# Beyond Insects: Emerging Arthropod Reservoirs of Current and Novel Entomopathogens for Insect Pest Management – A Review

**[1]Babatunde A. Kelly, [1]Taiye A Jemilaiye, [2]Funmi Olatujoye, [2]Olayinka E. Omoseyin, [1]Marcus O. Bello, [1]David O. Adegbola, [1]David O. Ajibola, [1]Esther L. Jacob, [1]Oluwabunmi G. Adeusi,**

**[1]Department of Microbiology, Adekunle Ajasin University Akungba-Akoko, Nigeria**

**[2]Department of Microbiology, Wesley University Ondo, Nigeria**

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## ABSTRACT

With the increasing resistance of insects to chemical pesticides, regulatory pressure on chemical pesticides, and the need for environmentally friendly and benign pest management systems, efforts are geared towards the search for new entomopathogens. Traditional bioprospecting focuses on diseased insects in sourcing for microbes to be used in formulating biopesticides, yet a wide range of non-insect arthropods—copepods, arachnids (spiders and mites), myriapods (millipedes, centipedes), and other arthropod lineages—harbour microbial pathogens (fungi, oomycetes, microsporidia, bacteria, and viruses) with potentials to control insect pests. This manuscript reviews the ecological rationales, discovery methods, promising taxa, safety considerations, and the processes for sourcing novel entomopathogens from alternative arthropod reservoirs other than insects. We gave evidence that spider-pathogenic fungi (e.g., Gibellula spp.), entomophthoralean fungi associated with springtails and mites (e.g., Pandora, Conidiobolus), oomycetes found on arthropods in aquatic environment (e.g., Lagenidium, Leptolegnia), as well as microsporidia linked with aquatic crustaceans (copepods) all represent yet-to-be-fully explored resources for both crop and veterinary pest management. We outline an integrated discovery framework which combines high-throughput field sampling, specialized culture strategies, functional screening in Galleria mellonella and target pests, multi-omics (amplicon, shotgun metagenomics, metabolomics), and genome mining for virulence/secondary metabolite clusters. Finally, we highlight a regulatory and formulation roadmap to de-risk development while safeguarding non-targets and aquatic ecosystems.

**Keywords:** entomopathogens; alternative reservoirs; arachnids; copepods; microsporidia; biological control

## INTRODUCTION

Insect are known to be sometimes detrimental to economic crops, livestock and human health systems. Controlling them appears arduous majorly due to their resistance to chemical insecticides which has continued to escalate. Biological control using entomopathogens of fungal, viral, nematodal and bacterial origins do provide an alternative to deal with this anomaly due to their distinct modes of action as well as lower resistance risk (Bihal et al., 2023; Qin et al., 2023). When sourcing for biocides to be used as candidates of biopesticides, researchers do focus on morbid insects and the edaphic environment. However, non-insect arthropods have equally been known to host rich, sometimes highly specialized pathogen communities that have been taxonomically clarified and re-evaluated in recent years (Hajek et al., 2024, 2025; Gryganskyi et al., 2024). Systematically mining these alternative reservoirs could expand the spectrum of candidate agents for controlling economically important insects and arachnids such as mosquitoes, locusts and ticks. These new candidates can equally yield new metabolites (e.g., destruxins, proteases, chitinases) with bioinsecticidal value (Barelli et al., 2022; Kuhnert et al., 2022; Vasquez et al., 2024).

### Rationale For Exploring Non-Insect Arthropod Reservoirs of Entomopathogens

Non-insect arthropods are known to share ecological niches, food webs, and fauna with target pest insects. Predation, scavenging, and shared substrates (leaf litter, soil, biofilms, aquatic habitats) create reservoirs where host-jump or closely related pathogens can be found (Bass et al., 2021; Pavanelo et al., 2023). In the aquatic environment, oomycetes like Lagenidium and Leptolegnia parasitize aquatic arthropods and mosquito larvae, thriving in ephemeral ponds and rice fields (Kaczmarek et al., 2021; Sila et al., 2023). Earlier research even traced Lagenidium giganteum to mixed collections of copepods and mosquito larvae (Scholte et al., 2004).

Away from aquatic environment, the known spider-pathogenic Hypocreales (e.g., Gibellula) and Entomophthorales together infect spiders and mites; their infection process (cuticle penetration, toxin secretion) mirrors that of an insect entomopathogenic fungus thus suggesting transferable virulence determinants (Joseph et al., 2024; Sacco et al., 2023; Evans et al., 2025).

In the case of Collembola and mites, Entomophthoromycotina (e.g., Conidiobolus, Pandora) shows a narrow host ranges yet a relatively broad arthropod coverage including springtails and mites—taxa sharing soils with many crop pests (Gryganskyi et al., 2024; Sacco et al., 2023).

Myriapods such as millipedes act as hosts to actinomycetes and fungi with antagonistic activities and biosynthetic capacity for metabolites potent against insects as well as their symbionts (e.g., Streptomyces volatiles) (Stulanovic et al., 2025).

## Potential Entomopathogen Groups from Alternative Arthropod Hosts

### Oomycetes from aquatic arthropods

Lagenidium giganteum is a mosquito-parasitizing oomycete capable of producing biflagellate zoospores that actively seek larval hosts; re-isolations from African rice fields demonstrate current relevance (Sila et al., 2023). Biosafety studies do indicate variable non-target effects, thus necessitating modern tiered toxicological and risk assessment (Nestrud et al., 1994). They are known to produce hydrolases which facilitate infection (Bell & Sayre, 1989).

Aedes and Culex larvae have been reported to succumb to Leptolegnia chapmanii. The agent often exhibits temperature-dependent efficacy and compatibility with other Integrated Pest Management practices. (Kaczmarek et al., 2021).

Oomycete infections found in planktonic copepods also indicates an overlooked reservoir; these parasites share aquatic niches with mosquito larvae, warranting targeted baiting and metagenomic surveillance (García et al., 2018; Bass et al., 2021).

### Entomophthorales from arachnids, mites and springtails

Erynioideae is a subfamily within the family Entomophthoraceae (order Entomophthorales) comprising obligate arthropod-pathogenic fungi. Members within this subfamily are highly evovled entomopathogens capable of causing rapid, often explosive epizootics in their hosts. Genera such as Pandora, Furia, Zoophthora, Strongwellsea include around a hundred arthropod pathogens with many narrow host ranges. Using modern phylogenomics, their taxonom, potential biocidal usage and host breadth can be better revised and understood including records from mites and Collembola (Gryganskyi et al., 2024; Hajek et al., 2024; Sacco et al., 2023).

### Spider-pathogenic Hypocreales

The Gibellula spp which is a strict araneopathogens is getting increasingly reported from diverse climates. The recent isolation and culture of Gibellula floridensis using modern methods has made researchers to better understand its mode-of-action and metabolite discovery (Joseph et al., 2024; Evans et al., 2025). New species continue to be discovered inside this group thus implying chemical/virulence novelty.

### Bacterial symbionts and actinomycetes from myriapods and mites

Actinomycetes (e.g., Streptomyces) associated with myriapods produce a combination of both insecticidal and certain antifungal volatiles. In addition, chitinolytic bacteria from both marine and terrestrial arthropod niches (e.g., Curtobacterium) exhibit antifungal activity as well as chitinase repertoires relevant to insect cuticle degradation (Vasquez et al., 2024; Lai et al., 2024). Such microbes may act directly as entomopathogens or as synergists/formulation adjuvants.

## DISCOVERY AND SCREENING PIPELINE

### Field sampling strategy

The first steps towards culturing new biocides involves identifying suitable habitats where the hosts are present. Different habitats possess differing qualities which support the growth of different hosts and biocides depending on their physiological and metabolic needs. Ecological habitats can be big or small in size including leaf litter, riparian zones, temporary ponds, rice fields, bromeliad phytotelmata, cave drip zones, and under-leaf microhabitats frequented by spiders and mites. The diverse arthropod hosts (spiders, mites, copepods, millipedes) can be sampled together with environmental substrates (soil, detritus, biofilms) (Joseph et al., 2024; Bass et al., 2021) as all of these can house the biocides of interest.

### Timing, seasonality and microclimate

Once a suitable habitat has been located, it is preferable to target humid, cooler morning periods for optimal spore discharge especially for fungi within the Entomophthorales group. As regards the season, it is advisable to target the rainy season and for sampling to be done during the post-rain windows for oomycete zoospores (García et al., 2018; Gryganskyi et al., 2024).

### Isolation techniques and culture-dependent enrichment

Most entomopathogens according to literature possess a relatively longer regeneration time which gives other microbes especially the saprobes an earlier start when being cultured. By the time the entomopathogens are ready to grow, the nutrient source would have been depleted and the culture space occupied. As a result, baiting procedure through the direct use of sentinel hosts (mosquito larvae, Galleria mellonella larvae, Tenebrio molitor pupae) in situ and ex situ to lure and attract pathogens from arthropod washes and microhabitats (Giammarino et al., 2024). This attraction reduces contaminants and when the baits are cultured directly, it eliminates the growth of unwanted microbes to a large extent.

Generally, entomopathogens sometimes require specialized media to be able to grow. For example, semi-selective plates are required for culturing oomycetes and certain entomopathogenic fungi. Media might require supplementation using antibiotics to suppress the growth of unwanted bacteria colonies. For some strains, oil overlays might be needed for optimal growth.  -supplemented PDA/SDA; oil overlays for hydrophobins). Some cultures also require incubation at 95–100% relative humidity (RH) using a saturated-moisture chamber to ensure maximal water availability and prevent desiccation stress. This near-saturation humidity level is critical for optimal sporulation, germination, and pathogen development, especially for entomopathogenic fungi and other moisture-dependent microbes (Kaczmarek et al., 2021; Gryganskyi et al., 2024).

### Molecular Approaches for The Identification of Novel Entomopathogens

Identifying novel entomopathogens greatly depends on molecular biology techniques, which usually offer a much higher taxonomic resolution compared to morphological or ecological characteristics alone. The identification process which solely relies on phenotypic traits alone is usually insufficient, particularly for microbial groups with similar morphological features or cryptic species complexes which so far has not been extensively studied. Therefore, confirmation using molecular methods is essential to reliably establish the novelty of any putative entomopathogen (Hajek & St. Leger, 1994).

Polymerase chain reaction (PCR)-based amplification and sequencing of conserved genetic markers—such as ITS rDNA for fungi, 16S rRNA for bacteria, and COI for insect-associated protists—provide definitive insights into phylogenetic placement and species boundaries. Multi-locus sequence typing (MLST) and whole-genome sequencing (WGS) have further improved taxonomic resolution, enabling differentiation among closely related strains and detection of previously unrecognized diversity (Kepler et al., 2014). Genomic techniques are currently the gold standard for characterizing novel taxa and confirming their evolutionary distinctiveness for many entomopathogenic fungi (Araújo & Hughes, 2016).

All things considered, reliable and repeatable identification is ensured by combining molecular technologies with traditional taxonomy. Molecular characterization is essential for verifying the originality of entomopathogens isolated from insect hosts since the designation of novel species depends on clear genetic evidence.

## ADVANCED FORMULATION

Next-generation formulations are primarily designed for protection and persistence. Key advancements that can be employed for novel entomopathogens include Nanoformulations which entails the usage of nanoparticles (e.g., chitosan, zeolite, or titania-based) as carriers which significantly shields the active microbial component (spores, conidia, or nematodes) from degradation by UV light, temperature extremes, and desiccation. These carriers can also provide a controlled-release system, synchronizing the EP release with the target pest's activity cycle and thus extending the product's effective window in the field (Wu et al., 2023).

Oil-Based and Emulsion Formulations is another significant formulation method that can still be used for emerging fungal biopesticides, formulations such as inverted emulsions (water-in-oil) enhance spore tolerance to dry and warm conditions, improving germination and penetration of the insect cuticle (Batta, 2016). Oil-based adjuvants can also improve foliar persistence for entomopathogenic nematodes.

The use of biodegradable carriers like hydrogels, gums (e.g., gum katira biogel), and biopolymers (e.g., cellulose or gellan gum) can also be employed as it offers a safe, environmentally responsible way to package and protect EPNs and microbial spores, replacing less eco-friendly synthetic alternatives.

Solid Formulations such as freeze and spray-drying technologies are being optimized to produce solid microbial formulations that offer extended shelf life and easier transportation, crucial for commercial viability (Shapiro-Ilan & Lewis, 2024).

## DELIVERY STRATEGIES FOR NOVEL ENTOMOPATHOGENS

Innovation in the use of EPs has been sparked by the drive for precision agriculture, especially for pests that are hard to reach. The majority of delivery strategies attempt to maximize target coverage, minimize waste throughout the procedure, and assure correct application and successful interaction with target pests. Drones and sophisticated precision spraying equipment have been used in the application of EPs in order to achieve this goal.

Additionally, a relatively new and specialized method called "living insect bombs" releases live insect hosts that have already been infected with EPNs. Effectively serving as mobile delivery systems, these "infected hosts" transport the subsequent generation of entomopathogens into obscure environments (such as wood-boring tunnels or beneath bark) that are inaccessible to traditional sprays (Gumus et al., 2015).

For soil and root pests, techniques like seed coating and the use of granular soil applications ensure EPNs or fungal spores are delivered directly into the rhizosphere (root zone), their natural habitat, where they can effectively intercept soil-dwelling pests.

To enhance immediate efficacy and broaden the target spectrum, EPs are increasingly being formulated for use in Integrated Pest Management (IPM) programs, often in synergy with compatible, low-dose chemical insecticides or other BCAs like parasitoids (Koller et al., 2023; Ma et al., 2025).

## BIOSAFETY, NON-TARGET RISK, AND ONE-HEALTH FRAMING

Usage of entomopathogens in pest control is considered safe and nonlethal to the environment. However, a lot of toxicological and safety analyses is required to guarantee and ensure this not just on the biocides but also on the formulation and delivery materials required. Every new candidate to be considered must be subjected to these tests. Rigorous tiered testing is essential especially for aquatic systems (fish, amphibians, crustaceans) and beneficial arthropods. Recent One-Health analyses underscore the need for exposure modelling and case surveillance for EPF/oomycete bioproducts (de Sousa et al., 2025; Nestrud et al., 1994).

## REGULATORY AND STEWARDSHIP ROADMAP

A crucial pathway that is still complicated and varied worldwide is the regulatory roadmap for New Biocontrol Agents (BCAs). Even the comparatively older entomopathogens still experience these complex regulatory process which are initially created for chemical pesticides due to their toxicity. One major issue associated with the regulatory framework of BCAs is the absence of specific, uniform rules for live creatures, which usually leads to expensive and time-consuming regulatory processes that hinder commercialization and farmer uptake (EFSA, 2025; Ravensberg, 2011). Creating simplified, risk-based assessment matrices that concentrate on environmental safety, non-target effects, and human health concerns unique to the BCA's mode of action is part of the reform movement that can facilitate the timely approval and usage of novel BCAs obtained from noninsect sources (Wu et al., 2023). At the same time, stewardship is crucial, emphasizing the proper use of BCAs by creating farmer-friendly e-learning materials, increasing regulators' and farmers' ability thus guaranteeing quality promoting the successful long-term integration of BCAs into Integrated Pest Management (IPM) systems (Hoeschle-Zeledon et al., 2013).

## CONCLUSIONS AND FUTURE DIRECTION

Alternative arthropod reservoirs are fertile ground for discovering next-generation entomopathogens and synergists. Integrating ecological sampling with multi-omics, genome mining and pragmatic screening can accelerate the identification of strains with suitable virulence, specificity and manufacturability. Priorities include moving beyond simple insect toxicity towards multi-functional biological agents that are deeply integrated into sustainable agriculture. Others areas of focus are geared towards overcoming current limitations including environmental instability, slow action and narrow host range. Although narrow hast range can be advantageous in cases where specific pests are targeted in the midst of other beneficial species.

Formulations and deliver methods can also be improved upon with modern formulation science and discovery of new delivery materials. The future of entomopathogens (EPs), is set to be dramatically accelerated and transformed by Artificial Intelligence (AI) and its sub-fields, like Machine Learning (ML) and Deep Learning (DL). AI’s ability to analyze massive, complex biological and ecological datasets will address key bottlenecks in the discovery, optimization, production, and deployment of these biological control agents.

AI will assist with Predicting Virulence and host specificity by analyzing the genomes and transcriptomes of novel EP isolates (e.g., fungi, bacteria, viruses) against large databases of known virulent factors and insect immune responses. It will also assist with Novel Toxin Identification as DL models can be trained to analyze the vast metabolome data of EPs (e.g., secondary metabolites from Metarhizium or Beauveria) to virtually screen and prioritize novel bioactive micropeptides and toxins with insecticidal properties. AI can also be useful in.Optimizing Multi-Role Agents in the case of dual-role pathogens (biopesticide and biofertilizer/antagonist). Process Optimization and Mass Production can also be AI driven. Key environmental parameters such as temperature, dissolved oxygen and media composition can be monitored in mass production bioreactors for EP fungi (spore production) or Entomopathogenic Nematodes (EPNs) thus ensuring consistent quality, scalability, and cost-efficiency (Ravensberg, 2011).

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