Review article

Biopesticide potentials of entomopathogenic microorganisms in the control of Malaria vectors

Adeniyi, K. A[1*]., Osodi, F. A[2]., Ibeh, E. O[3]., Muhammed, I. O[4]., Rufai, A. M[5]., and Oyibo-Usman, K. A[6].

[1]Department of Biological Sciences, Federal University Dutse, Dutse, Jigawa State, Nigeria
[2]Bioresources Development Centre, National biotechnology development agency Abuja
[3]Veritas University Abuja, Nigeria
[4]Kogi State Polytechnic, Lokoja, Nigeria
[5]Department of Zoology, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, Oke- Baale, Osogbo, Nigeria
[6]Zoology Unit. Department of Biological Sciences, Federal University Gusau, Zamfara, Nigeria;

Submitted: November, 2022; Accepted: December, 2022; Published: December, 2022

SUMMARY

Although chemical insecticide application against mosquito vectors has been the key element in malaria vector management programmes, their efficacy and potency have been greatly threatened by the evolution of insecticide-resistant mosquitoes. These situations have pushed the malaria vector control experts towards the discovery and development of new novel environmentally friendly bio-pesticide agents with distinct potential to combat the challenges associated with the use of synthetic insecticides. The uses of biological agents that are environmentally friendly against the malarial vector, Anopheline mosquito, are one of the vital parts of the recently launched malaria eradication programme. Entomopathogenic bacteria and fungi are some microorganisms that have the potency to grow on or in insects, eventually killing them. These microorganisms have been well documented to control larvae of both agricultural, forestry and medically important insect pest. In the current study, it is worthy of note that Bacteria isolates including *Pseudomonas aeruginosa, Bacillus polymyxa, Bacillus thuringiensis,* as well as, Fungi isolates such as *Beauveria* spp and *Metarhizium* spp have been well reported to possess a variety of metabolites with mosquito-larvicidal and -adulticidal potencies. An attempt has been made in this current review to explore the recent development of Entomopathogenic bacteria and fungi in malaria vector control. Findings from the present review thus suggest proper exploitation of these microorganism communities in the development of novel integrated control against the malarial vector.

Keywords: Biopesticide, Entomopathogenic bacteria, Malaria vector, Fungi isolates

***Corresponding author email**: adeniyi.k@fud.edu.ng
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INTRODUCTION

Malaria, a mosquito-borne infectious disease of humans and some other animals, remained the most severe public health problems worldwide main health challenges of the tropical [1,2] . It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the most vulnerable groups [3]. According to the 2021 World Malaria Report, nearly half the world's population lives in areas at risk of malaria transmission in 87 countries and territories [4, 5]. In 2020, malaria caused an estimated 241 million clinical episodes, and 627,000 deaths. An estimated 95% of deaths in 2020 were in the WHO African Region [6]. Malaria occurs mostly in poor, tropical and subtropical areas of the world. Africa is the most affected (Figure 1) due to a combination of factors including efficient mosquito (*Anopheles gambiae* complex) characterized with significant vectorial capacity, predominant and presence of virulent parasite species, *Plasmodium falciparum and* favourable Local weather conditions [7,8].

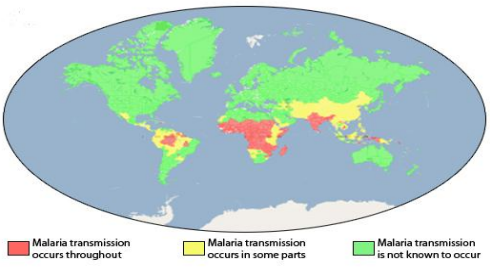

Figure 1: Global distribution of malaria. The disease transmission occurs throughout the year in major part of Africa [9].

The malaria parasites responsible for human malaria include *Plasmodium falciparum. Plasmodium vivax, Plasmodium ovale* spp., *Plasmodium malariae* and *Plasmodium knowlesi*, which is transmitted by the female *Anopheles* mosquito [10]. Humans get infected with malaria when an infected female *Anopheles* mosquito transmit the malaria parasite into the blood stream of a susceptible host after previously sucking blood from an infected host [11, 12].

The use of microbial biopesticides has received several attentions in the control of insect pests. These microbes can either eliminate or reduce the effect of the insect pest on the host to the barest minimum • these microbes are known as the entomopathogenic microorganisms. Entomopathogenic microorganisms are a wide array of microbes that have the capacity to grow either on or in insect pest, thereby causing diseases to the insect. However, the growth of these organisms on or in insect pests can result in high mortality among the insect populations at every developmental stage including the adult stage.

For decades, the control of malaria vector has solely relied on the application of chemical insecticides and repellant to combat the growth and development of mosquito vector; both at the larval or adult stage [12, 13]. Unfortunately the use of chemical insecticides, the ever-growing increase in the population of insecticide-resistant mosquito vectors in malaria-endemic region has undermined the efficacy of chemical insecticides [14]. This global concern prompted the search for alternative technologies and the Integrated Vector Management (IVM) are now focused on controlling the malaria vector (*Anopheles* mosquitos) either at the early developmental stage and/or at adult stage using microbial control approach, namely fungi and bacteria [15].

Entomopathogenic fungi have some characteristics that make them one of the most ideal biopesticides for malaria control. Fungi isolates are proven to be highly effective against insecticide-resistant mosquito strains [16-18]. Fungal isolates are potent that they also infect mosquitoes on contact and their applications have been deployed into conventional insecticides, including indoor residual sprays [19], and treatments for netting or cloth [20]. However, unlike most chemical insecticides that kills or knock down viable mosquitoes within 24 h exposure [21], fungal biopesticides can take a week or more before the effects are shown in the mosquitoes exposed [22, 20]. It is believed that fungal biopesticides are expected to reduce selection for new resistance phenotypes [23, 24].

Entomopathogenic bacteria have also been proven to be effective in the malaria control approach. Malaria control approach are now shifting attention towards the use of genetically engineered entomopathogenic bacteria to either inhibit the development of the malaria parasite with the mosquito (*Anopheles*) vector or target the vector itself [25-29].

Wolbachia is among the promising entomopathogenic bacteria to be associated with *Anopheles gambiae* [30]. According to Ramirez *et al*. [28], *Chromobacterium* sp. Panama (*C.* sp_P) isolated from the midgut of field-collected *Aedes aegypti* has unique properties that can eliminate larvae and adults of multiple mosquito species. It exerts in vitro anti-plasmodium and anti-dengue virus activity depicting that it could be an ideal pathogen for developing tools in the fight of present and future mosquito borne diseases [28].

Virulent Nature of Entomopathogenic Fungi on Insect Pest

Fungi is a large group of microorganisms constituting more than 500 species that can parasitize insects including malaria vectors. Many of the microorganism species found in this group are capable of reducing host insect populations [31, 32]. According to Faria [33], many products produced from fungi can serve as biopesticides. Among the entomopathogenic fungi capable of secreting toxins that are pesticidal in nature are *Metarhizium, Beauveria, Verticillium, Nomuraea, Entomophthora,* and *Neozygites* [34].

When it comes to the virulence of fungal entomopathogens, there are four steps involved: adhesion, germination, differentiation and penetration. However, each of the step is influenced by a range of integrated external and intrinsic factors, which determine the pathogenicity. It is reported that the success of the infection of entomopathogenic fungi depends majorly on the ability of a fungus to adhere and penetrate the host integument [35]. Entomopathogenic fungi infect their hosts by penetrating through the cuticle, thereby producing toxins, affecting the hemolymph, and develop by utilizing the nutrients present in the hemocoel to prevent insect immune responses [36]. The application of entomopathogenic fungi as an insecticide alternative could be very useful for insecticide resistant management [37].

Functions of the Fungal Community in *Anopheles* Mosquitoes

Anopheles mosquitoes are colonized by a huge range of fungal microorganisms that may affect mosquito biology and vectorial capacity. It is reported that *Anopheles* mosquitoes are colonized by a huge range of fungal microorganisms that may affect mosquito population, biology, and vectorial

capacity. According to Steyn *et al.* [38], some fungi coexisting in mosquito microbiota are non-pathogenic while some are pathogenic in nature [39]. Even though there are fewer reports on fungal species isolated from the natural microbiome community of *Anopheles* populations, fungi pathogens that are capable of infesting *Anopheles* vectors have been extensively studied [40]. According to Dada e*t al*. [41], entomopathogenic fungus, *Beauveria bassiana*, can interact with the gut bacteria in *Anopheles stephensi* to speed up the mortality rate in the mosquito population. Also, the report of Kikankie *et al*. [16] showed that both laboratory and wild-caught *Anopheles arabiensis* were found susceptible to the infection caused by *Beauveria bassiana* regardless of their insecticide susceptibility.

The study of Kikankie *et al*. [16] also showed that *B. bassiana* spores greatly reduced the lifespan of all mosquito colonies tested. The mineral oil formulations of the fungus *B. bassiana* resulted in reduced longevity of wild *An. Arabiensis, An. Funestus and An, gambiae* [42]. The similar study of Mnyone *et al*. [43] showed great reduction in *An. gambiae* survival when exposed to the co-formulations of both *M. anisopliae* and *B. bassiana* fungus. The same trend was reported in *An. gambiae* exposed to dried conidia of *Metarhizium anisopliae* [44] and in both *An. gambiae* and *An. funestus* exposed to low doses of conidia by direct contact [45, 46]. *Metarhizium anisopliae* infected wild *An. gambiae* s.l. and reduced mosquito lifespan upon contact on *M. anisopliae*-impregnated black cotton sheets [20].

Under laboratory conditions, fang e*t al*., [47] reported that, following the infection of the mosquitoes, these fungi species can auto-disseminate between the mosquito vectors through mating. In addition to their report, it was shown that age and blood feeding status of the mosquito did not affect the susceptibility of the mosquito to fungal infection [43]. These studies are helpful as they will enhance the propagation of the fungal infection in mosquito populations when the natural and eco-friendly malaria vector control based on these pathogens is implemented.

Different mosquito life stages and activities such as flight ability, fecundity, blood-feeding [48, 49] and host-seeking behavior [50] have all been reported to be negatively affected when exposed to *B. bassiana* and *M. anisopliae*. All these studies attest and highlight the potential of fungi isolates as biopesticides in the control of malaria vectors.

However, other fungi isolates have been reported to contribute to the pathogen's growth and development in mosquitoes. However, it was shown that the presence of *Pe. chrysogenum* in the *An. gambiae* midgut does not show any negative effect in mosquito survival, and it increases the mosquito susceptibility to *Plasmodium* infection by suppressing the immune system of the host [51]. Anonymously, *Leptosphaerulina* sp. was reported to activate the immune system to induce the production of melanin deposits on the fat body in *Anopheles gambiae* [52].

The Bacterial Community and Its Associated Bacteriophages in *Anopheles* Mosquitoes

During the period of aquatic development, bacteria have been of the primary sources of nutrition for the population of mosquito larvae [53]. Several studies including the study of Minard *et al*. [54] have shown that host huge bacteria communities differ

according to the mosquito sex, living environment, and developmental stage. Endosymbionts have been postulated as a promising candidate to develop paratransgenesis approaches [55]. This prompts the urgent need to keenly understand the bacterial spectrum in controlling malaria vectors.

Functions of Bacteria in *Anopheles* Mosquitoes

The Common bacteria microbiota infecting *Anopheles* species mosquitoes is detailed in Table 1.

Even though further studies are needed to demonstrate the role of bacteria in controlling *Anopheles* mosquitoes, previous literatures have attempted to shed more light. It has been previously reported that bacterial communities have been investigated to impede *Plasmodium* development in *An. gambiae* [56]. Also, an interaction of distinct bacteria with the pyrethroid resistance in *An. gambiae* has been reported [57]. Bacteria of *Anopheles* vectors interfere with both the vector competence and physiology of their bearers. Numerous reports have shown an overall inhibitory effect of the bacterial communities on *Plasmodium* parasites in *Anopheles* species. In the study of Damiani *et al*. [58], It was revealed that the intestinal bacterial communities can regulate the expression of the thioester-containing protein (TEP1) via an RNA interference (RNAi) mechanism to inhibit *P. yoelii* development in *An. dirus*.

Other bacterial species, including *Escherichia coli* (strains H243, HS5); *Pseudomonas aeruginosa; Pseudomonas sutzeria; Ewingella americana, Serratia marcescens; Xanthomonas malthophila; Cedecea lapageia; Enterobacter cloacae; Enterobacter amnigenus, S. aureus; Comamonas spp.; Bacillus pumilus*; *Chromobacterium sp. Csp_P*, and *Methylobacterium* were shown to have negative effects on the development of *P. falciparum, P. vivax*, and *P. berghei in An. gambiae* [59-64], *An. stephensi* [65, 61, 66, 67], *An. albimanus* and *An. coluzzii* [67].

Serratia ureilytica is another bacteria species that has been reported to inhibit the development of *P. falciparum* or the rodent parasite *P. berghei* by secreting a lipase capable of killing the parasite at different developmental stages [68]. In contrast to the report of Gao *et al*. [68], that the bacterium *Asaia bogorensis*, was shown to increase the midgut pH and subsequently stabilize the *Plasmodium berghei* gametogenesis by alkalizing the mosquito midgut. The genome of *Asaia* strain isolated from *An. stephensi* was investigated and it was revealed that this bacterial strain had the most predicted regulatory proteins, suggesting its ability to adapt to frequent changes in the environment of the mosquito gut [69].

In addition to all these reports, *Wolbachia* infection was demonstrated to greatly decrease the intensity and prevalence of sporozoite infection in *An. gambiae* s.I [70]. Not only does *Wolbachia* infection affects *Plasmodium* development, it also affects the egg-laying capacity of mosquito populations [71]. Reintroduction of *Pseudomonas putida, Pantoea* sp., and *Serratia marcescens* into *An. arabiensis* through sugar feeding, resulted also in significant inhibition of *P. falciparum* infection, with *S. marcescens* and *P. putida* exhibiting the strongest anti-parasite activity [72]. Experimental infection of *Chromobacterium violaceum* in insecticide-resistant *An. coluzzii* females was demonstrated to greatly decrease mosquito survival, blood-feeding and to affect fecundity and hatching rate [18].

Table 1: Common bacteria microbiota infecting *Anopheles* species mosquitoes.

Bacterial family	Bacteria species	*Anopheles* species	References
Acetobacteraceae	*Asaia sp.; Asaia bogorensis*	*An. stephensi; An. gambiae*	73 74
Bacillaceae	*Bacillus* sp.	*An. Darling*	47
Bifidobacteriaceae	*Bifidobacterium*	*An. Lesteri*	75
Brevibacteriaceae	*Brevibacterium* sp.	*An. Darling*	47
Enterobacteriaceae	*Enterobacter* sp.*; Enterobacter cloacae*	*An. darlingi; An. gambiae; An. funestus; An. Arabiensis*	76 77 78
Microbacteriaceae	*Leucobacter* sp.	*An. Darling*	47
Pseudomonadaceae	*Pseudomonas putida; Pseudomonas rhodesiae*	*An. dirus; An. Arabiensis*	75 61 72
Rickettsiaceae	*Wolbachia* sp.	*An. maculatus; An. sinensis;An. gambiae* *An. funestus; An. arabiensis*	30 70,79 80
Weeksellaceae	*Cryseobacterium meninqosepticum*	*An. Stephensis*	81
Xanthomonadaceae	*Stenotrophomonas* sp.	*An. Darling*	47
Chromobacteriaceae	*Chromobacterium violaceum*	*An. Coluzzi*	63,82
Enterobacteriaceae	*Serratia ureilytica*	*An. Stephensi*	83

Infection of *Anopheles coluzzii* with the Entomopathogenic Bacteria *Chromobacterium* sp. Burkina (C.sp_BInfection of Anopheles coluzzii with the Entomopathogenic Bacteria *Chromobacterium* sp. Burkina (C.sp_B) is explained in figure 1 and 2. For a long time, there has been a serious concern over the application of bacteria in the control of malaria vectors. In many studies, it has been revealed that many strains of bacteria under investigation are yet to meet the expectation due to some practical and functional limitations [84]. According to the report of Tetreau *et al*. [85], Bacteria strains such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) show no residual persistence post application.

However, some members of bacteria from the genus *Chromobacterium* sp. such as *Chromobacterium vaccinii* and *Chromobacterium* sp. Panama (Csp_P) are among the promising microbial-based tools for the control of malaria vectors. They have been demonstrated to possess insecticidal

activity across different species of mosquitoes including *Aedes Aegypti* and *Anopheles gambiae* s.s. [63]. In addition to the report of Ramirez *et al*. [63], the report of Caragata *et al*. [86] also revealed that a non-live preparation of Csp_P was a highly effective larval mosquito biopesticide.

In aim to control malaria vector, Gnambani *et al*. [87] isolated a new strain of *Chromobacterium* sp. from Burkina Faso formerly known as *Chromobacterium violaceum* [87]. Under laboratory condition, this strain of *Chromobacterium* demonstrated high mortality infection in the insecticide-resistant malaria vector *Anopheles coluzzii*. This Burkinabe new strain significantly decreased mosquito blood feeding propensity and fecundity [87].

The result of the study of Gnambani *et al*. [87] revealed that the exposure of L3-larvae insecticides resistant *Anopheles coluzzii* to different concentrations of Burkina Faso local and new strain of C.sp_B resulted in increased mortality rates within mosquito populations. It has been previously reported that the same strain of C.sp_B showed a high virulence against adult *Anopheles* mosquitoes [87]. The ability of C.sp_B to kill the larvae of mosquitoes could be the direct result of a mosquitocidal factor or systemic infection through dissemination into the hemolymph. Alternatively, C.sp_B colonization of the midgut might cause mortality indirectly by altering the vital functions of the mosquito [63]. The production of the hydrogen cyanide, violacein, siderophores, and chitinases could be cited among the potential virulence factors that may contribute to mosquitocidal effect of this bacteria strain [88].

In addition, some strains *Chromobacterium* can form biofilms in vitro, though it is not yet tested if biofilm formation occurs within the mosquito midgut. Gnambani *et al*. [87] also reported another entomopathogenic effect of C.sp_B, which was shown in the significant reduction of insemination rates of females. The insemination rates generated by crossing of infected females and males was similar to those obtained by Helinski *et al*. [89] in large cages with the classical Sterile Insect Techniques by lower irradiation dose [90]. With C.sp_B infected males, the sperm secretion can also be negatively affected by the bacterial treatments. The alteration of energy reserves and reduction of mating capacity from C.sp_B infected mosquitoes can also be the reason for the low insemination rates.

Furthermore, the result of Gnambani *et al*. []82 revealed that entomopathogenic bacteria C.sp_B uninfected *An. coluzzii* offspring were greatly bigger than infected ones. It has been previously reported by Ramirez *et al*. [63] and Gnambani *et al*. [82] that pigments and peptides as siderophores, hydrogen cyanide, violacein, and secreted chitinases could impede the fitness of adult mosquitoes.

Figure 1 *Anopheles coluzzii* mosquitoes feeding upon a cotton ball soaked with 6% glucose containing *Chromobacterium violaceum* [82]

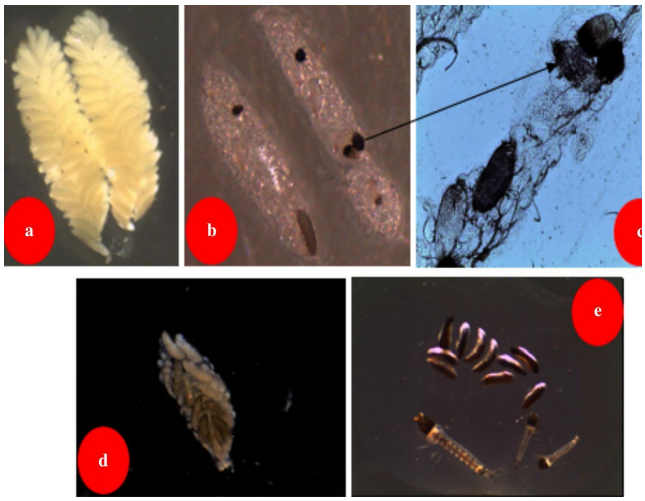

Figure 2 Impact of *C. violaceum* infections on ovarian follicles and fertilized egg maturations in *An. coluzzii* mosquitoes. Legend: (a) Eggs of an uninfected female; (b-d) Follicles and fertilized eggs of infected female with *C. violaceum*; (e) Non-viable eggs and larvae of an infected female [82].

Lethal Effects of a Fungal Biopesticide in the Control of Malaria Vectors

Even though chemical insecticides have recorded huge success in the control of malaria vectors (*Anopheles*) for decades, many malaria vectors have developed resistance to chemical insecticides including *Anopheles arabiensis* [91]. However, some studies have revealed that many mosquitoes vector that are resistant to chemical insecticides are fully susceptible to fungus infection. In order to attest these claims, Blanford *et al*. [92] conducted a research on the lethal effects of a fungal biopesticide in the control of malaria vectors. In their efforts to assess the impact of the fungal biopesticide on biopesticide on mosquitoes resistant to chemical insecticides, two bioassays were run against thirteen colonies of *Anopheles* mosquitoes comprising three different species (*An. gambiae* s.s., *An. arabiensis* and *An. funestus*). The colonies were assessed for their resistance to four compounds (an organophosphate, organochloride, pyrethroid and carbamate) at discriminatory doses prescribed by WHO [93] using a standard cylinder assay.

Following the standard WHO cone test methodology [93]. Blanford *et al*. [92] exposed adult female *Anopheles stephensi* mosquitoes to clay tiles sprayed with an oil formulation of spores of the entomopathogenic fungus *Beauveria bassiana*. In their reports, it was reported that once mosquito vectors are infected with fungus, they are less inclined to feed. They reported that the effect on *Anopheles* mosquito vectors appear severe as the fungal infection progresses but can contribute to great reductions in host feeding as early as the day two of treatment, essentially accelerating the transmission blocking effects of the fungus. In addition to their reports, their study revealed that the infection of fungus increases mosquito metabolic rate and reduces flight stamina and flight propensity. Also, fungal induced reductions in flight performance and elevated metabolic rate have been shown previously in other insects [94] and poor flight performance has been keenly associated with reductions in the mobile energy reserves of the host [94].

The study of Blanford *et al*. [92] on the evaluation of multiple mosquito strains and species covering diverse mechanisms and expressions of insecticide resistance showed that insecticide resistance confers no cross-resistance to fungal pathogens in the key African malaria vectors. Their results agree with the previous study of Ranson *et al*.,[14] and contrasts the situation with chemical insecticides where there are major problems of cross-resistance, undermining the potential of

many resistance management strategies [96, 14]. The study of Blanford *et al*. [92] also revealed that the co-exposure to fungus and insecticide can increase the susceptibility of otherwise resistant mosquitoes to existing chemical insecticides Blanford *et al*. [92]

In addition to the effect of fungi biopesticide in the control of malaria vectors, Heinig *et al*. [89] assessed the impact of realistic diurnal temperature variation on fungal virulence. Their results revealed that across the mean temperatures associated with malaria transmission, the fungal pathogen *B. bassiana* has the ability to kill two of the primary malaria vectors from Africa and Asia (*An. gambiae* and *An. stephensi* respectively) well within the estimated extrinsic incubation period (EIP) for both *Plasmodium falciparum* and *Plasmodium vivax* malaria. Their reports agree with some previous studies, highlighting the significance of thermal ecology for understanding insect-pathogen/parasite interactions and predicting the success of microbial biocontrol agents Blanford *et al*. [92].

Insecticidal Efficacy of *Metarhizium anisopliae* Derived Chemical Constituents against Disease-Vector Mosquitoes

As far as insect pest management is concerned, entomopathogenic fungi, bacteria, and nematodes has been one of the most effective control measures for insect pests [9798]. In a report of the studies of Bojke *et al*. [99] and Zhang *et al*. [100], entomopathogenic fungi are excellent source of secondary metabolites that are useful in the biopesticide development. One of the notable entomopathogenic fungi that can be used as biopesticide is *Metarhizium anisopliae*; the seconday metabolites of this fungus are effective in the control of *Aedes aegypti* as reported by Li *et al*. [101], Islam *et al*. [102] and Vivekan and han *et al.* [103]. Apart from the report of *Metarhizium* sp. as biopesticides, other fungi including Beauveria [97], Fusarium [99,104], and *Lagenidium giganteum* [105] have all been reported to have negative effect against insect pests. In an effort to investigate the efficacy of entomopathogenic fungi against disease-vector mosquitoes, Vivekan and han *et al*. [103] conducted a study on the toxicity of secondary metabolites extracted from *Metarhizium anisopliae* strains against *Anopheles Stephensi* [106]. In their result, it was reported that the mortality of *An. stephensi* larvae varied from 10.33 to 85.33%, for pupae, 8.33 to 70.33%, and adult (from 4.33 to 58.66%). They reported that the larvae of *An. stephensi* were more susceptible than the pupae and adult when exposed to the crude metabolites of *M. anisopliae*. They added ttat the secondary metabolites of *M. anisopliae* showed high toxicity towards the larvae, pupae and adults of *Anopheles stephensi* mosquitoes at 24 h post treatment under laboratory conditions. Similar results have earlier been reported by Vivekan and han *et al.* (2022). Also, the studies of Morales-Rodriguez and Peck [97], Bojke *et al*. [104], all reported that *Metarhizium anisopliae*, *Aspergillus flavus*, *Fusarium oxysporum*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Beauveria bassiana* (Figure 2), *and Fusarium moniliforme* toxins have been shown to secrete remarkable mosquitocidal efficacy on larvae, pupae, and adult mosquitoes.

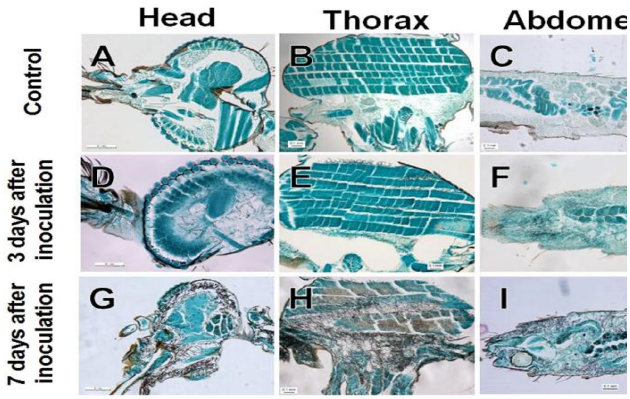

Figure 2: Images of uninfected and ***Beauveria bassiana*** conidial infected mosquito body parts. (**A‧C) Control:** ***Anopheles stephensi female*** adult without fungal infection. (**D‧I) Fungal infection of body parts in *An. stephensi female adults at (D‧F) an*** early stage of infection (3 days after inoculation) and (**G‧I) a later stage of infection (7 days after inoculation).** (**A,D and G) head; (B,E and H) thorax; and (C,F and I) abdomen. Mosquito tissues and organs were stained** with fast green, and fungal propagules were specifically stained (in black) with Grocott stain [107].

Table 2: List of some fungi entomopathogenic microorganism strains investigated as biopesticides for the control of mosquito vectors.

Entomopathogenic Microorganisms Used	*Anopheles* species	Developmental Stages	Results	References
Metarhizium anisopliae ethyl acetate crude extract	*Anopheles stephensi*	Larvae, pupae, and adult	High toxicity towards the larvae, pupae and adults of *Anopheles stephensi* mosquitoes.	103
Metarhizium anisopliae	*Anopheles stephensi*	Larvae	Larvae of *A. aegypti, An. stephensi* were found susceptible in laboratories and fields.	108
Beauveria sp.	*Aedes aegypti*, *Anopheles stephensi*	Larvae, pupae, adult	The introduction of *Beauveria* sp. caused high mortality among the mosquito populations tested.	97

Beauveria bassiana	*Anopheles stephensi*	Adult	Increased the mosquito metabolic rate and reduced flight propensity and flight stamina. Also, the fungus infection induced reductions in flight performance.	48.
Beauveria bassiana	*Anopheles gambiae*, *Anopheles stephensi*,	Adult	Killed two of the major malaria vectors from Asia and Africa (*An. stephensi* and *An. gambiae*, respectively) well within the estimated extrinsic incubation period (EIP) for both *Plasmodium falciparum* and *P. vivax* malaria.	18

Challenges with the use of Entomopathogenic Microorganism as Biopesticide against Mosquito vector

Despite intensive efforts to develop entomopathogenic microorganisms as biocontrol agents against malaria vectors, the strains under investigation have not met expectations due to some functional and practical limitations. Bacteria such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) infections, for instance, show no residual persistence post application. *Beauveria bassiana* is very promising fungi with residual potency. However, only a few studies address the effect of different microbial agents on malaria vectors. Most of these studies are only experimentally approached without any further practical applications. Besides, products based on these strains would require repeated production and application. Owing to the specificity of the action, microbes may control only a portion of the pests present in a field and may not control other type of pests present in treated areas.

Until now, several studies have been conducted on the efficacy of entomopathogenic microorganisms including bacteria and fungi in the control of mosquito malaria vectors. However, several studies including the studies of Blandford *et al*. [48], Heinig *et al*. [18], and Vivekan and han *et al*. [103] have reported the success of entomopathogenic fungi, particularly *Metarhizium anisopliae and Beauveria* sp. in controlling malaria vectors. In their reports, it was revealed that *Beauveria bassiana* infection contributed to great reductions in host feeding in *Anopheles* mosquitoes. Also, in their studies, it was revealed that the infection of *Anopheles* mosquitoes with fungus increases mosquito metabolic rate and reduces flight stamina and flight propensity. It is believed that the transmission of malaria parasite depends greatly on the flight ability and the host seeking behavior of *Anopheles* mosquitoes. So, with the reduction in the flight ability and host seeking behavior, the trend of transmission in *Anopheles* mosquitoes can be easily reduced.

However, the use of spores and mycelia of fungi recorded more success than the extracellular metabolites in mosquitoes larvae. Using selective media, the Deuteromycete fungus *Metarhizium anisopliae* is broad host • range insect pathogens that can be isolated from most soil without any hassle Heinig *et al*. [18] reported that the invasion of conidia depend on some factors including moisture, temperature and specific nutrients in the host cuticle. But the aggressiveness of the fungal isolate varies according to strain and different levels of proteolytic, lipolytic and chitinolytic activity.

On the other hand, entomopathogenic bacteria, according to some reports can also cause significant mortality in *Anopheles* mosquito populations [63, 87]. According to their reports, *Chromobacterium violaceum* is capable of causing reduction of insemination rates and fecundity in females *Anopheles* mosquitoes, particularly *Anopheles coluzzii*. The transmission of malaria parasite (*Plasmodium* sp.) also depends significantly on the reproduction rates of malaria vectors. The decrease in the malaria vector populations will cause significant reduction of the outbreak of malaria diseases among susceptible hosts.

CONCLUSION

This review has elucidated recent scholars' contribution
to the entomopathogenic effect of some microbial organisms namely Bacteria and Fungi on malaria vectors. This review shows that some entomopathogenic microorganisms, particularly fungi and bacteria have the potentials to inhibit and impede several activities of malaria vectors such as the host-seeking behavior, flight ability, insemination rates and fecundity. In this review. Bacteria strains such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* are yet to show persistence in post application. It has become a global fact that in the fight against malaria a 'magic bullet' does not exist. The disease can only be controlled by the coordinated deployment of as many weapons as possible. More efforts are needed to exploit these faunas and their metabolites in the development of novel pesticidal agents against the malaria vector, *Anopheles* mosquitoes.

ACKNOWLEDGEMENTS

Our profound gratitude goes to Prof. I. C. J. Omalu of the Department of Animal Biology, Federal University of Technology, Minna for the contribution to the success of this work.

Conflict of Interest: None

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