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Review Article

Scope of entomopathogenic fungi in combating insecticide resistance and management of mosquitoes: A review

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ABSTRACT: The increase in insecticide resistance in mosquitoes is a threat to current vector control strategies. Prior studies showed that repeated exposure to insecticides has led to increased vector resistance and consequently, an increase in cases of human morbidity and mortality. So, using safer alternatives to synthetic insecticides is essential. The potential of Entomopathogenic Fungi (EMF) as sustainable tools for the control of mosquitoes has recently been demonstrated by experimental investigations. Entomopathogenic fungi having the capacity to infect and kill arthropods are safer alternatives to insecticides to tackle the growing problem of rising insecticide resistance in mosquitoes. However, the information and research on entomopathogenic fungi for mosquito control is inadequate, unorganized and lacks contemporary evaluations. Therefore, the objective of the current review is to gather and update the information currently available about the most important entomopathogenic fungi that impact mosquitoes. The mode of action of different entomopathogenic fungi and their efficacy against mosquitoes is reviewed in this article.

KEYWORDS: *Beauveria bassiana*, insecticide resistance, *Lecanicillium lecanii*, *Metarhizium anisopliae*, mosquitoes

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INTRODUCTION

The gradual increase in insecticide resistance in mosquitoes and other vectors causing diseases hampers the effectiveness of current vector control methods (van den Berg *et al.*, 2021). Mosquitoes are found to be most resistant to pyrethroids**.** In addition, resistance to carbamates, organochlorines, and organophosphates has also been reported (Nwane *et al.*, 2009; Yadouleton *et al.*, 2011). As a result, the cases of mortality have also been increasing over the years from 2000 to 2020 as shown in Figures 1 and 2 (Global Partnership to Roll Back Malaria *et al.*, 2005; Malaria's Impact Worldwide, 2000; World Health Organization, n.d.).

Studies have found increased insecticide resistance at agricultural farmlands where farmers apply insecticides and pesticides more frequently and do switching. According to some reports, farmers' frequent switching to new insecticide classes when they felt ineffective and their inappropriate disposal of them led to the emergence of insecticide resistance (Abuelmaali *et al.*, 2013). The current vector control methods promote frequent contact between insecticides and resistant vectors because the same advised insecticides are continually used, which results in mosquito resistance over time (Hancock *et al.*, 2020). By extending their contact with treated materials without dying, these vectors can ingest higher insecticide dosages, thanks to the resistance alleles. The competitiveness of the vectors and other vectorial capacity parameters, such as biting frequency and density, are also impacted by the genes that generate insecticide resistance, according to earlier research (Hancock *et al.*, 2020; Kouadio *et al.*, 2023). In addition, metabolic resistance in the vectors can also develop upon exposure to urban pollution or other environmental toxins (Reid & McKenzie, 2016). Further research highlighted that the primary mechanism of pyrethroid resistance in mosquitoes is knockdown resistance (kdr) mutations involving the substitution of leucine by serine (L1014S) or phenylalanine (L1014F) at amino acid position 1014 (Medjigbodo *et al.*, 2022). Mosquitoes also exhibit insecticide resistance through the genes encoding for target proteins that interact with insecticides and result in structural point mutations, developing target site resistance. In addition, when cytochrome P450s, esterase and glutathione S-transferases are transcriptionally upregulated in mosquitoes, it results in metabolic resistance and elevated protein synthesis and enzyme activity (Ononamadu *et al.*, 2020).

Scientific groups are now required to concentrate on the creation of alternative environment-friendly solutions due

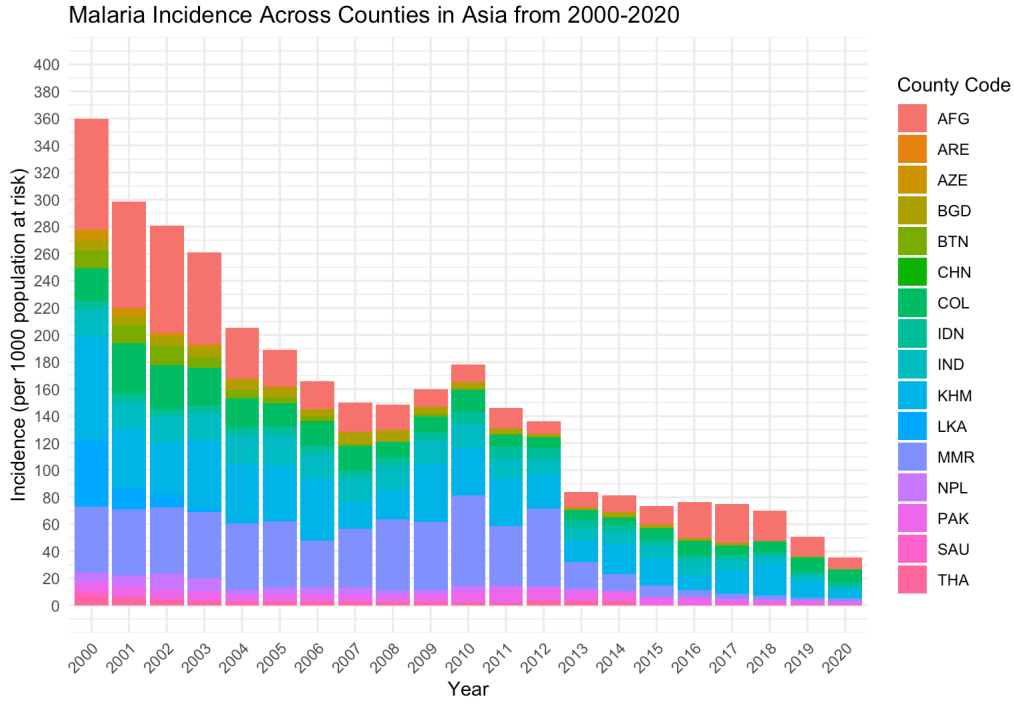


Figure 1. Cases of mortality caused by mosquito-borne diseases over the years from 2000-2020 across Asian countries (Data collected from (Malaria's Impact Worldwide, World Health Organization, n.d.) and the graphs were plotted using RStudio software (version 2024.04.2+764)).

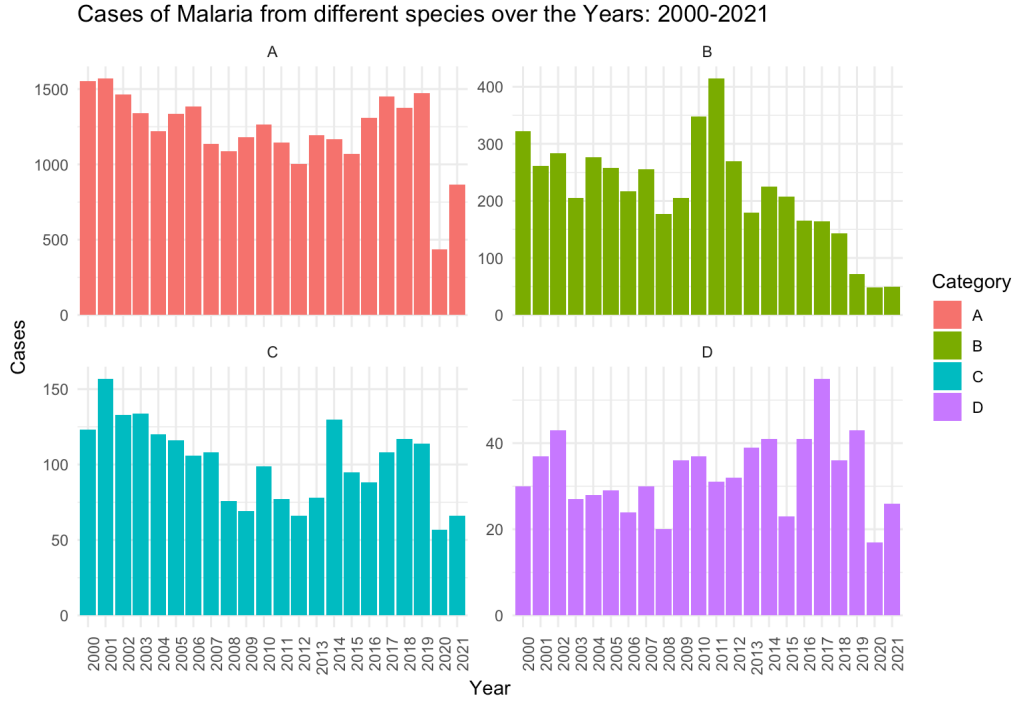


Figure 2. Cases of Malaria caused by different species (a) *Plasmodium falciparum*, (b) *Plasmodium vivax*, (c) *Plasmodium malaria*, (d) *Plasmodium ovale* from year 2000 to 2021 (Data collected from (Malaria's Impact Worldwide, World Health Organization, n.d.) and the graphs were plotted used RStudio software (version 2024.04.2+764)).

to the negative impacts of chemical pesticides on nontarget creatures, food safety, and the emergence of insect resistance (Sinha *et al.*, 2016). Thus**,** to find alternatives, microbial biopesticides have gained a lot of traction worldwide (Mascarin & Jaronski, 2016). A sustainable pest management approach relies heavily on the use of entomopathogenic fungi for biological plant protection. Numerous investigations and tests have shown that entomopathogenic fungi can be

used to manage mosquito vectors without endangering the environment (Accoti *et al.*, 2021; Kataki *et al.*, 2024). The primary characteristic of these entomopathogenic fungi is their ability to spread infection through direct contact and penetration. These fungi can go past the host's innate immune defence response by effectively penetrating the insect's cuticle and then reaching the hemocoel (Bava *et al.*, 2022; Tawidian *et al.*, 2019). The ability of entomopathogens to lessen the selection pressure for the development of pesticide resistance in insect populations has been widely documented in earlier studies. Experimental studies with entomopathogenic fungi have been quite successful, especially in honeybee research (Bava *et al.*, 2022). Therefore, to combat the growing insecticide resistance in mosquito species like *Anopheles gambiae*, *Culex quinquefasciatus*, *Aedes agypti*, etc. that cause numerous fatal diseases like malaria, dengue, filariasis, chikungunya, etc. each year that led to numerous cases of mortality and morbidity worldwide; the use of entomopathogenic fungi can be thought of as an alternative option. The potential of entomopathogenic fungi as next-generation agents for the control of malaria mosquitoes has recently been demonstrated by theoretical and experimental investigations (Hancock, 2009; Hancock *et al.*, 2008; Scholte *et al.*, 2005). However, there is a dearth of recent reviews and a relatively disorganized body of research on entomopathogenic fungi for mosquito control. Thus, the current overview's goal is to compile and update the knowledge currently accessible regarding the most significant entomopathogenic fungi that affect mosquitoes. This article is an attempt to provide an outline of entomopathogenic fungi and their mode of action that must be understood to use them as a potentially organic alternative to synthetic insecticides against mosquito population control.

Entomopathogenic fungi: An alternative to synthetic insecticides

Entomopathogenic Fungi (EPF) are parasitic microbes that have the capacity to infect and kill arthropods (Litwin *et al.*, 2020). Some of them are also utilized in Chinese medicine or biotechnological processes, but their primary usage is as biopesticides in ecological farming as a safe substitute for hazardous chemical insecticides (Jaihan *et al.*, 2016; Ríos-Moreno *et al.*, 2016). Oomycetes, Chytridiomycota, Microsporidia, Entomophtoromycota, Basidiomycota, and the most prevalent Ascomycota are the six classes into which these fungi are divided (Litwin *et al.*, 2020). Numerous investigations and tests have shown that entomopathogenic fungi can be used to manage mosquito vectors without endangering the environment (Farenhorst *et al.*, 2010; Kataki *et al.*, 2024; Khan *et al.*, 2012; Litwin *et al.*, 2020; Scholte *et al.*, 2004). Due to its high enzymatic activity, capacity to generate secondary metabolites, and favourable growth in culture media, EPF is also being investigated for application in other biotechnology domains, such as the biosynthesis of nanoparticles and the bioconversion of flavonoids or steroids, which are significant from an economic perspective (Gonzalez *et al.*, 2017; Litwin *et al.*, 2020; Rózalska *et al.*, 2016; Yosri *et al.*, 2018). Although Entomophthorales (such as Furia, Conidiobolus, Entomophagy, or Erymid) exhibit the highest insecticidal activity among the known EPF, they are not utilized as components of biopreparations because of technical challenges in growing in laboratory settings. The most widely utilized EPF are Ascomycota-related easily cultured saprotrophic fungi (Litwin *et al.*, 2020; Mascarin & Jaronski, 2016). Based on species from the genera Metarhizium, Beauveria, Paecilomyces, Isaria, and Lecanicillium, a variety of biopesticides have been employed all over the world. Because of their diverse range of activity, this fungus can infect many arthropod species and so, can be considered as an alternative to synthetic insecticides for vector control (Kataki *et al.*, 2024; Khan *et al.*, 2012; Litwin *et al.*, 2020; Mascarin & Jaronski, 2016). Moreover, entomopathogenic fungi are diverse organisms with a range of ecological functions. For instance, common soil-dwelling organisms of the genus Metarhizium and Beauveria regulate natural arthropod populations and establish intricate connections with plants. They are referred to as plant roots, stems, and leaves endophytes (Jaber & Enkerli, 2017).

Mode of action of entomopathogenic fungi

Usually, entomopathogenic fungi infect arthropod hosts when their spores come into touch with them. Spore adherence to arthropod shells initiates the infection process, which includes two stages: the first is dependent on hydrophobic and electrostatic forces, while the second necessitates the activity of enzymes and low-molecular-weight proteins known as hydrophobins (Skinner *et al.*, 2014. Unlike bacteria or viruses, they do not have to be ingested by an insect. Between 20°C and 30°C is the ideal temperature range for EPF development and germination. Another characteristic of the specific fungus strain is that spores can germinate at temperatures outside of this range (Litwin *et al.*, 2020; Skinner *et al.*, 2014. Fungal spores germinate in the presence of moderate temperatures and high relative humidity. They then use enzymatic breakdown and mechanical pressure to break through the insect's cuticle and enter its body. After entering the body, the fungi proliferate, infiltrate the tissues of the insect, emerge from the deceased insect, and generate further spores (Kanzok & Jacobs-Lorena, 2006; Kataki *et al.*, 2024; Litwin *et al.*, 2020; Scholte *et al.*, 2004). Following their entry into the hemocoel, the fungal hyphae begin to proliferate inside the arthropod. Some EPF can develop secondary hyphae that live in the host's tissues after blastospores reach the haemolymph of the host. The fungi now create secondary

compounds that paralyze the host and interfere with its physiological functions, primarily its immune responses (Donzelli *et al.*, 2016. The infection causes the insect's body to be destroyed by nutrient loss and mechanical harm to the internal organs caused by the growing hyphae (Donzelli *et al.*, 2016; Fan *et al.*, 2017; Mascarin & Jaronski, 2016). The fluid absorption by the fungus causes the insect's originally soft body to become stiff as the infection progresses. Initial signs of illness typically appear 7 days after infection (or possibly sooner, depending on the fungus species), however, the full infection process is somewhat lengthy and takes about 14 days. After the insect has been killed and all its nutrients have been used, the hyphae of the fungus emerge from the host's carcass through intersegmental areas and pores in the body (mouth hole, anus). After that, the fungus produces resting or infectious spores, which enable it to spread and infect more people (Skinner *et al.*, 2014).

Important species of entomopathogenic fungi for mosquito control

Beauveria bassiana

Agostino Bassi isolated *Beauveria bassiana* from silkworm cadavers for the first time in the 19th century. This organism can infect over 200 insect species across six orders and 15 families (Nakahara *et al.*, 2009). Beauveria is a filamentous species that belongs to the Deuteromycetes class. Some of the valuable species are *B. bassiana*, *B. brongniartii*, *B. amorpha*, and *B. caledonica*. *B. bassiana* is one species of this genus that grows naturally in soil all over the world. It is the cause of white muscardine disease and has pathogenic properties for numerous insect species (Islam *et al.*, 2021). In vulnerable insects, the spores of this fungus germinate and spread straight through the cuticle to the inside of the host. As the fungus spreads throughout the insect's body, it produces poisons, depletes its nutrients, and ultimately kills it. Beauveria and other fungal infections therefore infect insects upon contact rather than requiring ingestion by their host, in contrast to bacterial and viral pathogens caused by insects. White muscadine sickness gets its name from the fungus's growth through the softer parts of the cuticle after it has killed its host, which covers the insect with a layer of white mould (Bisandre *et al.*, 2023; Kataki *et al.*, 2024; Mascarin & Jaronski, 2016). It produces a range of toxins that lead to extrinsic infections as it multiplies vigorously (Chelico & Khachatourians, 2008; Naqqash *et al.*, 2016). The primary pathogenic agents of insect infections that are non-specifically absorbed via the insect's epidermis are fungi or conidia. Conidia germinate to produce hyphae under the right circumstances, after which they release a variety of insecticidal poisons (Lewis *et al.*, 2009; Wanchoo *et al.*, 2009). The majority of the *B. bassiana* toxins are small molecules and secondary metabolites, including calcium oxalate crystals, oxalic acid, beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, and other beauvericin analogs. Beauvericin, which is secreted by mycelia, is one of the most significant poisons among them. Furthermore, it has nematicidal activity too (Xu *et al.*, 2007). *Beauveria bassiana* can also be used as a synergist with other fungi and insecticides to increase its vector control efficacy as evident in some studies and research (Kataki *et al.*, 2024; Thakur *et al.*, 2022).

Metarhizium anisopliae

Metarhizium anisopliae is a significant entomopathogenic fungus that has long served as a model for research on the biological control of pest insects by fungi. It has been used in investigations on virulence and the biochemical mechanism of pathogenicity (Zimmermann, 1993). Although the fungus has co-evolved with insects, E. Metschnikoff discovered it on the cereal chafer *Anisoplia austriaca* and named it *Entomophthora anisopliae* in 1879 (Zimmermann, 1993). It seems that the primary enzyme responsible for this development is a serine protease (St. Leger *et al.*, 1996) that resembles subtilisin and starts the breakdown of proteins. During invasion, this protein is the main one released into the host's cuticle (Leger *et al.*, 1996). After this protease activity, exopeptidases like carboxypeptidases take over and liberate the host's specific amino acids for use in feeding (Woessner, 2013).

In the lab, the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have proven to be efficient against anopheline larvae (Hancock, 2009; Leger *et al.*, 1996; Scholte *et al.*, 2005). Both *Metarhizium anisopliae* and *Beauveria bassiana* fungal spores were simple to combine and apply to the water's surface when prepared in a synthetic oil (ShellSol T). Compared to formulations using mineral oil, dry powders, or 0.1% Tween 80, this formulation proved more effective against anopheline larvae. Following exposure to the water, ShellSol T also increased the fungus spores' persistence. Compared to the results of applying unformulated spores, the ShellSol T-formulated *Metarhizium anisopliae* and *Beauveria bassiana* spores dramatically decreased the percentage pupation of *An. gambiae* under Kenyan field circumstances by 39 to 50% (Kanaoka *et al.*, 1978).

Lecanicillium lecanii

Among the varied host ranges found in the genus Verticillium are nematodes, plants, fungi, and arthropods. Using rDNA sequencing, the genus was reclassified, and insect pathogens were grouped into the new genus Lecanicillium, which comprises *L. attenuatum*, *L. lecanii*, *L. longisporum*, *L. muscarium*, and *L. nodulosum*—all of which were previously categorized as *V. lecanii* (Gams & Zare, 2001). The EPF called *Lecanicillium lecanii* infects invertebrates by penetrating their outer layer of skin. Three stages make up the infection. The first is when the fungus spores adhere to the host's cuticle and

begin to germinate. The fungus then develops inside the insect body after a germ tube penetrates the insect's integument, covering the infected host with spores and hyphae in favourable humid conditions and ultimately leading to the infected host's death (Wang *et al.*, 2021). Secondary metabolite chemicals produced by *L. lecanii* are poisonous to insects. According to the secondary metabolite, chemicals include hydrolytic enzymes including lipases, chitinases, and proteases as well as toxic substances such as dipicolinic acid, vertilecanin-A1, decenedioic acid, and 10-hydroxy-8-decenoic acid (Hasan *et al.*, 2013). *Lecanicillium lecanii* is a commercially available fungus that targets thrips, aphids, and whiteflies under the trade names Mycotol and Vertalec. *Metarhizium anisopliae* is used to stop termites (marketed under the name Meta Guard), locusts (marketed under the name Biogreen), cockroaches (marketed under the name Bio-path), termites (marketed under the name Bio-Blast), sugarcane spittlebugs (marketed under the name Cobicant), cane grubs (marketed under the name Bio-Cane), and grasshoppers (marketed under the name Green muscle) (Arakere *et al.*, 2021). Upon encountering the host's integument, *L. lecanii* conidia sticks to the epicuticle and grows. Germinated conidia develop into germ tubes that can grow over the epicuticle's surface or directly penetrate the cuticle. Lysing the epicuticle and procuticle allows the germ tube to enter. This is done by the appressorium (penetration peg) applying mechanical pressure and by the secretion of enzymes called proteases, chitinases, and esterases, which are crucial for the cuticle penetration of the insect host and function as cuticle-degrading enzymes. The fungus spreads throughout the insect's body, depleting its resources and ultimately causing its death within 48 to 72 hours. *L. lecanii* produces bassianolide, a kind of mycotoxin (Kanaoka *et al.*, 1978).

For mosquito population management, entomopathogenic fungi like *Beauveria bassiana*, *Lecanicillium lecanii* and *Metarhizium anisopliae* present a viable substitute for chemical insecticides, especially considering the growing resistance to insecticides. Through natural biological processes, these fungi infect and kill mosquitoes, lowering the need for artificial chemical insecticides that fuel resistance. The capacity of these fungi to target mosquitoes at various stages of their lifecycle is one of their main advantages. For example, they can lower total population densities by infecting both adult and larval mosquitoes. These fungi also pose little threat to non-target creatures including beneficial insects, animals, and people because they are ecologically benign and have a high selectivity for their target insects. Because of this feature, they are perfect for Integrated Pest Management (IPM) programs that prioritize environmental preservation.

Due to their adaptability, these entomopathogenic fungi can be used in a variety of contexts. These can be sprayed to mosquito breeding grounds, resting places, or other high-density habitats as sprays, baits, or granules. Moreover, studies have shown that these fungi can be utilized to increase the efficacy of chemical insecticides at lower dosages or in combination with other biological agents (Alkhaibari *et al.*, 2018; Kataki *et al.*, 2024; Thakur *et al.*, 2022). By varying the selection pressures on mosquito populations, this combined strategy not only increases efficacy but also aids in delaying the establishment of resistance. The potential use of these fungi to combat insecticide-resistant mosquito strains is another significant advantage. Entomopathogenic fungi depend on physical and biological processes that are hard for mosquitoes to genetically adapt against, in contrast to chemical pesticides that target certain biochemical pathways. They are therefore a useful instrument for controlling resistance and maintaining long-term control initiatives.

CONCLUSION

Thus, to sum up, *Beauveria bassiana*, *Lecanicillium lecanii*, and *Metarhizium anisopliae* offer a viable and sustainable approach to mosquito control that tackles the growing issue of insecticide resistance as well as ecological problems. Their incorporation into current vector management initiatives could greatly improve the efficacy of initiatives to prevent diseases spread by mosquitoes while advancing human health and environmental safety. Their contribution to the worldwide battle against mosquito-borne diseases will be strengthened by ongoing research and development into the optimization of fungal formulations and delivery systems

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