Indian Journal of Experimental Biology
Vol. 64, July 2026, pp.641-650
DOI: 10.56042/ijeb.v64i07.25632

# Antibacterial activity of entomopathogenic fungi isolated from *Apis dorsata* (Giant Honeybee) and *Vespa affinis* (Lesser Banded Hornet) in Sri Lanka

Halpelage Pavani Sandeepani & Pamoda Bhasini Ratnaweera*

Department of Science and Technology, Faculty of Applied Sciences, Uva Wellassa University, Badulla 90000 Sri Lanka

*Received 14 November 2025; revised 10 February 2026*

The global rise in bacterial drug resistance highlights the urgent need for novel antibiotics. Entomopathogenic fungi (EPF) represent an underexplored yet promising source of antimicrobial secondary metabolites. This study reports, for the first time, the isolation of EPF from *Apis dorsata* and *Vespa affinis* in Sri Lanka and the evaluation of their crude extracts for antibacterial activity. EPF were isolated from surface-sterilized insect cadavers, cultured on PDA, and extracted with ethyl acetate. Molecular methods were used for fungal identification. Antibacterial activity of crude extracts was assessed by agar disc diffusion and bioautography against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, using methanol and gentamycin as negative and positive controls. Nine fungal isolates from *A. dorsata* and eight from *V. affinis* were obtained. The crude extract of *Talaromyces aculeatus* (BB1) from *A. dorsata* showed strong activity against *E. coli* (13 mm inhibition zone at 400 µg/disc; $P > 0.05$ vs. gentamycin) and additional activity against *S. aureus* in bioautography. *Aspergillus nomius* (VA7) inhibited *B. cereus*, while *Aspergillus niger* (BB10) was active against *S. aureus*. These findings reveal that EPF from *A. dorsata* and *V. affinis* are promising sources of novel antibacterial agents, with, particularly *T. aculeatus.*

**Keywords:** Bioautography, *Escherichia coli*, Gram-negative bacteria, Microbial drug resistance, Secondary metabolites

The emergence and global spread of drug-resistant pathogenic microorganisms have become a major public health concern[1,2]. Antimicrobial resistance in bacteria is primarily attributed to chromosomal mutations or horizontal gene transfer[3], often driven by the misuse and overuse of antibiotics[3,4]. Alarmingly, members of the Enterobacteriaceae family and *Pseudomonas* spp. have developed resistance to multiple available antibiotics[5]. This challenge is further exacerbated by the pharmaceutical industry's limited investment in novel antibiotic development, driven primarily by insufficient economic incentives, resulting in a fragile pipeline despite the early promise of the antibiotic era [6–8].

Among Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus* spp., and among Gram-negative bacteria, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterobacteriaceae*, and *Escherichia coli* are notable for their roles in antibiotic resistance[9]. The rapid emergence of such resistant strains undermines the therapeutic efficacy of existing antibiotics and poses a significant threat to modern healthcare systems[10]. Consequently, there is an urgent need for the discovery of novel antimicrobial agents.

Historically, many antibiotics, including Penicillin, Bacitracin, Erythromycin, and Gentamicin were discovered from microbial sources such as *Penicillium chrysogenum*, *Bacillus subtilis*, *Saccharopolyspora erythraea*, and *Micromonospora* spp., respectively[11,12]. These discoveries highlight the pivotal role of natural products in antibiotic development. Therefore, the exploration of untapped and diverse biological resources remains a promising strategy for identifying new antibacterial drug leads.

Entomopathogenic fungi (EPF), a diverse group of insect-associated fungal pathogens, represents an underexplored reservoir of bioactive metabolites[13]. Over 750 EPF species have been described, spanning more than 100 genera across major fungal lineages[14]. Despite their diversity, only limited studies have investigated the bioactive compounds they produce.

The present study aimed to isolate EPF from two relatively understudied insect hosts in Sri Lanka, *Apis dorsata* (giant honeybee) and *Vespa affinis* (lesser banded hornet), and to evaluate the antibacterial potential of their crude extracts. To the best of our knowledge, this is the first investigation exploring EPF associated with these insect species, with

*Correspondence:
E-mail: pamoda@uwu.ac.lk
E-mail: pamoda.b@gmail.com

molecular methods further employed to identify the isolated strains.

## Materials and Methods

### Isolation and identification of entomopathogenic fungi

Freshly deceased insect cadavers of *Apis dorsata* and *Vespa affinis* were collected from the premises of the Uva Wellassa University Library, Sri Lanka (8°10′4″E, 6°05′8″N) on 19th March 2019. Taxonomic identification of the collected specimens was confirmed by the Department of Entomology, National Museum, Colombo. The cadavers were transported to the Advanced Research Laboratory, Department of Science and Technology, Uva Wellassa University, Badulla, Sri Lanka, in sealed, sterilized polyethylene bags, and processed within 24 hours of collection.

Prior to fungal isolation, insect cadavers underwent surface sterilization. Briefly, cadavers were washed under running tap water for 5 minutes, immersed in 70% ethanol for 1 minute, followed by 5% sodium hypochlorite for 3 minutes. Finally, they were rinsed thoroughly with sterile distilled water and placed on sterilized paper towels inside a laminar flow cabinet to dry[15].

Each cadaver was homogenized in 1 mL of sterile distilled water using a sterile mortar and pestle. The homogenate was spread on Potato Dextrose Agar (PDA) plates supplemented with amoxicillin (10 mg/mL) to inhibit bacterial growth[16]. The plates were incubated at room temperature (30 °C) for several days under aseptic conditions. Emerging fungal colonies were sub-cultured repeatedly on antibiotic-free PDA plates to obtain pure cultures.

Colony morphology was documented through visual observation. Genomic DNA was extracted from pure fungal cultures using a published protocol[17]. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using the universal primers ITS1 and ITS4. Polymerase chain reaction (PCR) amplification and subsequent DNA sequencing were performed commercially. The obtained sequences were compared against the NCBI GenBank database using BLAST to identify the fungal isolates. Accession numbers were acquired from NCBI Genbank for the identified species.

### Extraction and antibacterial screening of fungal metabolites

Each purified fungal isolate was cultured on seven freshly prepared PDA plates. Following an incubation period of 3–5 weeks, depending on the growth rate of the fungus, the fungal mycelia along with the medium were cut into small fragments and extracted with 200 mL of ethyl acetate (EtOAc) for 24 hours at room temperature. The EtOAc extract was filtered through sterile cotton, and the filtrate was concentrated to dryness under reduced pressure to obtain crude fungal extracts[18].

The crude extracts were evaluated for antibacterial activity using the agar disc diffusion method at a concentration of 400 µg per disc, as per the guidelines of National Committee for Clinical Standards (NCCLS)[19]. Bioautographic analysis was also performed to assess the polarity and retention factors ($R_f$) of bioactive compounds[20]. Antibacterial assays were conducted against two Gram-positive bacterial strains, *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778), and two Gram-negative strains, *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 9027). These strains were obtained from the Microbiological Laboratory of the Industrial Technology Institute, Sri Lanka, and maintained at the Advanced Research Laboratory, Faculty of Applied Sciences, Uva Wellassa University, Badulla, Sri Lanka.

### Statistical analysis

The diameter of the inhibition zones was measured to assess antibacterial activity. Statistical comparisons of mean inhibition zones among extracts were performed using analysis of variance (ANOVA), followed by Tukey's multiple comparison test, using IBM SPSS Statistics software version 30.0.0.0 (171).

## Results

### Isolation and identification of the entomopathogenic fungi

A total of seventeen distinct entomopathogenic fungi (EPF) were isolated from freshly deceased insect cadavers of *Apis dorsata* and *Vespa affinis* (Fig. 1). Of these, nine morphologically distinct

Fig. 1 — Insect cadavers used for the isolation of entomopathogenic fungi (EPF): (a) *Apis dorsata* (giant honeybee) and (b) *Vespa affinis* (lesser banded hornet).

fungal isolates were obtained from *A. dorsata*, while eight were isolated from *V. affinis* cadavers. The purified EPF cultures derived from *A. dorsata* and *V. affinis* are presented in Fig 2 and 3, respectively, while their colony morphology and pigmentations are included in Table 1[21–30].

The isolated fungi were identified as belonging to eight genera: *Talaromyces*, *Penicillium*, *Nigrospora*,

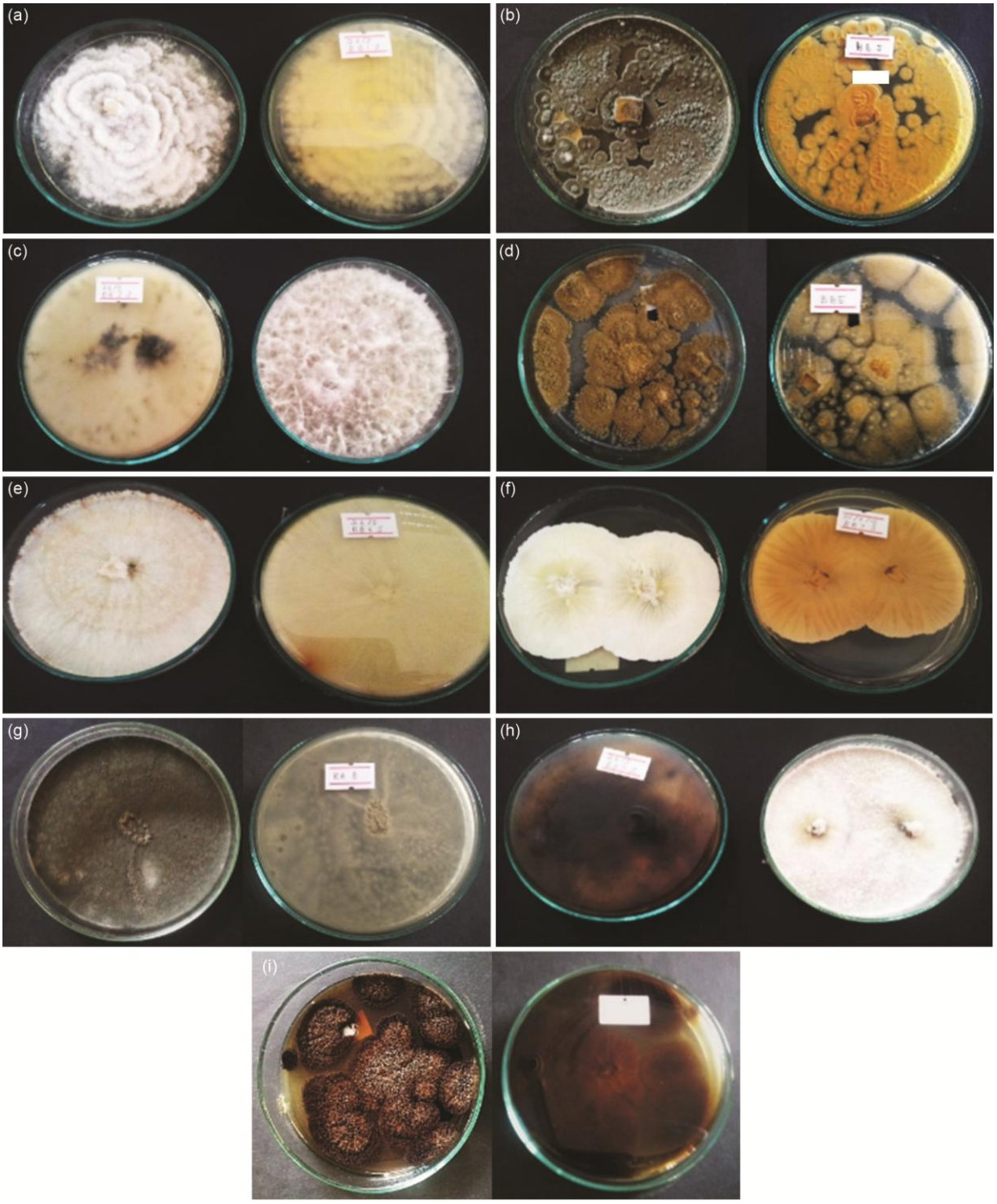


Fig. 2 — Pure cultures of EPF isolated from *Apis dorsata*: (a) *Talaromyces aculeatus* (BB1), (b) *Penicillium citrinum* (BB2), (c) *Nigrospora sacchari-officinarum* (BB3), (d) *Aspergillus flavus* (BB5), (e) *Aspergillus krugeri* (BB6), (f) *Aspergillus proliferans* (BB7), (g) *Aspergillus fumigatus* (BB8), (h) *Daldinia eschscholtzii* (BB9), and (i) *Aspergillus niger* (BB10).

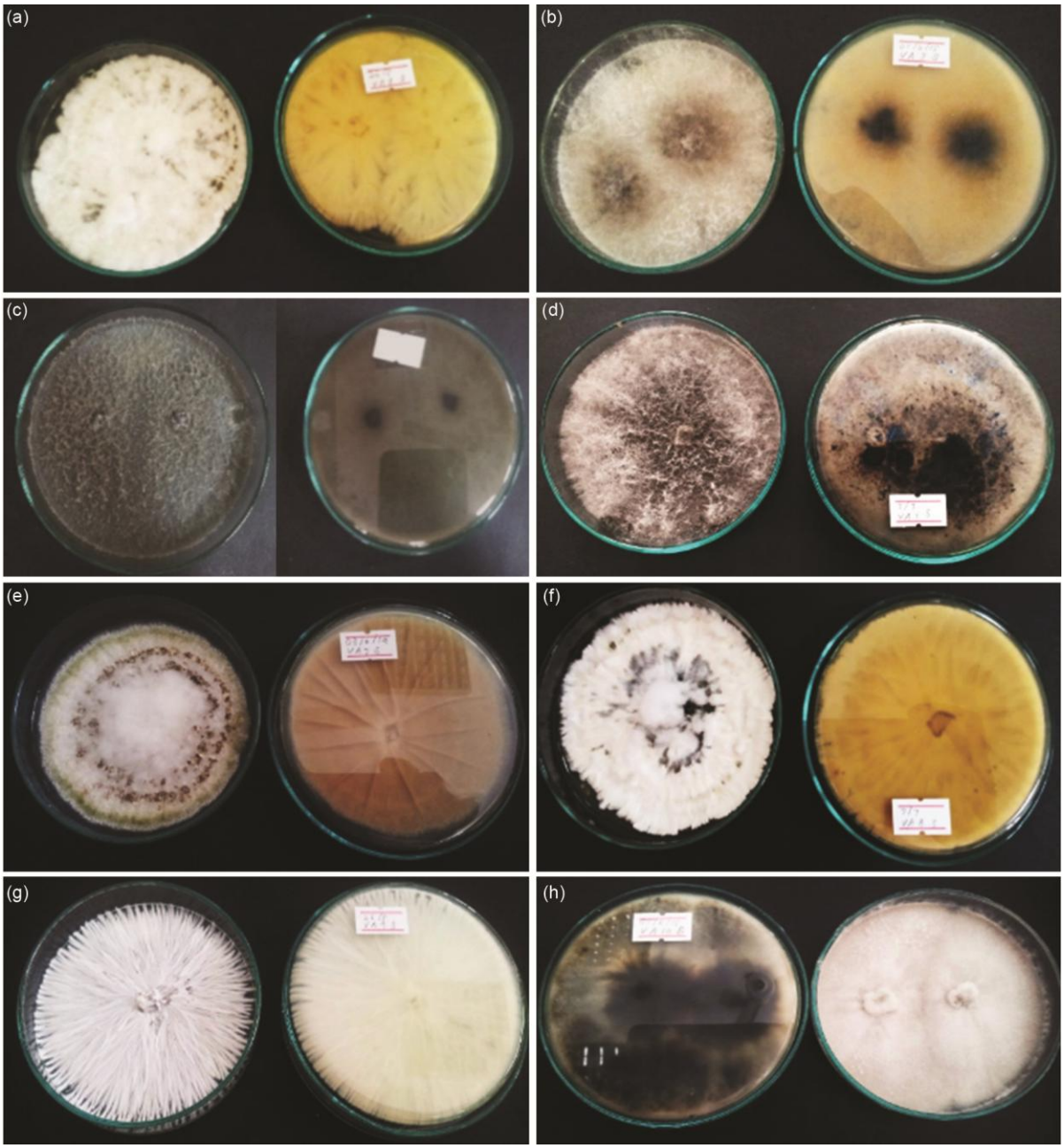


Fig. 3 — Pure cultures of EPF isolated from *Vespa affinis*: (a) *Xylaria adscendens* (VA2), (b) *Nigrospora oryzae* (VA3), (c) *Nigrospora oryzae* (VA4), (d) VA5, (e) *Aspergillus nomiae* (VA7), (f) *Pyrrhoderma noxium* (VA8), (g) VA9, and (h) *Aspergillus flavus* (VA10).

*Aspergillus*, *Daldinia*, *Xylaria*, *Panus*, and *Pyrrhoderma*. Taxonomically, *Talaromyces*, *Penicillium*, and *Aspergillus* species are classified under the class Eurotiomycetes, while *Nigrospora*, *Daldinia*, and *Xylaria* are members of the class Sordariomycetes. The genera *Panus* and *Pyrrhoderma* belong to the class Agaricomycetes.

Molecular identification of the isolates was conducted using ITS region sequencing, and the resulting sequences were deposited in the NCBI GenBank database. Fifteen fungal isolates identified using molecular data, along with their corresponding accession numbers, are listed in Table 2. Notably, the majority of isolates were assigned to the genus *Aspergillus*.

**Antibacterial activity of the isolated EPF**

The most promising antibacterial activity was exhibited by *Talaromyces aculeatus* (isolate BB1), obtained from *Apis dorsata*. Based on ITS sequence

Table 1 — Colony morphology and pigmentations of the isolated entomopathogenic fungi (EPF)

| Identification obtained from molecular data | EPF Code | Color | Colony surface | Colony margin | Pattern | Pigments |
|---|---|---|---|---|---|---|
| *Talaromyces aculeatus*[21] | BB 1 | pale white | cottony | wavy | concentric | light orange |
| *Penicillium citrinum*[22] | BB 2 | green | cottony | irregular | irregular | dark yellow |
| *Nigrospora sacchari-officinarum* | BB 3 | pale white | cottony | spreading | filamentous | black brown |
| *Aspergillus flavus*[23] | BB 5 | white with yellow spots | cottony | irregular | irregular spreading | dark yellow |
| *Aspergillus krugeri* | BB 6 | pure white | cottony | lobate | radiate | - |
| *Aspergillus proliferans* | BB 7 | white with light green centre | cottony | lobate | radiate | light yellow |
| *Aspergillus fumigatus*[24] | BB 8 | green | cottony | ciliate | irregular | green |
| *Daldinia eschscholtzii*[25] | BB 9 | white with green centre | velvety | ciliate | irregular | brown black |
| *Aspergillus niger*[26] | BB 10 | brown | velvety | radiate | radiate | brown |
| *Xylaria adscendens*[27] | VA 2 | pure white | velvety | lobate | radiate | - |
| *Nigrospora oryzae*[28] | VA 3 | whitish gray | cottony | ciliate | radiate | dark brown |
| *Nigrospora oryzae*[28] | VA 4 | gray | cottony | filamentous | radiate | black brown |
| *Unconfirmed identification* | VA 5 | ash | cottony | filamentous | radiate | black brown |
| *Aspergillus nomiae*[29] | VA 7 | pale white | cottony / sloppy | ciliate | radiate | light brown |
| *Pyrrhoderma noxium*[30] | VA 8 | pale white | cottony filamentous | lobate | radiate | black spots |
| *Unconfirmed identification* | VA 9 | pale white | cottony filamentous | sharp | radiate | - |
| *Aspergillus flavus*[23] | VA 10 | ash | cottony filamentous | ciliate | irregular | purple black |

[BB codes given for fungi isolated from *A. dorsata* and VA code given for fungi isolated from *V. affinis*]

Table 2 — Molecular identification of entomopathogenic fungi (EPF) isolates based on ITS region sequencing, along with the corresponding NCBI GenBank accession numbers

| EPF Code | Identification obtained from molecular data | Accession number |
|---|---|---|
| BB 1 | *Talaromyces aculeatus* | OR485910 |
| BB 2 | *Penicillium citrinum* | OR485911 |
| BB 3 | *Nigrospora sacchari-officinarum* | OR485912 |
| BB 5 | *Aspergillus flavus* | OR485913 |
| BB 6 | *Aspergillus krugeri* | OR485914 |
| BB 7 | *Aspergillus proliferans* | OR485915 |
| BB 8 | *Aspergillus fumigatus* | OR485916 |
| BB 9 | *Daldinia eschscholtzii* | OR485917 |
| BB 10 | *Aspergillus niger* | OR485918 |
| VA 2 | *Xylaria adscendens* | OR504181 |
| VA 3 | *Nigrospora oryzae* | OR504182 |
| VA 4 | *Nigrospora oryzae* | OR504183 |
| VA 7 | *Aspergillus nomiae* | OR504184 |
| VA 8 | *Pyrrhoderma noxium* | OR504185 |
| VA 10 | *Aspergillus flavus* | OR504186 |

[BB codes given for fungi isolated from *A. dorsata* and VA code given for fungi isolated from *V. affinis*]

analysis and BLAST results from the NCBI GenBank database, this isolate shared 99.6% sequence identity with previously reported *T. aculeatus* strains (accession numbers KJ413369.1 and OP164426.1). Morphologically, *T. aculeatus* produced white

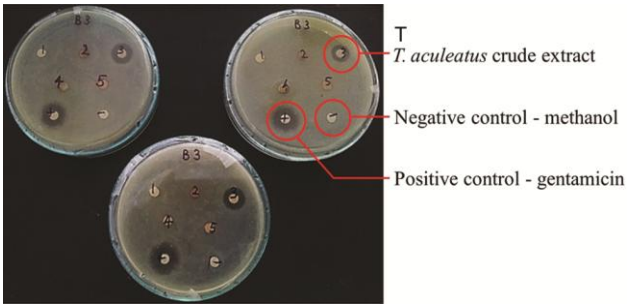


Fig. 4 — Triplicate disc diffusion agar assays showing the antibacterial activity of *Talaromyces aculeatus* (BB1) ethyl acetate crude extract against *Escherichia coli* at a concentration of 400 µg/disc.

mycelia with a concentric ring pattern and wavy margins and secreted an orange pigment into the surrounding medium within 10 - 15 days of incubation on PDA at room temperature (30 °C).

This isolate demonstrated significant antibacterial activity ($P < 0.05$) against *Escherichia coli*, with a mean inhibition zone diameter of $13.00 \pm 1.00$ mm (Fig. 4). Among all fungal crude extracts tested, ten showed activity against the Gram-positive bacterium *S. aureus*, and eight were active against *B. cereus*. Nine extracts exhibited activity against the Gram-negative bacterium *E. coli*. Notably, none of the fungal extracts showed inhibitory activity against *P. aeruginosa*. Table 3 summarizes the inhibition

Table 3 — Antibacterial activity of ethyl acetate crude extracts from entomopathogenic fungi isolated from *Apis dorsata* (EPF code: BB) and *Vespa affinis* (EPF code: VA) against selected bacterial pathogens at a concentration of 400 µg/disc

| EPF code | Fungal isolate | Fungal incubation period (days) | Mean diameter of the inhibition zone (mm±1.00mm) | | | |
|---|---|---|---|---|---|---|
| | | | *S. aureus* | *B. cereus* | *E. coli* | *P. aeruginosa* |
| BB1 | *Talaromyces aculeatus* | 16 | 8 $^{b}$ | 8 $^{b,c}$ | 13 $^{c}$ | 0 $^{a}$ |
| BB2 | *Penicillium citrinum* | 6 | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| BB3 | *Nigrospora sacchari-officinarum* | 8 | 0 $^{a}$ | 0 $^{a}$ | 7 $^{b}$ | 0 $^{a}$ |
| BB5 | *Aspergillus flavus* | 16 | 7 $^{b}$ | 6 $^{b}$ | 7 $^{b}$ | 0 $^{a}$ |
| BB6 | *Aspergillus krugeri* | 12 | 9 $^{b}$ | 7 $^{b,c}$ | 7 $^{b}$ | 0 $^{a}$ |
| BB7 | *Aspergillus proliferans* | 6 | 8 $^{b}$ | 9 $^{c}$ | 0 $^{a}$ | 0 $^{a}$ |
| BB8 | *Aspergillus fumigatus* | 8 | 0 $^{a}$ | 7 $^{b,c}$ | 6 $^{b}$ | 0 $^{a}$ |
| BB9 | *Daldinia eschscholtzii* | 8 | 7 $^{b}$ | 7 $^{b,c}$ | 7 $^{b}$ | 0 $^{a}$ |
| BB10 | *Aspergillus niger* | 6 | 9 $^{b}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA2 | *Xylaria adscendens* | 12 | 7 $^{b}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA3 | *Nigrospora oryzae* | 6 | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA4 | *Nigrospora oryzae* | 6 | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA5 | *Unconfirmed identification* | 16 | 7 $^{b}$ | 6 $^{b}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA7 | *Aspergillus nomiae* | 8 | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |
| VA8 | *Pyrrhoderma noxium* | 28 | 7 $^{b}$ | 0 $^{a}$ | 6 $^{b}$ | 0 $^{a}$ |
| VA9 | *Unconfirmed identification* | 16 | 0 $^{a}$ | 0 $^{a}$ | 6 $^{b}$ | 0 $^{a}$ |
| VA10 | *Aspergillus flavus* | 10 | 7 $^{b}$ | 6 $^{b}$ | 7 $^{b}$ | 0 $^{a}$ |
| +Ve | gentamycin, 10µg/disc | | 22.33 $^{c}$ | 20.84 $^{d}$ | 15.08 $^{c}$ | 16.11 $^{b}$ |
| -Ve | methanol | | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ | 0 $^{a}$ |

[Means that do not share the same letter are significantly different at $P < 0.05$ (Tukey's HSD)]

zone diameters for the antibacterial activity of ethyl acetate crude extracts from the isolated EPF strains, tested at 400 µg/disc against the four bacterial species. The incubation period for each fungal isolate prior to extraction is also provided in the same table.

The thin-layer chromatography (TLC) profiles developed with 100% ethyl acetate, along with the corresponding bioautography results for the EPF crude extracts, are presented in Fig 5. Based on the bioautography assay, the crude extract of *Aspergillus niger* (BB10) exhibited activity against *S. aureus*, while *A. nomiae* (VA7) was active against *B. cereus*. Notably, *T. aculeatus* (BB1) demonstrated activity against both *S. aureus* and *E. coli*. None of the other extracts showed detectable antibacterial activity in the bioautography assay. Further, according to the results of the bioautography assay (Fig. 5), the active compound in *A. niger* (BB10) was identified as a moderately non-polar compound, with a $R_f$ value around 0.8. The active compound in *A. nomiae* (VA7) was relatively more polar, with an $R_f$ value of around 0.5. In contrast, the active compound(s) in *T. aculeatus* (BB1) did not migrate with the 100% ethyl acetate solvent system, suggesting a higher polarity compared to the active metabolites from the *Aspergillus* species (BB10 and VA7).

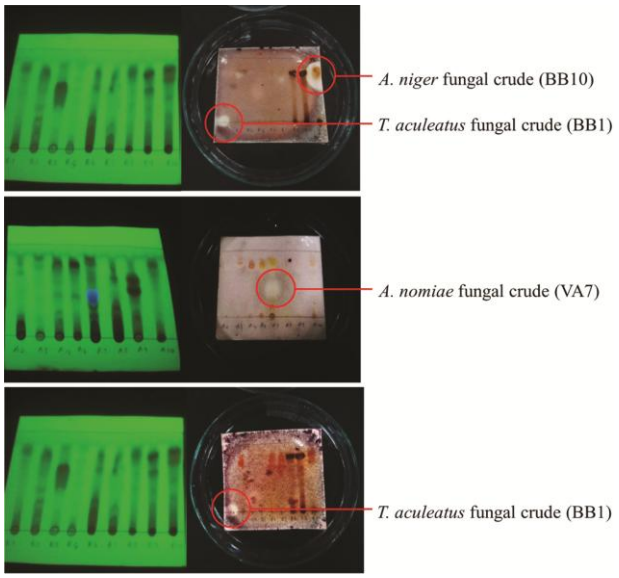


Fig. 5 — Thin-layer chromatography (TLC)-bioautography assay of ethyl acetate crude extracts from EPF isolated from *Apis dorsata and Vespa affinis*. Zones of inhibition are shown against (a) *Staphylococcus aureus*, (b) *Bacillus cereus*, and (c) *Escherichia coli*. Reference TLC plates (left) were developed using 100% ethyl acetate and visualized under UV light at 254 nm.

## Discussion

Entomopathogenic fungi (EPF) are recognized as promising sources of secondary metabolites with potential pharmaceutical applications, including the discovery of novel drug leads[31,32]. However, isolating these metabolites poses considerable challenges due to the ecological complexity of harvesting EPF from their natural environments and the difficulty of cultivating these insect-dependent fungi under laboratory conditions[33]. EPF are primarily classified within two major taxonomic groups: Hypocreales (Ascomycota) and Entomophthoromycota (Zygomycota). While Hypocreales species typically exhibit a broad host range, Entomophthoromycota are generally more host-specific. Despite these differences, both groups produce infective propagules, often as conidia or other asexual spores[33,34].

EPF are widely distributed across diverse ecosystems, thriving in various geographic and climatic conditions. They inhabit both cultivated and natural soils and are capable of infecting insect hosts at all developmental stages, including eggs, larvae, pupae, and adults[35]. In the present study, *Apis dorsata* (giant honeybee) and *Vespa affinis* (lesser banded hornet) were selected as host species for EPF isolation due to their ecological roles as pollinators and predators, their widespread availability, and frequent contact with microbe-rich environments such as plant surfaces, decaying organic matter, and other insects. Prior studies have also documented the susceptibility of bees and wasps to EPF infections[36,37]. In Thailand, *Streptomyces* spp. isolated from *A. dorsata* combs, bees, and pollen have demonstrated antibacterial activity against phytopathogenic bacteria[38]. However, the isolation of entomopathogenic fungi from *A. dorsata* and *V. affinis*, and the evaluation of their antibacterial activity against clinically relevant pathogens, highlights the novelty of the present study.

To facilitate the successful isolation of EPF, the culture medium (PDA) was supplemented with amoxicillin to inhibit bacterial contamination. This measure was critical in preventing the rapid overgrowth of bacteria, which could interfere with fungal isolation. A total of 17 distinct EPF species were successfully isolated from the cadavers of the two insect hosts. However, only 15 identities were confirmed by molecular data. Of these, five species, *Penicillium citrinum*[39], *Aspergillus flavus*[40], *A. niger*[41], *Nigrospora oryzae*[42], and *A. nomius* (syn. *A. nomiae*)[43], had previously been reported as EPF. Notably, the remaining isolates, including *Talaromyces aculeatus*, *Nigrospora sacchari-officinarum*, *Aspergillus krugeri*, *A. fumigatus*, *A. proliferans*, *Daldinia eschscholtzii*, *Xylaria adscendens*, and *Phellinus noxium*, have not previously been reported as EPF, suggesting new potential associations.

To minimize the isolation of saprophytic or environmental fungi and ensure the collection of true EPF, only freshly deceased insect cadavers were used. The cadavers were processed within 24 hours of collection and subjected to surface sterilization, a procedure designed to reduce the likelihood of isolating surface fungal invaders.

EPF are known to exhibit specific growth and nutritional requirements, and many do not sporulate well on standard artificial media. The selection of culture medium and incorporation of insect-derived components in the medium, will play a critical role in promoting the production of bioactive compounds by EPF[44]. While the current study utilized only PDA at room temperature, future studies employing multiple culture media with varying nutrient compositions may improve the isolation of additional, potentially cryptic, EPF species and promote the production of diverse bioactive compounds through the activation of silent genes.

Destruxins (DTXs) are a class of cyclic hexadepsipeptides predominantly derived from *Metarhizium* species, a well-known EPF genus. Among more than 40 known destruxins (DTX), DTXs A, B, and E are especially notable for their roles in fungal pathogenicity[32]. DTXs possess a range of bioactivities, including insecticidal, phytotoxic, antimicrobial, antiviral, antiproliferative, cytotoxic, and immunosuppressive effects[45]. Similarly, cyclic hexadepsipeptides such as bassianolide, also have been isolated from *Beauveria bassiana* and *Lecanicillium* spp. have exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria[46]. Certain entomopathogenic fungi such as *Metarhizium anisopliae* have exhibited antibacterial activity against clinically important pathogens[47]. These previous research demonstrates the importance of EPF as potential sources for biosynthesizing antibacterial secondary metabolites.

The present study focused on evaluating the antibacterial properties of EPF isolated from *A. dorsata* and *V. affinis* to assess their potential as sources of novel antimicrobial agents. Notably, the

crude extract from *T. aculeatus* exhibited significant antibacterial activity against *E. coli*, as confirmed by both agar disc diffusion and bioautography assays. Given the clinical importance of *E. coli* as a prevalent Gram-negative pathogen associated with infections across all age groups[48], and the increasing global threat posed by multidrug-resistant strains[49], this finding is particularly noteworthy. When compared with the standard antibiotic gentamicin (Fig 4), the antibacterial activity of the *T. aculeatus* extract was comparable, indicating its potential to biosynthesize a potent bioactive compound.

Previous studies have also highlighted the bioactive potential of *T. aculeatus*. For instance, the strain *T. aculeatus* DS-62013, isolated from saline soil in China, was reported to produce eleven azaphilone derivatives under various fermentation conditions, several of which exhibited antibacterial and enzyme inhibitory activities[50]. In addition, a recent investigation identified antibacterial polyketides from a deep-sea-derived *Talaromyces* species, with several compounds demonstrating potent activity against *E. coli* (minimum inhibitory concentration [MIC] 0.5 µg/mL) as well as other pathogenic microorganisms[51]. Collectively, these findings underscore the growing recognition of the *Talaromyces* genus as a prolific source of structurally diverse secondary metabolites with promising applications in drug discovery[52]. Although several studies have explored the bioactivities of *Talaromyces* species, isolates derived specifically from insects have not been reported. This distinction is important, as the biological niche can influence the biosynthesis of various bioactive secondary metabolites. Also, to the best of our knowledge, this study is the first to demonstrate the antibacterial activity of *T. aculeatus* against the Gram-negative pathogen *E. coli*.

The bioautography assays conducted in this study further demonstrated that the crude extracts of *T. aculeatus* and *A. niger* exhibited activity against Gram-positive *S. aureus*, while the extract of *A. nomiae* was active against *B. cereus*. It is also important to note that the observed activities could result from synergistic interactions among multiple compounds in the crude extracts. However, bioautography remains a valuable method for detecting novel antimicrobial compounds, including those present in minute quantities within crude mixtures[20]. This was evident in the case of the VA7 extract, which exhibited antibacterial activity only in the bioautography assay. In the present study, bioautography was used as a preliminary tool to assess the polarity and $R_f$ values of the bioactive compounds, thereby aiding in their subsequent isolation.

Song and his coworkers[53] reported the isolation of oligophenalenone dimers from *Talaromyces* sp., which exhibited inhibitory activity against *S. aureus*, with minimum inhibitory concentration (MIC) values ranging from 0.195 to 100 µg/mL. Additional literature further supports the antimicrobial potential of *A. niger* and *A. nomiae*. *A. niger* is known to produce malformins, cyclic pentapeptides with significant toxicity and broad-spectrum antibacterial activity[54]. Furthermore, *A. niger* demonstrated inhibitory activity against *S. aureus*, with an inhibition zone of 12 mm, as reported by Al-Shaibani[55]. However, reports on the antibacterial activity of *A. nomiae* are limited. Putra and his team have observed a mild inhibition (1.43 mm) of *B. cereus* by a sponge-derived *A. nomiae* isolate[56]. These findings, consistent with the results of the present study, highlight the potential of insect-associated fungi also as sources of bioactive compounds with antibacterial properties.

Interestingly, six out of the 15 identified fungal isolates identified in this study belong to the genus *Aspergillus*, a group well-known for producing a wide array of secondary metabolites with significant industrial and pharmaceutical applications[57,58].

To our knowledge, this is the first comprehensive investigation into the antibacterial properties of EPF isolated from *A. dorsata* and *V. affinis* in Sri Lanka. The findings confirm that these insect species harbor a diverse array of EPF capable of producing antibacterial compounds effective against both Gram-positive and Gram-negative bacteria. While many previously characterized EPF exhibit greater activity against Gram-positive organisms, this study demonstrates that isolates from *A. dorsata* and *V. affinis* also possess activity against Gram-negative bacteria, *E. coli*, suggesting a broader spectrum of bioactivity. Nevertheless, it is essential to acknowledge that these conclusions are based on crude fungal extracts. Further studies are necessary to isolate, purify, and structurally characterize the active compounds to fully assess their pharmacological potential. However, the current study demonstrates that EPF isolated from *A. dorsata* and *V. affinis* represent potential sources of antibacterial compounds, warranting further investigation.

## Conclusion

This study demonstrates that *Apis dorsata* and *Vespa affinis* harbor diverse entomopathogenic fungi (EPF) capable of producing antibacterial compounds with activity against both Gram-positive and Gram-negative bacteria. Among the isolates, *Talaromyces aculeatus* exhibited particularly strong activity against *Escherichia coli*, highlighting its potential as a source of novel bioactive metabolites. The findings expand current knowledge of insect-associated fungi and suggest that these fungi represent promising candidates for further chemical and pharmacological characterization aimed at developing new antimicrobial agents to address antibiotic resistance.

## Acknowledgments

The authors are grateful to Dr. Manori Gunatilake, Department of Entomology, National Museum, Colombo, Sri Lanka, for the authentication of the insect cadavers.

## Conflict of Interests

The authors have no relevant financial or non-financial interests to disclose.

## Ethical Statement

No ethical approval was required as this study did not involve human participants or laboratory animals.

## References

1 Tanwar J, Das S, Fatima Z & Hameed S. Multidrug resistance: An emerging crisis. *Interdiscip Perspect Infect Dis*, 2014 (2014) 541340.

2 World Health Organization, Antimicrobial resistance: global report on surveillance 2014. *World Health Organization*, (2014).

3 Munita JM & Arias CA. Mechanisms of antibiotic resistance. *Microbiol Spectr*, 4 (2016) 24.

4 Chang Y, Chusri S, Sangthong R, McNeil E, Hu J, Du W, Li D, Fan X, Zhou H, Chongsuvivatwong V & Tang L. Clinical pattern of antibiotic overuse and misuse in primary healthcare hospitals in the southwest of China. *PLoS One*, 14 (2019) e0214779.

5 Neu HC. The crisis in antibiotic resistance. *Science*, 257 (1992) 1064.

6 Abraham EP. The antibiotics. *Compr Biochem*, 11 (1963) 181.

7 Renwick MJ, Brogan DM & Mossialos E. A systematic review and critical assessment of incentive strategies for discovery and development of novel antibiotics. *J Antibiot (Tokyo)*, 69 (2016) 73.

8 World Health Organization, Antibacterial agents in clinical and preclinical development: an overview and analysis. *World Health Organization*, (2022).

9 Rossolini GM, Arena F, Pecile P & Pollini S. Update on the antibiotic resistance crisis. *Curr Opin Pharmacol*, 18 (2014) 56.

10 Piddock LJV. The crisis of no new antibiotics—what is the way forward? *Lancet Infect Dis*, 12 (2012) 249.

11 Rokem JS, Lantz AE & Nielsen J. Systems biology of antibiotic production by microorganisms. *Nat Prod Rep*, 24 (2007) 1262.

12 Butler MS & Buss AD. Natural products — The future scaffolds for novel antibiotics? *Biochem Pharmacol*, 71 (2006) 919.

13 Wagenaar MM, Gibson DM & Clardy J. Akanthomycin, a new antibiotic pyridone from the entomopathogenic fungus *Akanthomyces gracilis*. *Org Lett*, 4 (2002) 671.

14 Roberts DW & Humber R. Entomopathogenic fungi. In: *Infection Processes of Fungi*. Marcel Dekker, New York, (1984) 1.

15 Ratnaweera PB, Williams DE, de Silva ED, Wijesundera RLC, Dalisay DS & Andersen RJ. Helvolic acid, an antibacterial nortriterpenoid from a fungal endophyte, *Xylaria* sp. of orchid *Anoectochilus setaceus* endemic to Sri Lanka. *Mycology*, 5 (2014) 23.

16 Ratnaweera PB, Jayasundara JMNM, Herath HHMSD, Williams DE, Rajapaksha SU, Nishantha KMDWPN, de Silva ED & Andersen RJ. Antifeedant, contact toxicity and oviposition deterrent effects of phyllostine acetate and phyllostine isolated from the endophytic fungus *Diaporthe miriciae* against *Plutella xylostella* larvae. *Pest Manag Sci*, 76 (2020) 1541.

17 Kariyawasam GK, Mithrasena YJPK, Fernando THPS, Wijesundera RLC & Wijesundera WSS. A new cost-effective method for extracting genomic DNA from fungi. In: Papers, 5th annual sessions of Institute of Biochemistry, Molecular Biology and Biotechnology, (2012) 49.

18 Dissanayaka RK, Ratnaweera PB, Williams DE, de Silva CD, Wijesundera RLC, Andersen RJ & de Silva ED. Antimicrobial activities of endophytic fungi of the Sri Lankan aquatic plant *Nymphaea nouchali* and chaetoglobosin A and C, produced by the endophytic fungus *Chaetomium globosum*. *Mycology*, 7 (2016) 1.

19 National Committee for Clinical Laboratory Standards (NCCLS), Performance standards for antimicrobial disk susceptibility tests. Wayne, PA, USA, (2003).

20 Betina V. Bioautography in paper and thin-layer chromatography and its scope in the antibiotic field. *J Chromatogr* A, 78 (1973) 41.

21 Yilmaz N, Visagie CM, Houbraken J, Frisvad JC & Samson RA. Polyphasic taxonomy of the genus *Talaromyces*. *Stud Mycol*, 78 (2014) 175.

22 Houbraken JAMP, Frisvad JC & Samson RA. Taxonomy of *Penicillium citrinum* and related species. *Fungal Divers*, 44 (2010) 117.

23 Klich MA. *Aspergillus flavus*: The major producer of aflatoxin. *Mol Plant Pathol*, 8 (2007) 713.

24 Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latgé JP & Steinbach WJ. *Aspergillus fumigatus* and related species. *Cold Spring Harb Perspect Med*, 5 (2014) a019786.

25 Ng KP, Chan CL, Yew SM, Yeo SK, Toh YF, Looi HK, Na SL, Lee KW, Yee W & Kuan CS. Identification and characterization of *Daldinia eschscholtzii* isolated from skin scrapings, nails, and blood. *PeerJ*, 4 (2016) e2637.

26 Levetin E. An atlas of fungal spores. *J Allergy Clin Immunol*, 113 (2004) 366.

27 Vinu K, Krishna V, Himani S & Krishnappa M. HR-LCMS based mychochemical profiling of endophytic *Xylaria adscendens* (fr.) fr., in *Wendlandia thyrsoidea* (roth) steud. of Central Western Ghats. *Plant Arch*, 21 (2021) 881.

28 Jia W, Luo M, Wei T, Zhang H, Zeng Y & Jiang Y. *Nigrospora musae* and *N. oryzae* as new causal agents of broad bean leaf spot disease in China. *Crop Protection*, 117 (2024) 106567.

29 Rodrigues EL, Zalán Z & Bata-Vidács I. First report of *Aspergillus nomius* isolation from Hungarian apple: An emerging mycotoxigenic threat in Central Europe. *Acta Aliment*, 54 (2025) 498.

30 Panchalingam H, Adra C, Shapcott A & Kurtböke İ. Biological control of *Pyrrhoderma noxium*: an aggressive basidiomycete responsible for brown root rot of trees. *Microbiol Aust*, 46 (2025) 119.

31 Paschapur A, Subbanna ARNS, Singh AK, Jeevan B, Stanley J, Rajashekhar H & Mishra KK. Unravelling the importance of metabolites from entomopathogenic fungi in insect pest management. *Springer*, 17 (2021) 89.

32 Litwin A, Nowak M & Różalska S. Entomopathogenic fungi: unconventional applications. *Rev Environ Sci Biotechnol*, 19 (2020) 23.

33 Shah PA & Pell JK. Entomopathogenic fungi as biological control agents. *Appl Microbiol Biotechnol*, 61 (2003) 413.

34 Roy HE, Steinkraus DC, Eilenberg J, Hajek AE & Pell JK. Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts. *Annu Rev Entomol*, 51 (2006) 331.

35 Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F & Rehner SA. Entomopathogenic fungal endophytes. *Biological Control*, 46 (2008) 72.

36 Akkoç S, Karaca İ & Karaca G. Effects of some entomopathogen fungi on *Apis mellifera* L. and *Bombus terrestris* L. *Süleyman Demirel univ. fen bilim. enst. Derg*, 39 (2019) 157.

37 Poidatz J, Plantey RJL & Thiéry D. A *Beauveria bassiana* strain naturally parasitizing the bee predator *Vespa velutina* in France. *Entomol General*, 39 (2019) 73.

38 Promnuan Y, Promsai S & Meelai S. Antimicrobial activity of *Streptomyces* spp. isolated from *Apis dorsata* combs against some phytopathogenic bacteria. *PeerJ*, 8 (2020) 10512.

39 Ramayanti I, Herlinda S, Muslim A & Hasyim H. First report of entomopathogenic fungi from South Sumatra (Indonesia): pathogenicity to egg, larvae, and adult of *Culex quinquefasciatus*. *Biodiversitas*, 23 (2022) 5695.

40 Bhan S, Mohan L & Srivastava CN. Larvicidal toxicity of temephos and entomopathogenic fungus, *Aspergillus flavus* and their synergistic activity against malaria vector, *Anopheles stephensi*. *J Entomol Zool Stud*, 1 (2013) 55.

41 Qayyum MA, Saeed S, Wakil W, Nawaz A, Iqbal N, Yasin M, Chaurdhry MA, Bashir MA, Ahmed N, Riaz H, Bilal H, Hashem M & Alamri S. Diversity and correlation of entomopathogenic and associated fungi with soil factors. *J King Saud Univ Sci*, 33 (2021) 101520.

42 Moharram AM, Abdel-Galil FA & Hafez WMM. On the enzymes' actions of entomopathogenic fungi against certain indigenous and invasive insect pests. *Egypt J Biol Pest Control*, 31 (2021) 51.

43 Poulsen M, Hughes WOH & Boomsma JJ. Differential resistance and the importance of antibiotic production in *Acromyrmex echinatior* leaf-cutting ant castes towards the entomopathogenic fungus *Aspergillus nomius*. *Insectes Soc*, 53 (2006) 349.

44 Lee SY, Nakajima I, Ihara F, Kinoshita H & Nihira T. Cultivation of entomopathogenic fungi for the search of antibacterial compounds. *Mycopathologia*, 160 (2005) 321.

45 Liu BL & Tzeng YM. Development and applications of destruxins: A review. *Biotechnol Adv*, 30 (2012) 1242.

46 Sahab AF. Antimicrobial efficacy of secondary metabolites of *Beauveria bassiana* against selected bacteria and phytopathogenic fungi. *J Appl Sci Res*, 8 (2012) 1441.

47 Ravindran K, Sathishkumar G, Rajkuberan C & Sivaramakrishnan S, Antibacterial activity of indigenous entomopathogenic fungus *Metarhizium anisopliae* against clinically isolated human pathogens. *Int J Pharm Pharm. Sci*, 6 (2014) 179.

48 Begier E, Rosenthal NA, Gurtman A, Kartashov A, Donald RGK & Lockhart SP. Epidemiology of invasive *Escherichia coli* infection and antibiotic resistance status among patients treated in US hospitals: 2009–2016. *Clin Infect Dis*, 7 (2021) 565.

49 Shlaes DM, Sahm D, Opiela C & Spellberg B. The FDA reboot of antibiotic development. *Antimicrob Agents Chemother*, 57 (2013) 4605.

50 Ren J, Ding SS, Zhu A, Cao F & Zhu HJ. Bioactive azaphilone derivatives from the Fungus *Talaromyces aculeatus*. *J Nat Prod*, 80 (2017) 2199.

51 Wu Z, Li XM, Yang SQ, Wang BG & Li X. Antibacterial polyketides from the deep-sea cold-seep-derived fungus *Talaromyces* sp. CS-258. *Mar Drugs*, 22 (2024) 204.

52 Zhai MM, Li J, Jiang CX, Shi YP, Di DL, Crews P & Wu QX. The bioactive secondary metabolites from *Talaromyces* species. *Nat Prod Bioprospect*, 6 (2016) 1.

53 Song F, Dong Y, Wei S, Zhang X, Zhang K & Xu X. New antibacterial secondary metabolites from a marine-derived *Talaromyces* sp. Strain BTBU20213036. *Antibiotics*, 11 (2022) 222.

54 Kobbe B, Cushman M, Wogan GN & Demain AL. Production and antibacterial activity of malforming C, a toxic metabolite of *Aspergillus niger*. *Appl Environ Microbiol*, 33 (1977) 996.

55 Al-Shaibani ABA, Al-Shakarchi FI & Ameen RS. Extraction and characterization of antibacterial compound from *Aspergillus niger*. *J Al-Nahrain Univ*, 16 (2013) 167.

56 Putra IPYA, Ariantari NP, Wibowo JT, Leliqia NPE, Dwija IBNP, Wulandari NMW, Dwijayanti NK, Putri NPAE & Dewi KTN. *Phyllospongia foliascens*-derived fungi: Molecular identification, antimicrobial activity and secondary metabolite profile of their methanolic extracts. In: *BIO Web of Conferences*, 176 (2025) 02003.

57 Keller NP. Fungal secondary metabolism: regulation, function and drug discovery. *Nat Rev Microbiol*, 17 (2019) 167.

58 Frisvad JC & Larsen TO. Chemodiversity in the genus *Aspergillus*. *Appl Microbiol Biotechnol*, 99 (2015) 7859.