Scientific Reports

https://doi.org/10.1038/s41598-026-46458-1

Article in Press

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

Received: 25 October 2025

Accepted: 26 March 2026

Published online: 04 April 2026

Cite this article as: Shahbaz M., Chong K.P., Abdullah S. *et al.* Entomopathogenic fungi as potential biological control agents against basal stem rot in oil palm plantations of Sabah, Malaysia. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-46458-1

Muhammad Shahbaz, Khim Phin Chong, Syahriel Abdullah, Kishneth Palaniveloo & Jaya Seelan Sathiya Seelan

Entomopathogenic Fungi as Potential Biological Control Agents Against Basal Stem Rot in Oil Palm Plantations of Sabah, Malaysia

Muhammad Shahbaz[1], Khim Phin Chong[2], Syahriel Abdullah[1, 5], Kishneth Palaniveloo[3, 4], Jaya Seelan Sathiya Seelan[1, *]

[1]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400, Jalan UMS, Kota Kinabalu, Sabah, Malaysia

[2]Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400, Jalan UMS, Kota Kinabalu, Sabah, Malaysia

[3]Institute of Ocean and Earth Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[4]Centre for Natural Product Research and Drug Discovery (CENAR), Level 3, Research Management & Innovation Complex, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[5]Borneo Research on Algesia, Inflammation and Neurodegeneration (BRAIN) Group, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Malaysia

[*]Corresponding author: seelan80@ums.edu.my (J.S.S.S)

Abstract

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 oof 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 calendulinus*, *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 µL/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 – 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 76.02–79.76% at 300 µL/mL and 67.86–75.57% at 200 µL/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.

Key words: antagonistic activity, dual culture, growth inhibition, pathogen and sustainable agriculture.

Introduction

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 billionr in Malaysia alon [4,5]. The pathogenicity of Ganoderma spp. is associated with the secretion of lignocellulolytic enzymes, including ligninases, cellulases, proteases, laccases, as well as bioactive metabolities 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 temperature, humidity, and soil characteristics, which enhance pathogen survival and infection efficiency [8]. 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 [11-13].

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 [9, 10,14].

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 pathogens [15]. 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 activities [18]. 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 assay [20].

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 to 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 processing [21]. 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 growth [22].

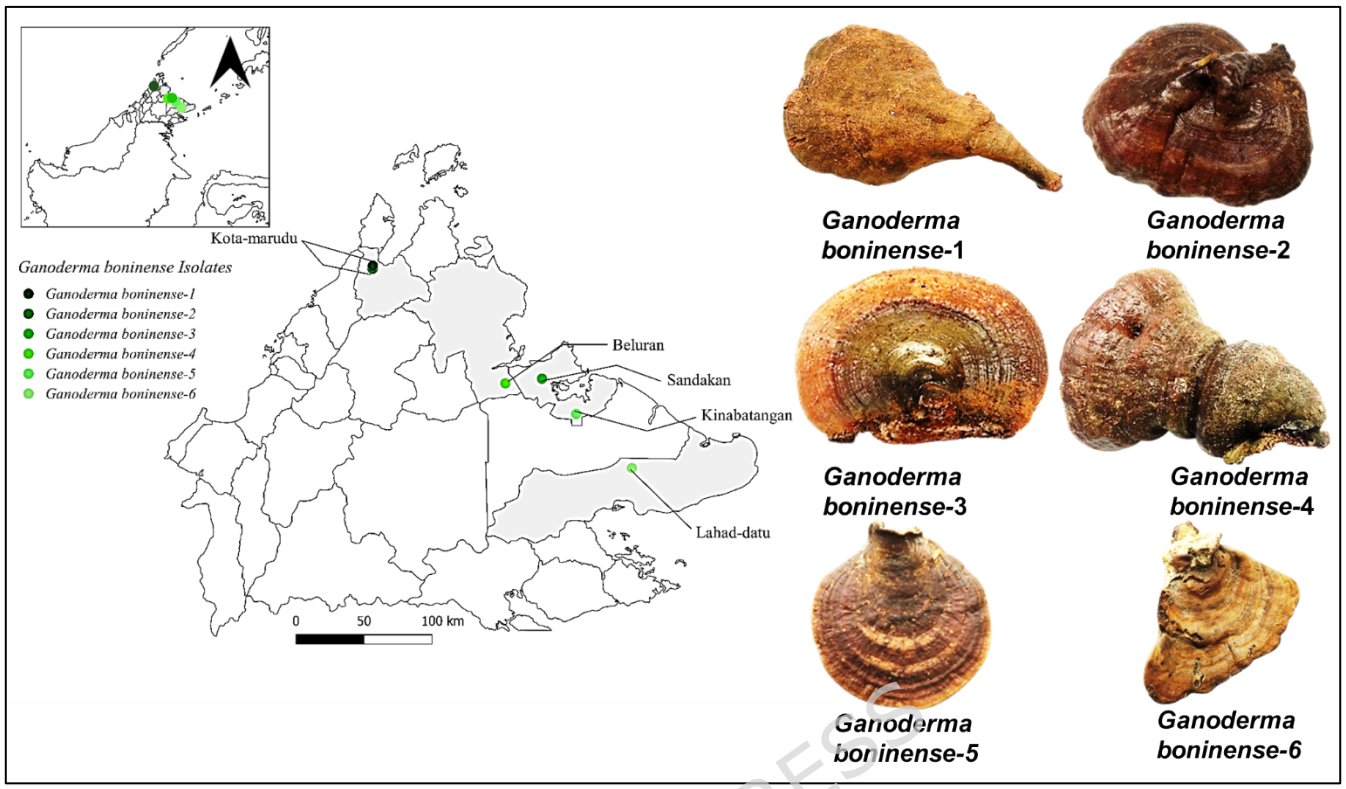


Fig. 1. *Ganoderma* fruiting bodies collected from different areas of oil palm plantations in Sabah, Malaysia. Kota Marudu = *Ganoderma boninense*-1 and *Ganoderma boninense* -2; Beluran = *Ganoderma boninense*-3; Sandakan = *Ganoderma boninense*-4; Kinabatangan = *Ganoderma boninense*-5; Lahad Datu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

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 mins 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 microscope [24].

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.

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 (S1Table 1 and 2) were used to construct phylogenetic tree based on parameters as proposed by Bich et al. [27] and Zhang et al. [29]. Using RAxML-HPC2 on ACCESS v. 8.2.12, 1000 nonparametric bootstrap iterations were used to conduct Maximum Likelihood (ML) analysis [30]. 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 0.725 [31]. Finally, XSEDE v. 3.2.7a was used to conduct Bayesian Inference (BI) analysis using MrBayes. Five million generations of four Markov chains were executed, with a tree sample taken every 1000 generations. Bootstrap values (BS) of ≥70% and posterior probabilities (PP) of ≥90% were considered to indicate significant support. Phylogenetic trees were generated using FigTree v1.4.4 and edited with Adobe Illustrator v27.6.1 [33].

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 days [34]. Nystatin oral suspension (100,000 units/mL; T.O. Chemicals, Thailand) was used as the positive control. The tested concentrations of 200 µL/mL (equivalent to 20,000 units/mL) and 300

µL/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 water [35]. 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

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

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 (2F&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.

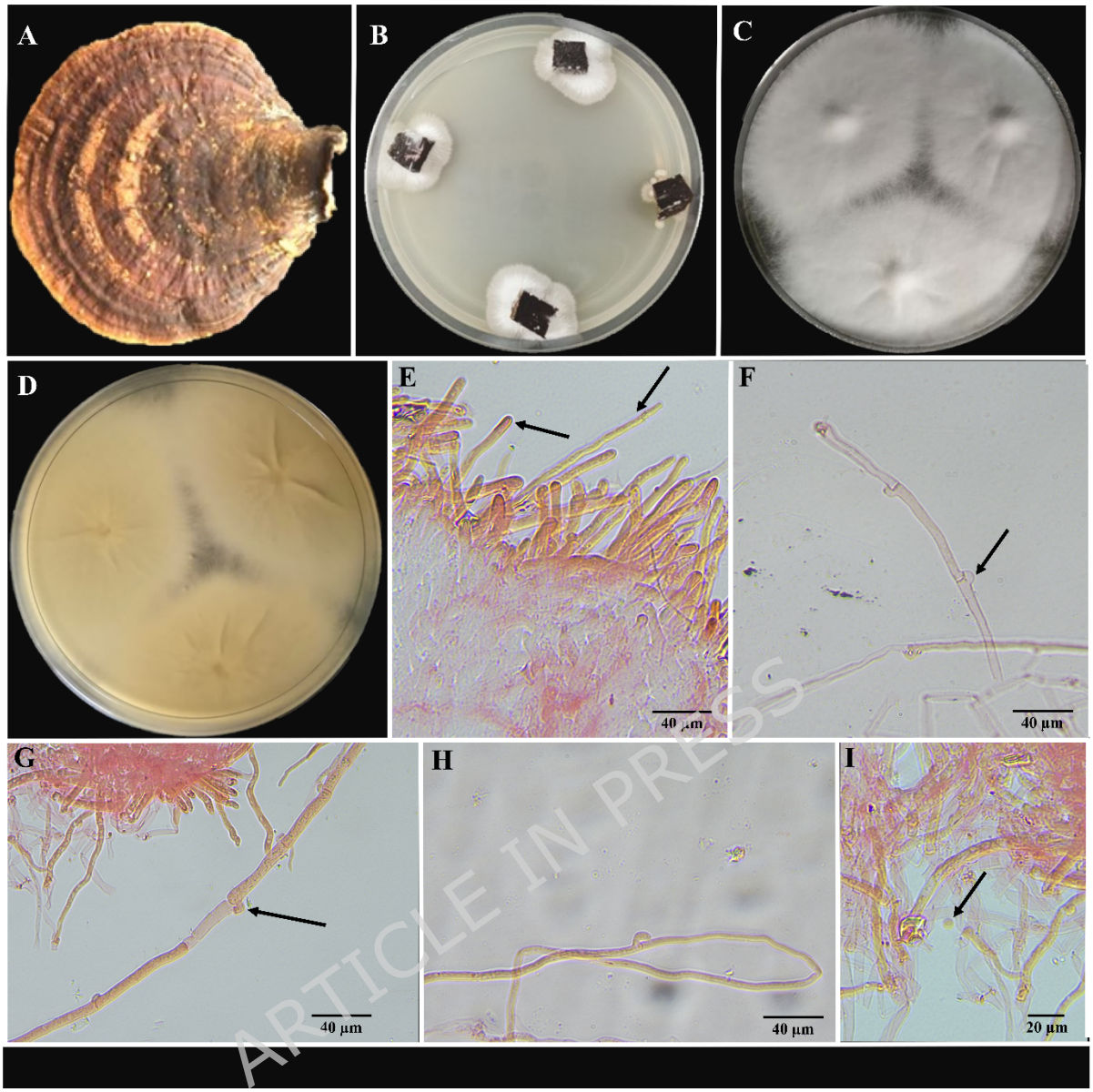


Fig. 2. Morphological identification of *Ganoderma boninense*. (A) Fresh basidiocarp of *Ganoderma boninense*, **(B)** Isolation, **(C)** Front Colony, **(D)** Reverse colony (Cells of crust hymeniderm and hyphae, **(F-H)** Hyphae showing clamp connection and **(I)** Basidiospores.

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).

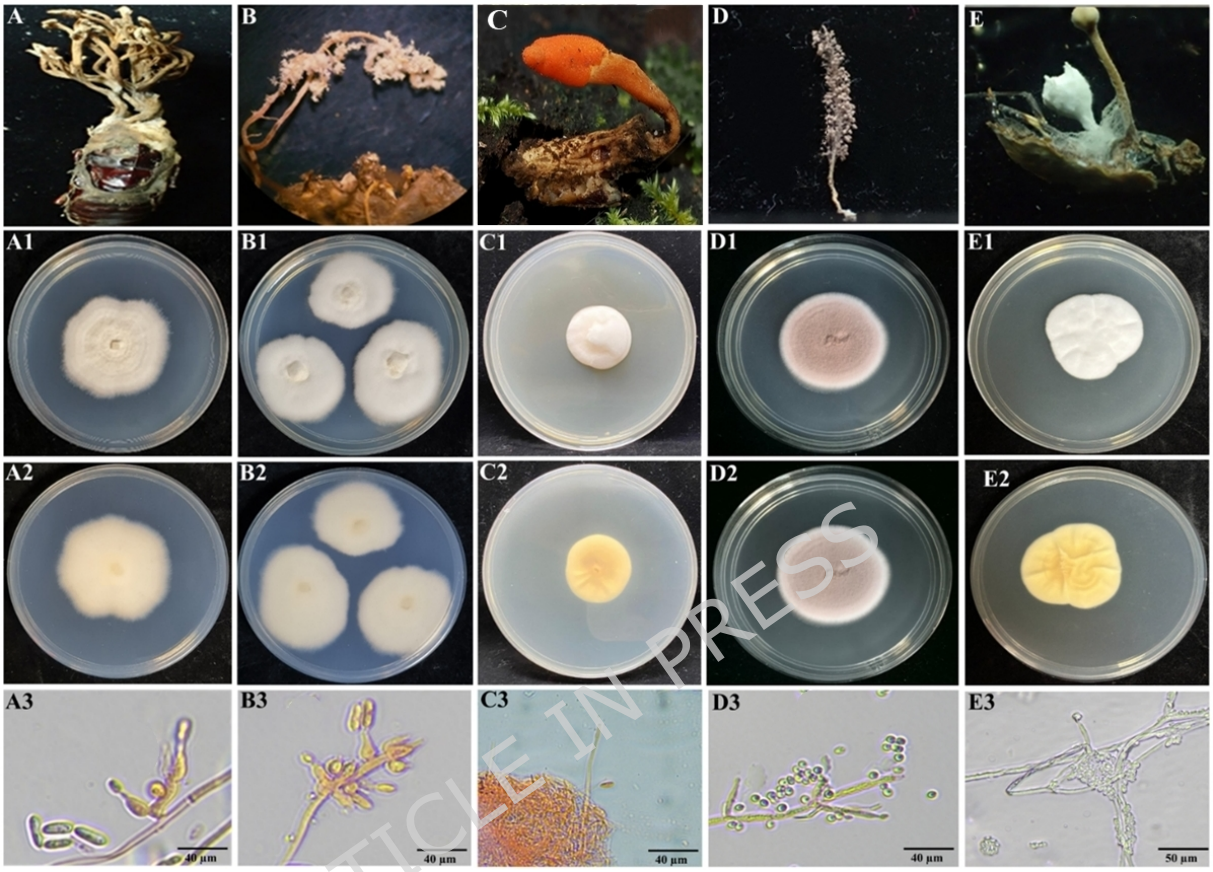
Cordyceps jakajanicola was obtained from naturally infected cicada that exhibited stromata emerging from host body (Fig. 3A). The culture front colony appeared as dense, cottony, white with a concentric ring while cream to pale-yellow pigmentation was observed in reverse colony (Fig. 3A1 & A2). Phialides 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. 3 E1 & E2). The phialides are simple, unbranched, and tapering structures while conidia are



elliptical, single-celled, formed in slimy heads (Fig. 3E3).

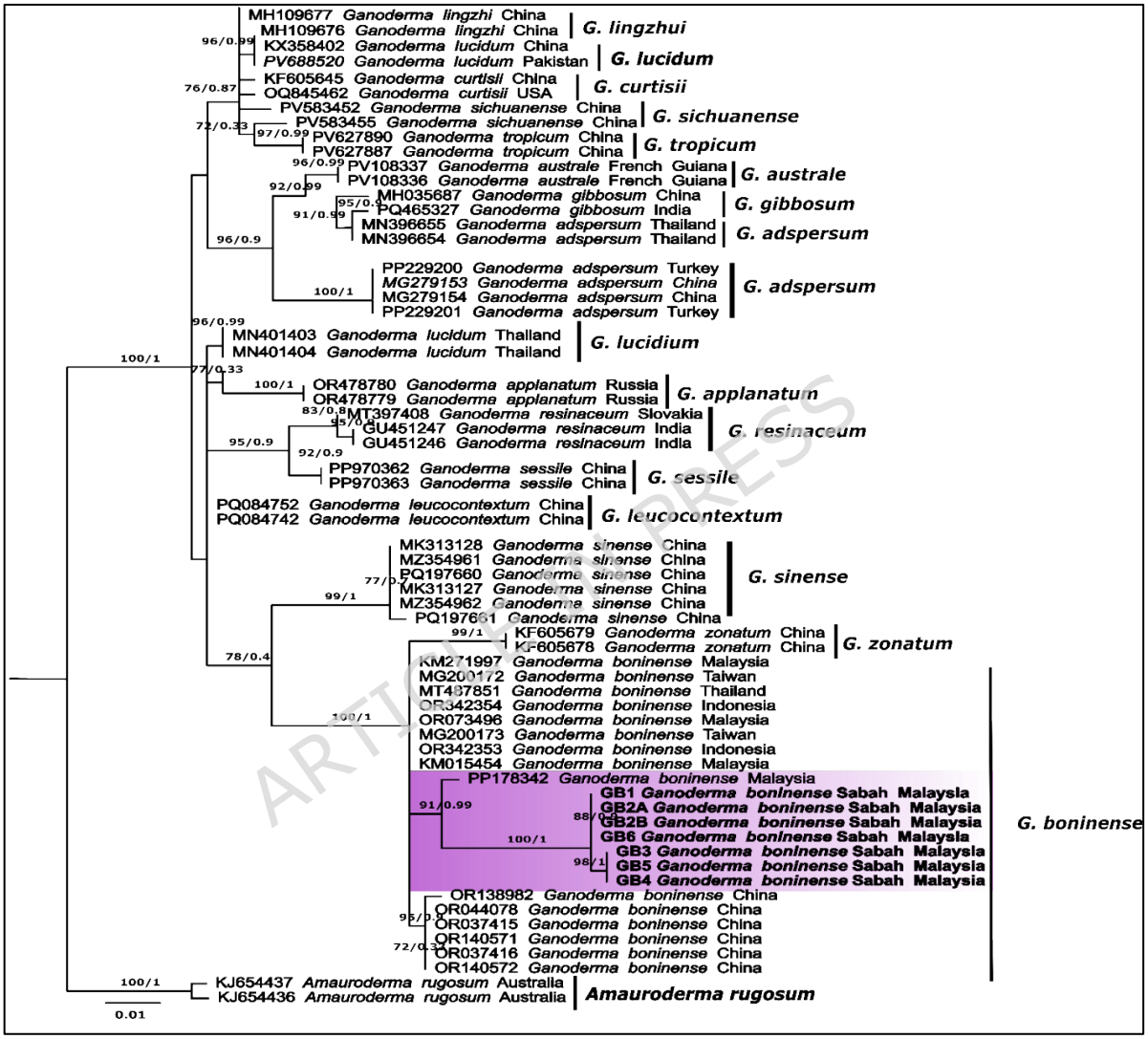
Fig. 3. Morphological description of Entomopathogenic fungi. **(A)** *Cordyceps jakajanicola* infected cicada, **(A1)** *C. jakajanicola* culture front colony on PDA, **(A2)** Culture reverse colony, **(A3)** Phialides and conidia. **(B)** *C. tenuipes* infected pupa of Lepidoptera buried in soil, **(B1 & B2)** *C. tenuipes* culture front and reverse colony, **(B3)** Phialides and conidia**. (C)** *Blackwellomyces calendulinus* infected cocoon, **(C1 & C2)** *B. calendulinus* 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.

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: 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 sec) (Fig. 4).

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 ≥ 70%) and Bayesian



posterior probabilities (BPP ≥ 0.70).

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* (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).

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 ≥ 70%) and Bayesian posterior probabilities (BPP ≥ 0.70).

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 – 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 was measured as 76.02% and 77.22% when Nystatin (300 µL/mL) applied. Similarly, moderate inhibition of 57.89% and 66.46% was recorded when *C. jakajanicola* was tested (Fig. 7).

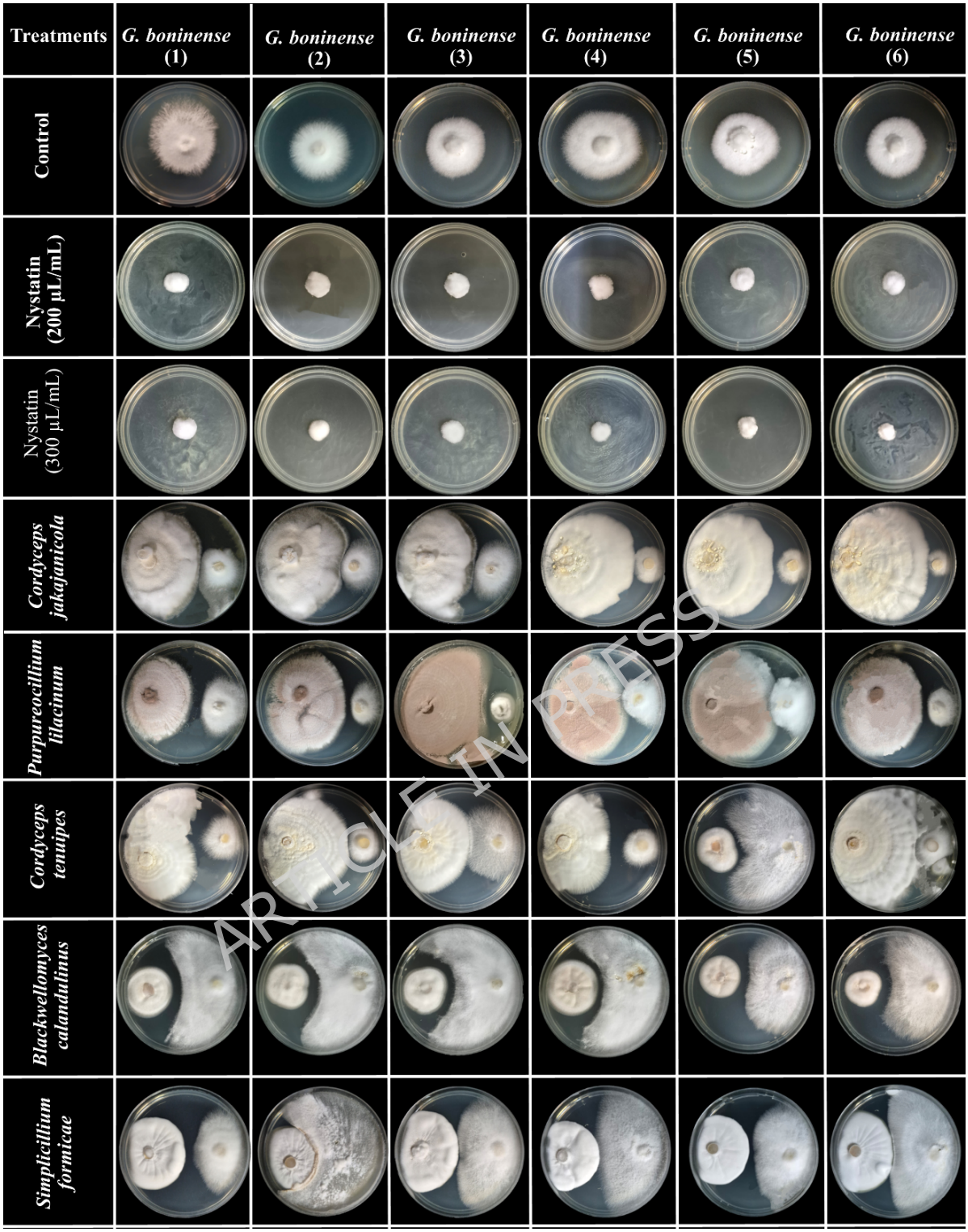


Fig. 6. Dual culture assay was performed to evaluate the antagonistic activity of entomopathogenic fungi (*Cordyceps jakajanicola* (BORH(F)03653), *Cordyceps tenuipes* (BORH(F)03654), *Blackwellomyces calendulinus* (BORH(F)03655), *Purpureocillium lilacinum* (BORH(F)03656) and two isolates of *Simplicillium 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.

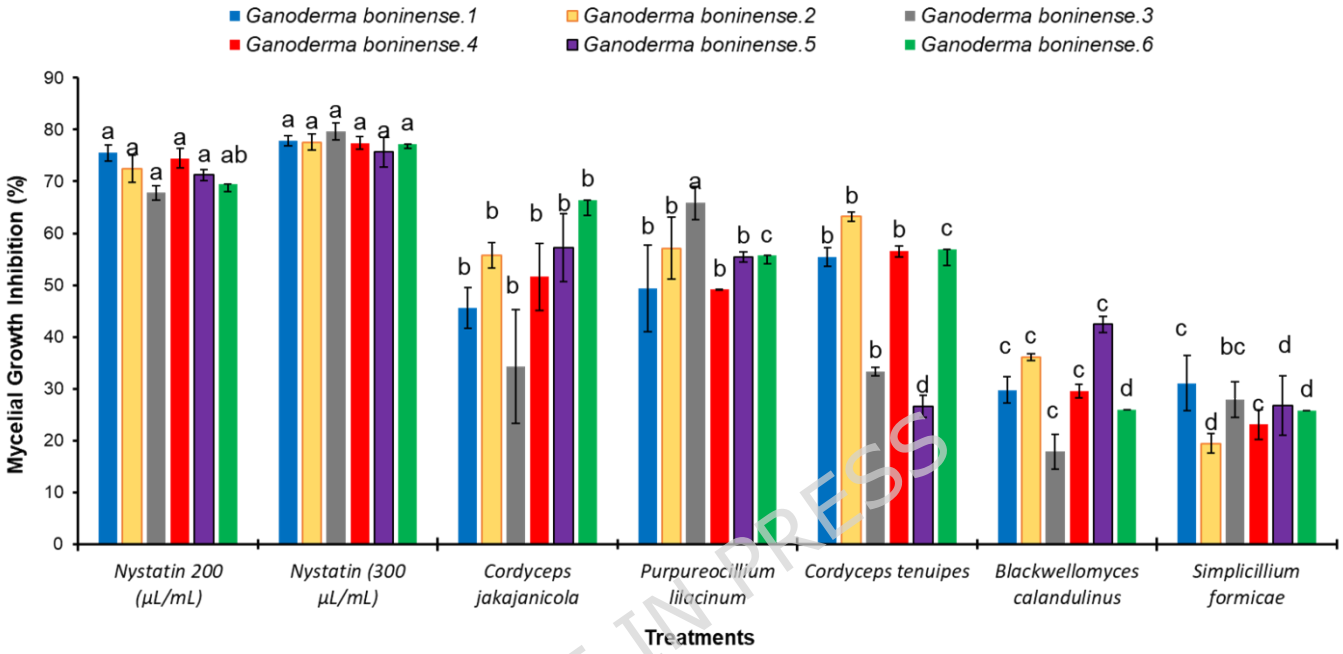


Fig. 7. Antagonistic activities of Entomopathogenic fungi against six isolates of *Ganoderma boninense* collected from different location in Sabah. Different letters in the figure show significant difference among the treatment. (Mean ± standard error, n=3).

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).

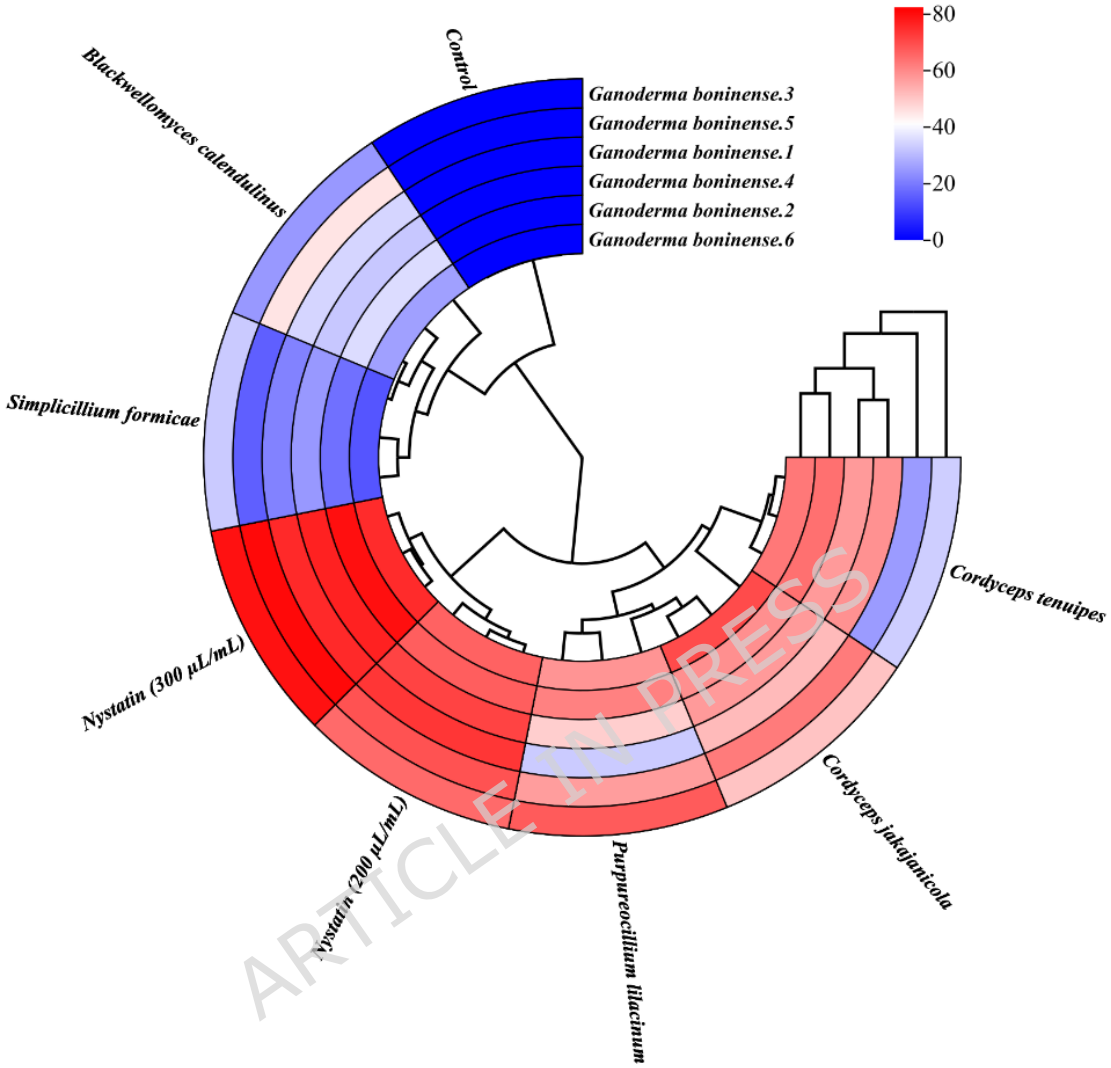


Fig. 8. The heatmap illustrates the inhibitory effects of various treatments against *Ganoderma boninense* isolates, with colour intensity representing measured inhibition values ranging from 0 to -80. Red tones signify stronger inhibition or less negative values, while blue indicates weaker inhibition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

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 (Fig. 9). Applications of Nystatin at concentrations of 200 µL /mL and 300 µL /mL caused shrinkage, slight deformation and anomalies, including surface roughening, fragmentation, and collapse respectively. Structural damages such 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 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 vitro [41]. 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 phytopathogens [46]. Neverthless, 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 growth [46]. 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 supress *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 conditions [49]. 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* [50]. *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* [51]. Similarly, *Beauveria bassiana* crude extract inhibited plant pathogenic fungi, including *Alternaria solani*, *Glomerella cingulata*, *Rhizopus oryzae*, *Chrysoporium tropicum*, *Rhizoctonia solani*, *Myrothecium roridum*, and *Fusarium oxysporum* [52].

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 [47,48]. The comparable in vitro inhibition of *G. boninense* by *Trichoderma harzianum* reported previously [53] 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 supress *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 microscopy [58], supporting the hypothesis that differential metabolities 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 efficiency [60]. Importantly, EPF are generally biocompatible and pose lower ecological risks to non-target organisms, though ecological risk assessment remains essential prior to large-scale application [61]. 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.

Acknowledgements

The authors are thankful for the Postgraduate Research Grant (UMSGREAT), LPK2413 and LPK2501 grants to support during the field and laboratory work. The authors would like to extend their gratitude of Sabah Biodiversity Conservation (SaBC) for issuing permit (JKM/MBS.1000-2/2 JLD. 20 (146) and (JKM/MBS.1000-2/2 JLD.20 (221) for fungi collections. We are also appreciating the support of the Sabah Forestry Department and Yayasan Sabah.

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. **Syahriel 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.

Funding The research was supported by grants UMSGREATE, LPK2413, and LPK2501. No additional funding was received for the preparation of this manuscript.

Declarations

Competing interests the authors state no competing interests.

Conflict of interest the authors do not have any conflict of interest.

Ethical approval current research work does not require any ethical approval.

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), PZ161640 (BORH(F)03640), PZ161641 (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).

Additional information

Supplementary information: Supplementary Fig. S1: The scanning electron microscopy (SEM) images demonstrate the morphological alterations in the hyphal structure of six G. boninense isolates (l–6) following treatment with various antifungal agents and biocontrol fungi, compared to the untreated control.

References

1. Afifi EH, Martin JJJ, Wang Q, Li X, Liu X, Zhou L, et al. Fatty acid and lipid metabolism in oil palm: from biochemistry to molecular mechanisms. *Int J Mol Sci.* **26**(6):2531 (2025).
2. Rahman MH, Naito D, Moeliono M, Mitani Y, Susaeta AI. Oil palm- and rubber-driven deforestation in Indonesia and Malaysia (2000–2021) and efforts toward zero deforestation commitments. *Agrofor Syst.* **99**(1):20 (2025).
3. Che Hussian CHA, Seman-Kamarulzaman AF, Othman NW, Nor Muhammad NA, Jalinas J, Zainal Abidin CMR, et al. Global research trends in oil palm pests and their potential threat: a bibliometric analysis. *Int J Pest Manag.* 1–22 (2025).

4. Baharim MSA, Adnan NA, Izzuddin MA, Laurence AL, Karsimen MK, Arof H. Modelling water use efficiency (WUE) for estimating the severity of *Ganoderma boninense*-derived basal stem rot disease in oil palm. *J Plant Pathol.* 1–14 (2024).
5. Olaniyi ON, Szulczyk KR. Estimating the economic damage and treatment cost of basal stem rot striking the Malaysian oil palms. *For Policy Econ.* **116**:102163 (2020).
6. Ramzi AB, Me MLC, Ruslan US, Baharum SN, Muhammad NAN. Insight into plant cell wall degradation and pathogenesis of *Ganoderma boninense* via comparative genome analysis. *PeerJ.* **7**:e8065 (2019).
7. Alexander A, Dayou J, Abdullah S, Phin CK. Differential expression and profile of oil palm root-sterols composition related to *Ganoderma boninense* infection. *ASM Sci J.* **12**(13):40–47 (2019).
8. Jazuli NA, Kamu A, Chong KP, Gabda D, Hassan A, Abu Seman I, et al. A review of factors affecting *Ganoderma* basal stem rot disease progress in oil palm. *Plants.* **11**(19):2462 (2022).
9. Anothai J, Chairin T. Development of integrated factor modeling: inhibiting *Ganoderma* lignocellulosic enzymes while promoting *Trichoderma* sporulation for enhanced plant disease control. *Physiol Mol Plant Pathol.* **133**:102382 (2024).
10. Abdul Majid N, Ramli Z, Md Sum S, Awang AH. Sustainable palm oil certification scheme frameworks and impacts: a systematic literature review. *Sustainability.* **13**(6):3263 (2021).
11. Asmawi AA, Adam F, Azman NAM, Rahman MBA. Advancements in the nanodelivery of azole-based fungicides to control oil palm pathogenic fungi. *Heliyon.* **10**(18) (2024).
12. Karunarathna SC, Patabendige NM, Lu W, Asad S, Hapuarachchi KK. An in-depth study of phytopathogenic *Ganoderma*: pathogenicity, advanced detection techniques, control strategies, and sustainable management. *J Fungi.* **10**(6):414 (2024).
13. Islam T, Danishuddin, Tamanna NT, Matin MN, Barai HR, Haque MA. Resistance mechanisms of plant pathogenic fungi to

fungicide, environmental impacts of fungicides, and sustainable solutions. *Plants.* **13**(19):2737 (2024).

14. Zakaria L. Basal stem rot of oil palm: the pathogen, disease incidence, and control methods. *Plant Dis.* **107**(3):603–615 (2023).
15. Singh KA, Nangkar I, Landge A, Rana M, Srivastava S. Entomopathogens and their role in insect pest management. *J Biol Control.* **38**(1) (2024).
16. Shahbaz M, Palaniveloo K, Tan YS, Palasuberniam P, Ilyas N, Wiart C, et al. Entomopathogenic fungi in crops protection with an emphasis on bioactive metabolites and biological activities. *World J Microbiol Biotechnol.* **40**(7):217 (2024).
17. Ghorui M, Chowdhury S, Burla S. The science behind entomopathogenic fungi: mechanisms and applications. In: *Entomopathogenic Fungi: Prospects and Challenges.* Singapore: Springer Nature (2024):**3**–35.
18. Kim, J. J., Goettel, M. S., & Gillespie, D. R. (2007). Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Biological Control*, **40**(3), 327-332.
19. Saidi, A., Mebdoua, S., Mecelem, D., Al-Hoshani, N., Sadrati, N., Boufahja, F., & Bendif, H. (2023). Dual biocontrol potential of the entomopathogenic fungus *Akanthomyces muscarius* against *Thaumetopoea pityocampa* and plant pathogenic fungi. *Saudi Journal of Biological Sciences*, **30**(8), 103719.
20. Yurnaliza, Y., Aryantha, I. N. P., Esyanti, R. R., & Susanto, A. (2014). Antagonistic activity assessment of fungal endophytes from oil palm tissues against *Ganoderma boninense* Pat. 257-267.
21. Aliyu TH. The effect of sodium hypochlorite and ethanol as seed sterilants on cowpea infected with cowpea mottle virus. *Niger J Pure Appl Sci.* **31**:3122–3127 (2018).
22. Santiago KAA, Wong WC, Goh YK, Tey SH, Ting ASY. Pathogenicity of monokaryotic and dikaryotic mycelia of *Ganoderma*

boninense revealed via LC–MS-based metabolomics. *Sci Rep.* **14**(1):5330 (2024).

23. Vivekanandhan P, Bedini S, Shivakumar MS. Isolation and identification of entomopathogenic fungus from Eastern Ghats of South Indian Forest soil and their efficacy as biopesticide for mosquito control. *Parasitol Int.* **76**:102099 (2020).
24. Chen W, Hu Q. Secondary metabolites of *Purpureocillium lilacinum*. *Molecules.* **27**(1):18 (2021).
25. Garibaldi A, Bertetti D, Martino I, Gullino ML. First report of *Botrytis* blight caused by *Botrytis cinerea* on *Plectranthus scutellarioides* in Italy. *Plant Dis.* **108**(3):805 (2024).
26. Badaluddin NA, Jamaluddin SNT, Ihsam NS, Sajili MH, Khalit SI, Mohamed NA. Molecular identification of isolated fungi from Kelantan and Terengganu using internal transcribed spacer (ITS) region. *J Agrobiotechnol.* **9**(1S):222–231 (2018).
27. Bich GA, Castrillo ML, Kramer FL, Villalba LL, Zapata PD. Morphological and molecular identification of entomopathogenic fungi from agricultural and forestry crops. *Floresta e Ambiente.* **28**(2):e20180086 (2021).
28. Lu Q, Wang P, Ali A, Zang LS. Molecular identification and virulence of four strains of entomopathogenic fungi against *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J Econ Entomol.* **115**(3):731–738 (2022).
29. Zhang L, Fasoyin OE, Molnár I, Xu Y. Secondary metabolites from hypocrealean entomopathogenic fungi: novel bioactive compounds. *Nat Prod Rep.* **37**(9):1181–1206 (2020).
30. Gu L, Su T, An MT, Hu GX. The complete chloroplast genome of *Oreocharis esquirolii* (Gesneriaceae): structural features, comparative and phylogenetic analysis. *Plants.* **9**(12):1692 (2020).
31. Cheak CC, Shin TY, Sathiya Seelan JS. Discovery of a rare medicinal mushroom, *Lignosus hainanensis* (Polyporaceae, Basidiomycota) in Sabah (Northern Borneo), Malaysia. *Malayan Nat J.* **76**(3) (2024).

32. Wang J, Chen F, Xiao X, Yang X, Xia W. A comprehensive protocol for Bayesian phylogenetic analysis using MrBayes: from sequence alignment to model selection and phylogenetic inference. *Bio-protocol.* **15**(8):e5276 (2025).
33. Naidu Y, Idris AS, Madihah AZ, Kamarudin N. In vitro antagonistic interactions between endophytic basidiomycetes of oil palm (*Elaeis guineensis*) and *Ganoderma boninense*. *J Phytopathol.* **164**(10):779–790 (2016).
34. Slamenova D, Horvathova E. Cytotoxic, anti-carcinogenic and antioxidant properties of the most frequent plant volatiles. *Neoplasma.* **60**(1):343–354 (2013).
35. Venkatesh Babu G, Perumal P, Muthu S, Pichai S, Sankar Narayan K, Malairaj S. Enhanced method for high spatial resolution surface imaging and analysis of fungal spores using scanning electron microscopy. *Sci Rep.* **8**(1):16278 (2018).
36. He D, Fu C, Xue Z. Optimization study of direct morphology observation by cold field emission SEM without gold coating. *Micron.* **109**:53–57 (2018).
37. Khoo YW, Chong KP. *Ganoderma boninense*: general characteristics of pathogenicity and methods of control. *Front Plant Sci.* **14**:1156869 (2023).
38. Fadly F, Safni I, Lubis K. The potency of antagonistic microbes as plant growth-promoting on oil palm seedling infected with basal stem rot disease (*Ganoderma boninense*). In: *IOP Conf Ser Earth Environ Sci.* **782**(4):042060 (2021).
39. Raza MU, Abasi F, Shahbaz M, Ehsan M, Seerat W, Akram A, et al. Phytomediated silver nanoparticles (AgNPs) embellish antioxidant defense system, ameliorating HLB-diseased ‘Kinnow’ Mandarin plants. *Molecules.* **28**(5):2044 (2023).
40. Sharma R, Sharma P. Fungal entomopathogens: a systematic review. *Egypt J Biol Pest Control.* **31**(1):57 (2021).
41. Pavic, A., Radakovic, N., Moric, I., Stankovic, N., Opsenica, D., & Senerovic, L. (2024). Long-chain 4-aminoquinolines inhibit

filamentation and increase efficacy of nystatin against *Candida albicans* infections in vivo. *npj Biofilms and Microbiomes*, **10**(1), 146.

42. van den Bosch, F., & Gilligan, C. A. (2008). Models of fungicide resistance dynamics. *Annu. Rev. Phytopathol.*, **46**(1), 123-147.
43. Yin, Y., Miao, J., Shao, W., Liu, X., Zhao, Y., & Ma, Z. (2023). Fungicide resistance: Progress in understanding mechanism, monitoring, and management. *Phytopathology®*, **113**(4), 707-718.
44. Sharma A, Sharma S, Yadav PK. Entomopathogenic fungi and their relevance in sustainable agriculture: a review. *Cogent Food Agric.* **9**(1):2180857 (2023).
45. Litwin A, Nowak M, Różalska S. Entomopathogenic fungi: unconventional applications. *Rev Environ Sci Biotechnol.* **19**(1):23–42 (2020).
46. Alexander A, Dayou J, Abdullah S, Chong KP. The changes of oil palm roots cell wall lipids during pathogenesis of *Ganoderma boninense*. In: *IOP Conf Ser Earth Environ Sci.* **77**(1):012014 (2017).
47. Archer L, Crane JH, Albrecht U. Trunk injection as a tool to deliver plant protection materials—an overview of basic principles and practical considerations. *Horticulturae.* **8**(6):552 (2022).
48. Zakaria L. Basal stem rot of oil palm: the pathogen, disease incidence, and control methods. *Plant Dis.* **107**(3):603–615 (2023).
49. Alexander A, Khai R, Lo S, Chong KP. The effectiveness of selected biological control agents in controlling *Ganoderma boninense*. *J Sustain Sci Manag.* **16**:128–137 (2021).
50. Darlis, D., Jalloh, M. B., Chin, C. F. S., Basri, N. K. M., Besar, N. A., Ahmad, K., & Rakib, M. R. M. (2023). Exploring the potential of Bornean polypore fungi as biological control agents against pathogenic *Ganoderma boninense* causing basal stem rot in oil palm. *Scientific Reports*, **13**(1), 10316.
51. Ravindran, K., Chitra, S., Wilson, A., & Sivaramakrishnan, S. (2014). Evaluation of antifungal activity of *Metarhizium anisopliae* against plant phytopathogenic fungi. In *Microbial diversity and*

biotechnology in food security (pp. 251-255). New Delhi: Springer India.

52. Parine, N. R., Devinder Kumar, D. K., Khan, P. A. A., & Varaprasad Bobbarala, V. B. (2010). Antifungal efficacy of secondary metabolites from entomopathogenic fungi *Beauveria bassiana*.Cabidigitallibrary.org 855-856.

53. Siddiquee S, Yusuf UK, Hossain K, Jahan S. In vitro studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense*. *J Food Agric Environ.* **7**(3–4):970–976 (2009).

54. Wei DP, Wanasinghe DN, Hyde KD, Mortimer PE, Xu J, Xiao YP, et al. The genus *Simplicillium*. *MycoKeys.* **60**:69 (2019).

55. Si B, Wang H, Bai J, Zhang Y, Cao Y. Evaluating the utility of *Simplicillium lanosoniveum*, a hyperparasitic fungus of *Puccinia graminis* f. sp. *tritici*, as a biological control agent against wheat stem rust. *Pathogens.* **12**(1):22 (2022).

56. Li W, Luo T, Li J, Zhang J, Wu M, Yang L, et al. Antifungal activity and biocontrol potential of *Simplicillium lamellicola* JC-1 against multiple fungal pathogens of oilseed rape. *J Fungi.* **9**(1):57 (2022).

57. Elsherbiny EA, Taher MA, Abd El-Aziz MH, Mohamed SY. Action mechanisms and biocontrol of *Purpureocillium lilacinum* against green mould caused by *Penicillium digitatum* in orange fruit. *J Appl Microbiol.* **131**(3):1378–1390 (2021).

58. Jiang M, Chen S, Lu X, Guo H, Chen S, Yin X, et al. Integrating genomics and metabolomics for the targeted discovery of new cyclopeptides with antifungal activity from a marine-derived fungus *Beauveria felina*. *J Agric Food Chem.* **71**(25):9782–9795 (2023).

59. Ong CE, Ahmad R, Goh YK, Azizan KA, Baharum SN, Goh KJ. Growth modulation and metabolic responses of *Ganoderma boninense* to salicylic acid stress. *PLoS One.* **16**(12):e0262029 (2021).

60. Ramli NR, Mohamed MS, Seman IA, Zairun MA, Mohamad N. The potential of endophytic bacteria as a biological control agent for *Ganoderma* disease in oil palm. *Sains Malaysiana.* **45**(3):401–409 (2016).

61. Reinbacher L, Bacher S, Praprotnik E, Grabenweger G. Standard non-target tests for risk assessment of plant protection products are unsuitable for entomopathogenic fungi—a proposal for a new protocol. *J Soils Sediments.* **21**(6):2357–2368 (2021).