. Morphological description of Entomopathogenic fungi. (A) *Cordyceps jakajanicola* infected cicada, (A1) *C. jakajanicola* culture front colony on PDA, (A2) Culture reverse colony, (A3) Phialides and conidia. (B) *C. tenuipes* infected pupa of Lepidoptera buried in soil, (B1 & B2) *C. tenuipes* culture front and reverse colony, (B3) Phialides and conidia. (C) *Blackwellomyces calandulinus* infected cocoon, (C1 & C2) *B. calandulinus* culture front and reverse colony on PDA, (C3) Fungal hyphae and conidia. (D) Beetles infected with *Purpureocillium lilacinum*, (D1 & D2) *P. lilacinum* culture front and reverse colony on PDA, (D3) Phialides and conidia. (E) Spider parasitised *Simplicillium formicae*, (E1 & E2) *S. formicae* culture front and reverse colony, (E3) Phialides and conidia.

(BORH(F)03656) and *Simplicillium formicae* (BORH(F)03640, BORH(F)03641) formed identical clades with their respective species with maximum likelihood analysis value 85% and 100% and Bayesian posterior probabilities (BPP) 0.99 and 1 respectively (Fig. 5).

Antagonistic activity against *Ganoderma boninense*

The antifungal activity of all EPF and Nystatin to against the six isolates of *G. boninense* is exhibited (Fig. 6). Among all applied treatments, Nystatin exhibited the highest antifungal potential with 76.02% – 79.76% inhibition at the concentration of 300 µL/mL, followed by 67.86% – 75.57% at 200 µL/mL concentration against all six isolates of *G. boninense*. Similarly, among the EPF, *C. jakajanicola* and *P. lilacinum* performed best with mycelial growth inhibition of 38.6–70.37%, and 32.76–70.18 respectively. *Cordyceps tenuipes* and *B. calandulinus* exhibited inhibition within the range of 23.81–64.41% and 13.64–45.54% respectively against the six isolates of *G. boninense*. *Simplicillium formicae* had the least inhibition between 13.73 and 39.34%.

Isolate-specific responses to antifungal treatments

The variability in treatments effectiveness was determined by evaluating the isolate-specific responses towards applied treatments. *Ganoderma boninense*-1 and *G. boninense*-2 achieved over 72% inhibition when Nystatin applied, while 55.11% and 65.36% inhibition was observed in case of *C. tenuipes*. For *G. boninense*-3, highest inhibition of 79.76% was recorded at Nystatin 300 µL/mL and 66.07% when *P. lilacinum* was applied. The weakest inhibition of 21.43% was recorded when *G. boninense*-3 was treated with *B. calandulinus*. In the case of *G. boninense*-4, highest inhibition of 77.51% and 55.03% was measured when this isolate was treated with Nystatin (300 µL/mL) and *Cordyceps tenuipes*, respectively. The minimum inhibition of 23.08% was measured for *S. formicae* against *G. boninense*-4. For *G. boninense*-5 and *G. boninense*-6 maximum mycelial growth inhibition

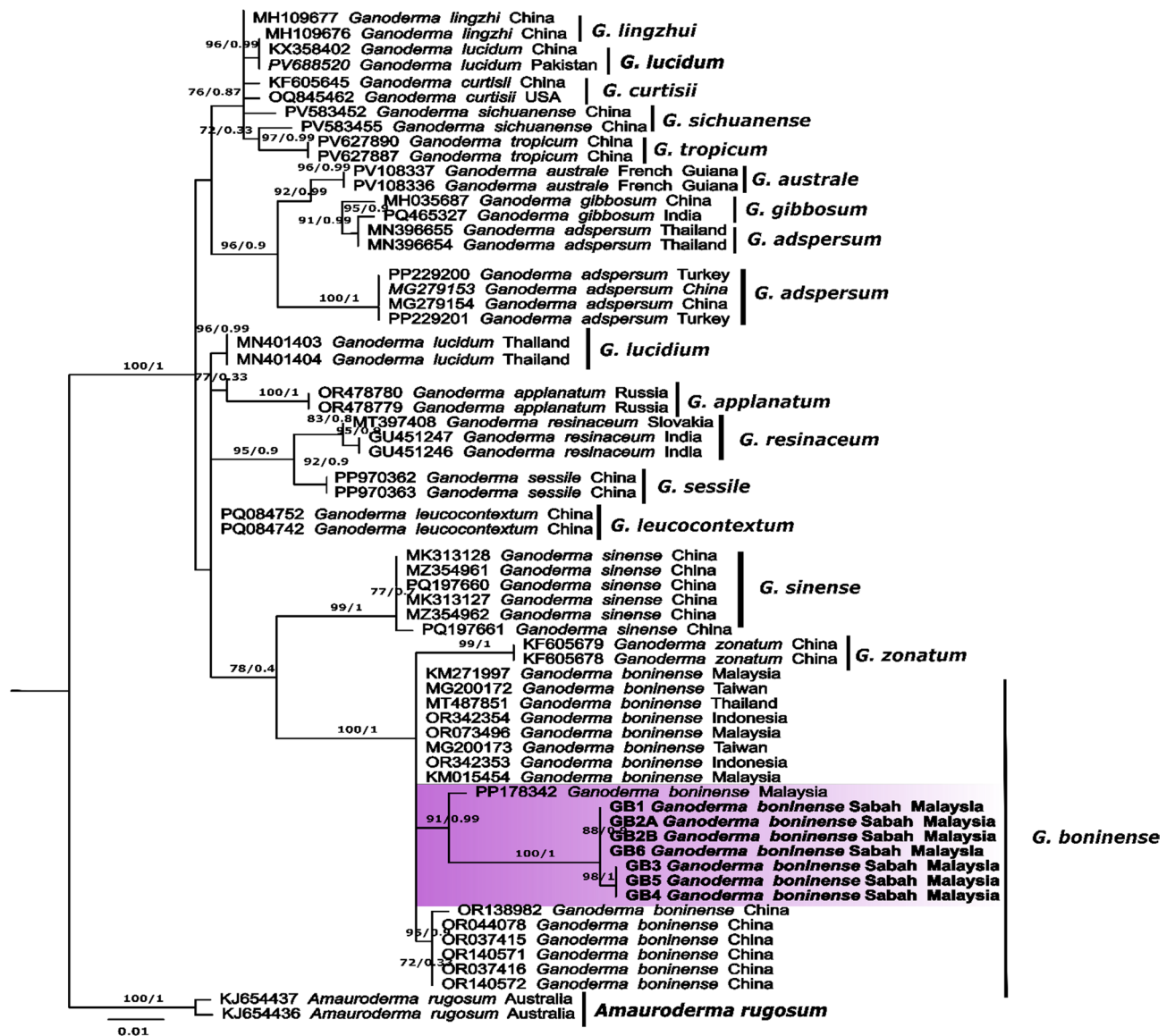


Fig. 4. Phylogenetic tree (ITS) of the six isolates of *Ganoderma boninense* (highlighted) and related species. Numbers at the major nodes revealed the maximum likelihood bootstrap values (MLB $\geq 70\%$) and Bayesian posterior probabilities (BPP ≥ 0.70).

was measured as 76.02% and 77.22% when Nystatin (300 $\mu\text{L/mL}$) applied. Similarly, moderate inhibition of 57.89% and 66.46% was recorded when *C. jakajanicola* was tested (Fig. 7).

Heatmap analysis of antifungal activity

A heatmap was generated based on the percentage inhibition values obtained from the antagonistic assays to visualise and compare the inhibitory effects of all treatments against the six *G. boninense* isolates (Fig. 8). The stronger inhibition of EPF was indicated as red shades while blue shades depicted the weaker antifungal potential. Among the applied EPF, *Cordyceps tenuipes*, *Cordyceps jakajanicola*, and *Purpureocillium lilacinum* exhibited intense red shades because of significant antifungal potential. Moreover, *B. calendulinus* and *S. formicae* possess mixed responses in inhibiting the six isolates of *G. boninense*, showing more blue areas, suggesting the lower antifungal activity. Similarly, control treatments exhibited mostly intense blue shades, showing the no antifungal activities (Fig. 8).

Scanning electron microscopy (SEM) observations of hyphal damage

Scanning electron microscopy was performed to evaluate the structural damages in *G. boninense* hyphae due to applications of different treatments. All six isolates of *G. boninense* under untreated control samples possess smooth, continuous, and well-organised filamentous hyphal networks with dense interwoven mycelia. Applications of Nystatin at concentrations of 200 $\mu\text{L/mL}$ and 300 $\mu\text{L/mL}$ caused shrinkage, slight deformation and anomalies, including surface roughening, fragmentation, and collapse respectively. Structural damages such



Fig. 5. Concatenated Phylogenetic tree consists of ITS + TEF data set of genus *Cordyceps*, *Simplicillium*, *Purpureocillium*, and *Blackwellomyces* and their related species from GenBank. Different numbers at the nodes indicate the maximum likelihood bootstrap values (MLB $\geq 70\%$) and Bayesian posterior probabilities (BPP ≥ 0.70).

as hyphal thinning, rupture, and apparent disintegration were observed with the applications of *C. jakajanicola* and *C. tenuipes*. Effect of *Purpureocillium lilacinum* was observed in terms of moderate to strong distortion in hyphae such as irregularly shaped, with collapsed and fragmented regions while widespread disorganization and surface damage was noted when the six isolates of *G. boninense* was treated with *B. calandulinus*. Similarly, *Simplicillium formicae* caused hyphal degradation in all isolates of *G. boninense* (Supplementary Fig. S1).

Discussion

This study demonstrated that entomopathogenic fungi (EPF) possess varying degree of antagonistic activity against geographically distinct isolates of *Ganoderma boninense*, indicating their potential role as biological control agents in basal stem rot (BSR) management. Although the chemical antifungal nystatin showed higher inhibitory effects under in vitro conditions, the relevance of EPF lie not in outperforming synthetic fungicides, but in offering environmentally compatible and biological sustainable alternatives for long-term disease management. Even potent synthetic antifungals like Nystatin rarely achieve complete inhibition in

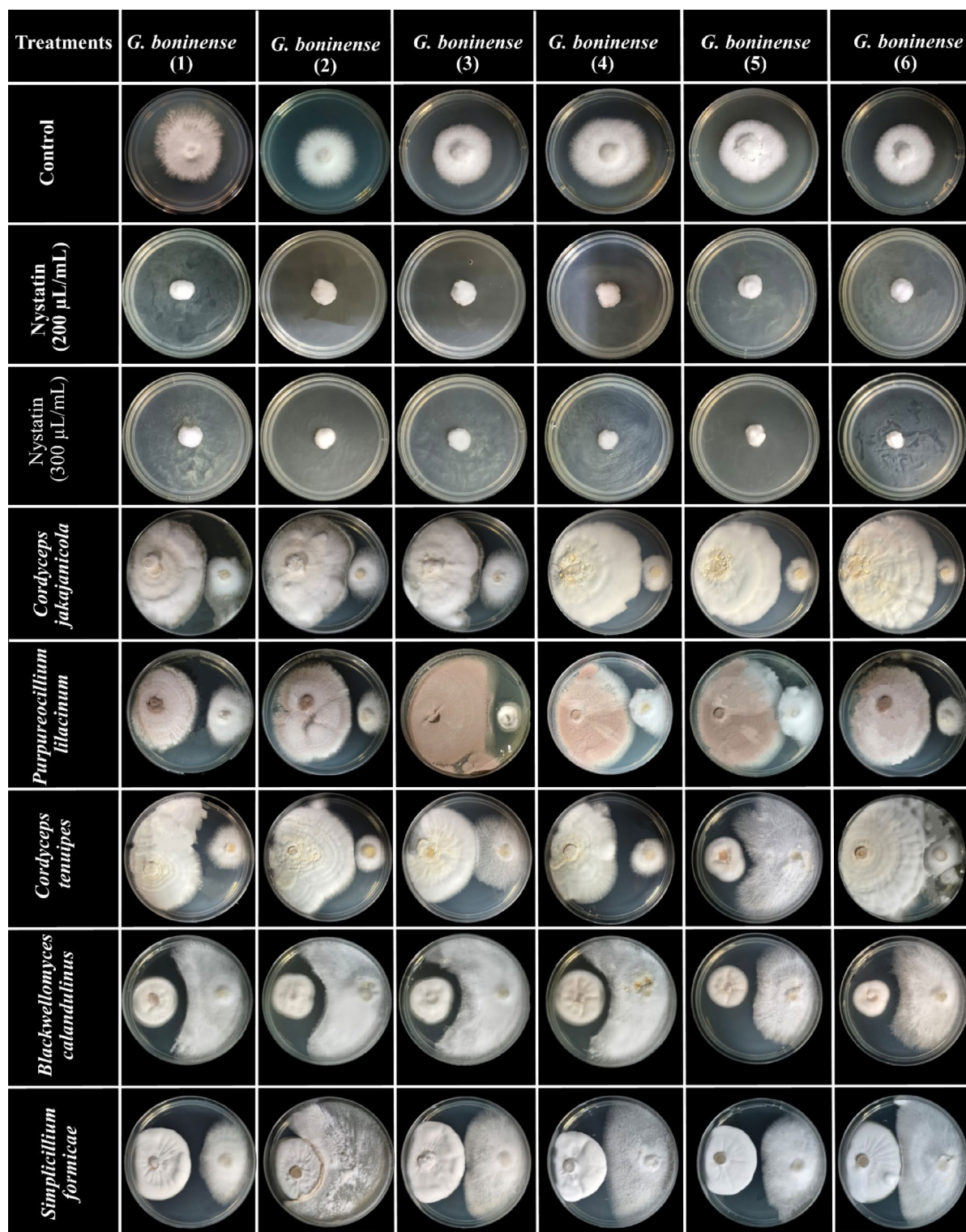


Fig. 6. Dual culture assay was performed to evaluate the antagonistic activity of entomopathogenic fungi (*Cordyceps jakajanicola* (BORH(F)03653), *Cordyceps tenuipes* (BORH(F)03654), *Blackwellomyces calandulinus* (BORH(F)03655), *Purpureocillium lilacinum* (BORH(F)03656) and two isolates of *Simplicillium formicae* (BORH(F)03640, against *G. boninense* isolates (1–6). Commercial antifungal agent Nystatin was used as a positive control (200 and 300 µL/mL), while untreated *G. boninense* cultures acted as the negative control. Restricted growth or overgrowth of entomopathogenic fungi over *G. boninense* isolates (1–6) is evidence of antagonistic interactions.

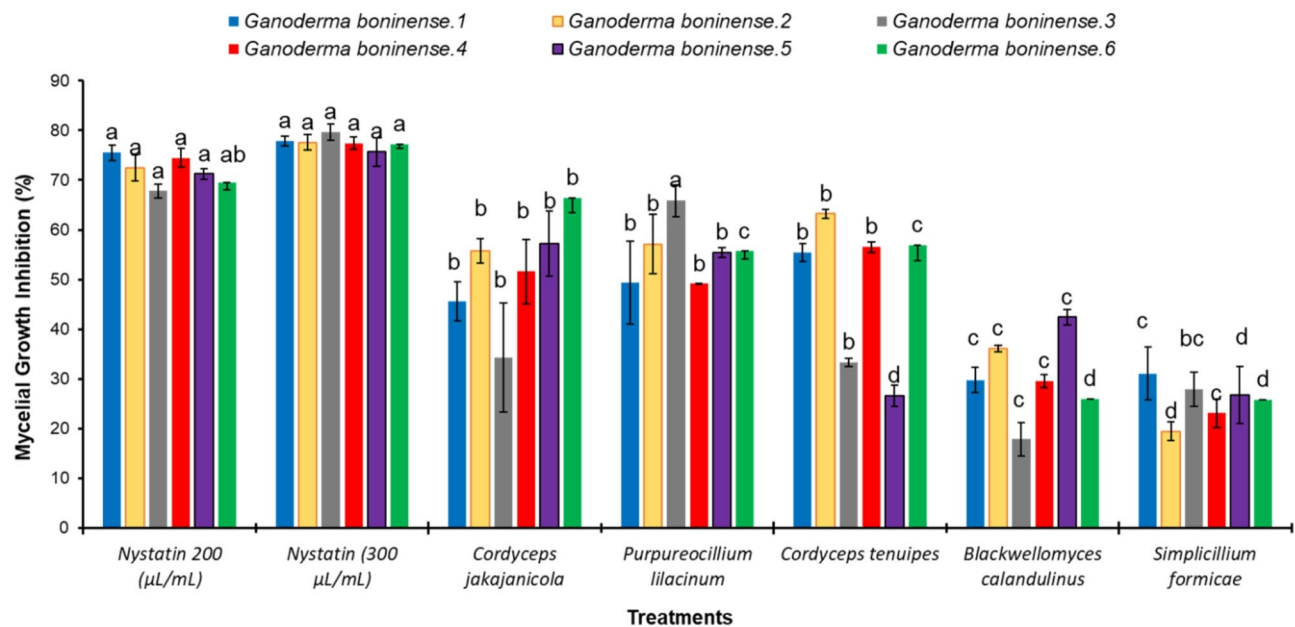


Fig. 7. Antagonistic activities of Entomopathogenic fungi against six isolates of *Ganoderma boninense* collected from different location in Sabah. Different letters in the figure show significant difference among the treatment. (Mean $\pm$ standard error, $n = 3$).

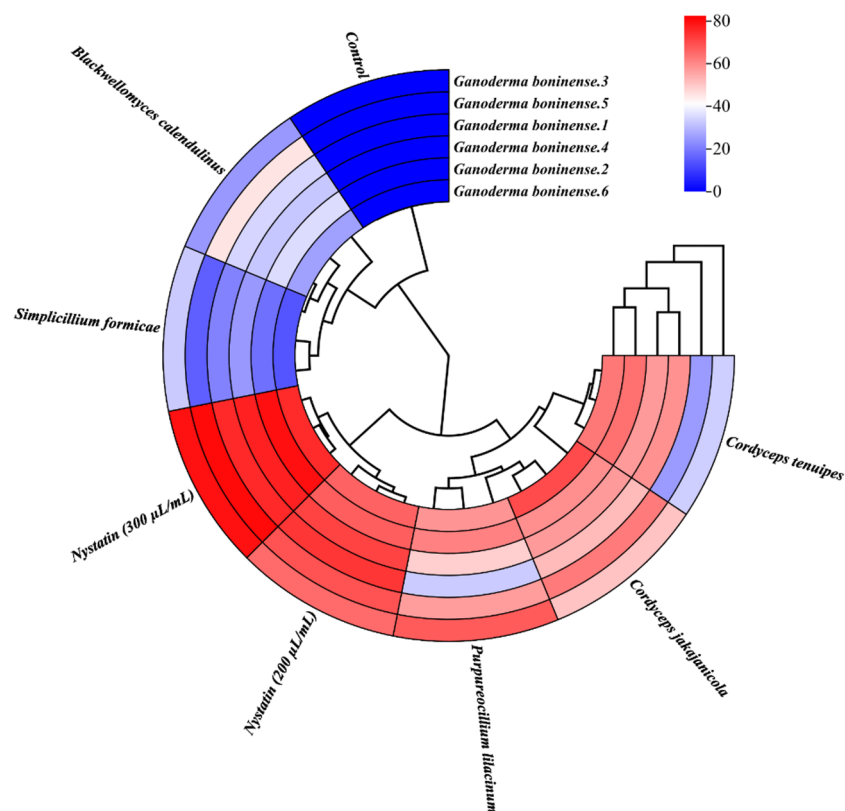


Fig. 8. The heatmap illustrates the inhibitory effects of various treatments against *Ganoderma boninense* isolates, with colour intensity representing measured inhibition values ranging from 0 to -80. Red tones signify stronger inhibition or less negative values, while blue indicates weaker inhibition or more negative values. Nystatin, used as a positive control at both 200 $\mu\text{L/mL}$ and 300 $\mu\text{L/mL}$ concentrations, displays a dominant red coloration across most isolates, confirming its strong antifungal activity.

vitro⁴¹. Partial survival may result from fungal heterogeneity or limited diffusion of the compound, allowing some hyphae to persist^{42,43}.

The use of EPF has traditionally focused on insect pest control due to their ability to penetrate insects' cuticles; however, their antifungal potential has gained increasing attention over the last two decades, particularly in the context of sustainable agriculture^{44,45}. Previous studies have reported antifungal activity of EPF such as *Beauveria bassiana* and *Metarhizium anisopliae* against various phytopathogens⁴⁶. Nevertheless, studies specifically addressing EPF antagonism against *G. boninense* remain limited, highlighting the significance of present investigations. The antifungal activity observed in EPF is commonly attributed to the production of secondary metabolites and hydrolytic enzymes, including chitinases, and lipases, which disrupt fungal cell walls and inhibit mycelial growth⁴⁶. Among the tested EPF, *P. lilacinum* and *C. jakajanicola* consistently exhibited stronger antagonistic responses compared to other EPF, suggesting specific-specific bioactive mechanism. Therefore, further investigations such as enzyme activity assays and comprehensive metabolomic analyses are necessary to elucidate and confirm the antifungal mechanisms employed by *C. jakajanicola* and *P. lilacinum* against *G. boninense*. Rather than direct fungicidal action, these fungi may suppress *G. boninense* through competition, antibiosis, and enzymatic degradation, making them suitable candidates for integration into biological control programs. Previous studies have demonstrated that biological control agents, including *Trichoderma* and *Bacillus* species, can reduce *G. boninense* infection and disease incidence under nursery and field conditions⁴⁷. Such findings support the ecological feasibility of fungal-based biocontrol approaches, even when in vitro inhibition levels are lower than those of synthetic antifungals. A recent study reported non-pathogenic polypore fungi inhibiting *G. boninense* up to 72.7% (PIRG) and 43–52% via VOCs, supporting the potential of fungal biocontrol, consistent with our findings for *C. jakajanicola* and *P. lilacinum*⁴⁸. *Metarhizium anisopliae* (TK09) showed antifungal activity against *Cladosporium herbarum* and *Fusarium oxysporum* in agar diffusion assays at concentrations of 500–1,200 µg mL⁻¹, with stronger inhibition observed against *C. herbarum*⁴⁹. Similarly, *Beauveria bassiana* crude extract inhibited plant pathogenic fungi, including *Alternaria solani*, *Glomerella cingulata*, *Rhizopus oryzae*, *Chrysosporium tropicum*, *Rhizoctonia solani*, *Myrothecium roridum*, and *Fusarium oxysporum*⁵⁰.

The application method is a critical factor in determining the success of biological control strategies. EPF-based formulations may be effectively applied through soil treatment during early infection stages or via trunk injection at later stage, offering flexibility that is often not achievable with chemical fungicides^{51,52}. The comparable in vitro inhibition of *G. boninense* by *Trichoderma harzianum* reported previously⁵³ further supports the biological relevance of the inhibitory trends observed in the present study, validating EPF as functional biocontrol agents rather than direct chemical substitutes.

The genus *Simplicillium* is known for its mycoparasitic activity, particularly against rust fungi^{54,55}. *S. lamellicola* has been reported to suppress *Botrytis cinerea*⁵⁶. Similarly, *P. lilacinum* produces diverse secondary metabolites such as leucinostatins, polyketides, and non-ribosomal peptides with antimicrobial properties^{24,57}. Studies using other EPF, including *Beauveria felina*, have demonstrated pathogen cell disruption and structural damage under scanning electron microscopy⁵⁸, supporting the hypothesis that differential metabolites profiles contribute to variable inhibition patterns among *G. boninense*⁵⁹.

Although *Cordyceps tenuipes*, *Blackwellomyces calendulinus*, and *Simplicillium formicae* exhibited comparatively lower inhibition, their activity remains biologically meaningful when viewed from an ecological and sustainability perspective rather than direct fungicidal efficiency⁶⁰. Importantly, EPF are generally biocompatible and pose lower ecological risks to non-target organisms, though ecological risk assessment remains essential prior to large-scale application⁶¹. Overall, the consistent antagonistic performance and environmental compatibility of *P. lilacinum* and *C. jakajanicola* support their candidacy as eco-friendly biological control agents for sustainable management of basal stem rot in oil palm.

Conclusion

The Present research work illustrates the antagonistic activity of EPF particularly *Cordyceps jakajanicola* and *Purpureocillium lilacinum*, against *Ganoderma boninense* under in vitro conditions, evaluating their eco-friendly biocontrol agents than synthetic fungicides. The findings highlight the present understanding of EPF beyond insect control to include plant disease management. Future study should focus on exploring the mechanisms through enzymes assays, metabolomic profiling, and green house or field study to validate their practical application in crop protection.

Data availability

The DNA sequence data generated in this study have been deposited in the GenBank database of the National Center for Biotechnology Information. The internal transcribed spacer (ITS) sequences were submitted under the accession numbers PZ161636 (BORH(F)03653), PZ161637 (BORH(F)03654), PZ161638 (BORH(F)03655), PZ161639 (BORH(F)03656), PZ196262 (BORH(F)03640), PZ196263 (BORH(F)03641), PZ161642 (BORH4448), PZ161643 (BORH4449), PZ161644 (BORH4450), PZ161645 (BORH4451), PZ161646 (BORH4452), and PZ161647 (BORH4453). The translation elongation factor 1-alpha (TEF1-α) sequences were deposited under the accession numbers PX504899 (BORH(F)03653), PX504900 (BORH(F)03654), PX504901 (BORH(F)03655), PX504902 (BORH(F)03656), PX233240 (BORH(F)03640), and PX233241 (BORH(F)03641).

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Author contributions

Muhammad Shahbaz and Jaya Seelan Sathiya Seelan : Sample collection, laboratory experiments (isolation, DNA extraction, sequencing), data curation, formal analysis, and writing—original draft preparation. Syahril Abdullah : Assistance in sample collection, morphological identification, data analysis, and visualization of *Ganoderma boninense*. Jaya Seelan Sathiya Seelan : Principal Investigator, overall project supervision, funding acquisition, resources, and writing—review & editing. Khim Phin Chong and Kishneth Palaniveloo : formal analysis, visualization, writing—review & editing.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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