# THE ENTOMOPATHOGENIC FUNGI FOR MALARIA VECTOR CONTROL

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## Abstract

Malaria is a global health problem and its burden is high in the sub-Saharan countries. The mainstay control of malaria is by use of chemical insecticides against malaria mosquitoes. However, resistance to pyrethroids has been reported in both *Anopheles gambiae* and *Anopheles funestus* in many malaria endemic countries in Africa. Additionally, parasite resistance has rendered previous antimalarial medicines ineffective in most parts of the world, threatening malaria control. These scenarios call for alternative strategies for malaria control. To date, many successful laboratory studies have demonstrated the potential of using entomopathogenic fungi against malaria mosquitoes.

**Keywords:** Entomopathogenic fungus, malaria, vector, Anopheles, conidia, biological control

## 1. INTRODUCTION

Malaria is endemic in 91 countries and territories, with the highest prevalence in sub-saharan Africa, where 43 countries are still endemic for the disease despite of the decline in infection rates from 22% in 2005 to 17% in 2010, and to 13% in 2015 (WHO, 2017). Malaria is transmitted to humans through bites of infected female Anopheles mosquitoes (WHO, 2017). Malaria parasite species that infect humans include: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium Ovale* and *Plasmodium knowlesi*. *Plasmodium vivax and P. falciparum* are the most life-threatening parasites responsible for most malaria-related deaths globally and dominant in most countries outside sub-Saharan Africa, respectively (WHO, 2017).

Females of *Anopheles gambiae* sensu lato (*An. gambiae and An. arabiensis*) and *An. funestus* are the principal vectors of malaria in most African countries (Coetzee, 2004; Okara *et al*., 2010). Plasmodium parasites undergo sexual reproduction inside the mosquito and need to develop in the insect for several days, generally 10 - 14 days (Koella *et al*., 2009), the duration within which symptoms appear in non-immune people after an infective mosquito bite (WHO, 2017). A malaria vector must therefore survive longer than the extrinsic incubation period of the parasite, for successful transmission of the Plasmodium pathogen (Beier, 1998). Malaria transmission can be blocked by shortening the life span of mosquitoes (MalERa C group, 2011). In the year 2016, there were 216 million (90%) cases of malaria in 91 countries, an increase of 5 million cases over 2015, and 445,000 malaria attributed deaths in the African continent (WHO, 2017), where 15 countries in sub-Saharan except India, carried 80% of the world malaria burden. Young children and pregnant women are the most vulnerable (Greenwood and Mutabingwa, 2002; WHO, 2010; 2017).

Control of malaria mosquitoes relies mainly on indoor residual spraying (IRS) and use of insecticide-treated bed nets (ITNs) while control of Plasmodium parasites especially the falciparum-malaria is by use of Artemisinin-based combination Therapy (ACT) and its derivatives (Enayati *et al*., 2010, WHO, 2017). However, sustainable use of chemicals is undermined by the following problems: resistance of parasites to antimalarial medicines and resistance of adult mosquito populations, environmental contamination and risks to human health

(Hargreaves *et al*., 2003; Kikankie *et al*, 2007, WHO, 2017) hence, threatening malaria control (WHO, 2009; 2017). These problems therefore have increased the interest in alternative and integrated implementation of vector control strategies to include biological control.

Entomopathogenic fungi, microorganisms that attack insects while using them as hosts for development of part of their life cycle (Delgado and Murcia, 2011), are non mobile organisms (Badii and Abreu, 2006) which have been in use for more than 100 years as biological control agents in research studies (Mora *et al*., 2017). Although several effective biological larvicides exist (Fillinger *et al*., 2003) there have been no biological agents effective against adult mosquitoes. To address this gap, several laboratory and small scale field studies have demonstrated the potential of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* to infect and kill adults of Anopheles, Aedes and Culicine mosquitoes (Farenhorst *et al*., 2009). A study that involved trials with cotton sheets impregnated with fungal spores and suspended in some rural Tanzanian houses showed that mosquitoes rested on the sheets and were infected with fungus. According to Scholte *et al.*, (2005), survival of mosquitoes decreased due to infection with the entomopathogenic fungi.

Furthermore, they estimated the number of infective mosquito bites per person per year and found that there was a drop from 262 bites to 64 bites. Increasing the coverage of mosquito resting sites could bring this number down to ten (Michalakis and Renaud, 2005). Additionally, theoretical studies have demonstrated that the relatively slow speed of kill of entomopathogenic fungi are sufficient to impact on transmission of malaria because the extrinsic incubation period of the Plasmodium parasite within the mosquito (10-14 days in high transmission settings) creates a window of several days for the fungus to act (Hancock, 2009; Koella *et al*., 2009).

A successful control of malaria vectors using the entomopathogenic fungus ensures that the host or mosquito contacts a treated surface and receives a sufficient dose of infectious conidia when doing so (Hughes *et al*., 2004), otherwise the vectors might escape the negative effects of fungal infection (Hancock, 2009). Several studies show that fungal infections have been induced by exposure of mosquitoes to various conidia-treated substrates (Garcia-Munguia *et al*., 2011).

Delivery tools are essential factors to be considered before entomopathogenic fungus can be integrated into a control programme. These could be used to target either host-seeking and or resting mosquitoes. Most houses in Africa are built with open eaves to allow flow of air within houses. However, it is through these eaves that mosquitoes enter houses (Charlwood *et al*., 2003; Njie *et al*., 2009).

Host-seeking mosquitoes could thus be targeted when entering a house through the eaves (Njie *et al*., 2009) or when attacking a host sleeping or resting under a bed net (Hancock, 2009).

## 2. LITERATURE REVIEW

### *2.1. Transmission of Malaria*

Malaria is a disease that involves an interaction between the *malaria* mosquitoes, the parasite, the hosts and the environment. Malaria is caused by protozoan parasites of the genus Plasmodium namely, *P. falciparum, P. vivax, P. malariae* and *P. ovale*. A fifth human parasite *P. knowlesi* was discovered with an animal reservoir (Cox-Singh *et al*., 2008; Luchavez *et al*., 2008). Plasmodium parasites are transmitted through bites of female adult mosquitoes belonging to the genus Anopheles (Diptera: Culicidae). Principal malaria vectors in most African countries are *An. gambiae, An. arabiensis* and *An. funestus*. Their dominance as malaria vectors is largely due to preference for human blood, high vector competence and high daily survival rates. *Anopheles gambiae* and *An. arabiensis* are sympatric; however, *An. arabiensis* is more widely distributed in drier areas whereas *An. gambiae* prefers humid areas (Okara *et al*., 2010).

#### *2.1.1. Behaviour of adult mosquitoes*

*Anopheles gambiae* and *An. funestus* are anthropophilic, biting almost exclusively humans (Constantini *et al*., 1999) although in West Africa, *An. gambiae* are less discriminating and will feed readily on other animals like horses and cattle (Bogh *et al*., 2001). Seasonal changes in *An. gambiae* populations tend to follow the seasonal pattern of rainfall (Gillies and De Meillon, 1968). *Anopheles funestus* is highly anthropophilic and endophilic making it susceptible to control by indoor residual spraying (Gillies and DeMeillon, 1968). *Anopheles arabiensis* on the other hand have a wide distribution in Africa. Their range and relative

abundance tend to be influenced by climatic factors, especially total annual precipitation. They tend to be more tolerant to high temperatures and are able to survive in drier conditions, which explains why they are found biting during the dry season (Coetzee *et al*., 2000). *Anopheles arabiensis* may feed on humans as well as on animals, depending on the availability of both hosts (Constantini *et al*, 1999; Tirados *et al*., 2006).

Survival and reproductive success of mosquitoes depend on a series of characteristic behaviours such as mating, foraging and oviposition (Takken and Knols, 1999). These behaviours are governed by internal and external factors to which the mosquitoes respond to in a particular manner. To locate food and oviposition sites successfully, insects must integrate visual and olfactory cues. Interactions between olfactory and visual cues guide the insects during this search behaviour. Vision is considered more important in diurnally active mosquitoes while physical and olfactory cues are important for nocturnal species (Takken and Knols, 1999).

Dispersal in insects fits five primary needs: finding a resting site, finding a mate, finding a nectar source, finding a host and finding an oviposition site. Mosquitoes mate within 24 – 48 hours after adult emergence. Mating occurs once in the entire life of most mosquito species. However, multiple mating has been reported in some Anopheles females. In Anopheles species, males form swarms and females entering the swarms are recognized by their lower wing-beat frequency (Brogdon, 1998).

Sugar is the basic food of adult mosquitoes, and it is the only nutrient consumed by male mosquitoes (Foster, 1995). The sugar meal provides energy for female mosquitoes during host seeking flight and enhances reproduction (Foster, 1995). Mosquitoes are believed to locate sugar sources by odours emitted by flowering plants (Foster and Takken, 2004). Female mosquitoes require a blood meal for their gonotrophic development. Each blood meal leads to the development of a batch of eggs. In most species, females take blood after mating while in others species, blood feeding precedes mating (Charlewood *et al*., 2003). Host-seeking behaviour of malaria vectors is influenced by human odour; emanations from skin, sweat and

breath greatly influence this behaviour (Constantini *et al*., 1999). Physical cues such as body heat and temperature also play a role in mosquito host seeking behaviour.

*2.1.2. Tools to interrupt transmission of malaria*

Measures against malaria can either target Plasmodium parasites or adult malaria vectors, *An. gambiae, An. arabiensis* and *An. funestus*.

*2.1.2.1 Antimalarial drugs and vaccines*

Antimalarial drugs play an important role in treating and controlling malaria. In the past five years, treatment of uncomplicated malaria had transformed to the use of artemisinin combination therapies (ACTs) (WHO, 2009). Efficacy of antimalarial drugs is however undermined by the emergence of drug resistant strains of Plasmodium parasites (Ntoumi, 2004; WHO, 2010). The highly effective artemisinin derivatives and their partner drugs are vulnerable to the same risk (Talisuna *et al*., 2004; Dondorp *et al*., 2010). Next to antimalarial drug use, development of vaccines is underway. Despite good progress with certain candidate vaccines (Abdulla *et al*., 2008), there is still no effective vaccine for malaria available to date (Winzeler, 2008).

*2.1.2.2 Use of insecticides against adult malaria vectors*

The objectives of malaria vector control are two-fold: to protect people against infective malaria mosquito bites by reducing vector longevity, vector density and human-vector contact and to reduce the intensity of local malaria transmission at community level and hence the incidence and prevalence of infection and disease (WHO, 2010). There are four classes of insecticides approved for use in public health interventions namely, Carbamates, organophosphates, pyrethroids and organochlorides (WHO, 2010).

Pyrethroids and an organochloride namely, Dichlorodiphenyltrichloroethane (DDT) are the most important and widely used insecticides against malaria vectors (Nauen, 2007). Insecticides act on the insect's central nervous system by blocking neuronal activity thus causing rapid paralysis and death. Resistance to these insecticides has been reported in key vector species such as *An. gambiae* (Ransom *et al*., 2009; Yadouleton *et al*., 2010), *An. funestus* (Brooke *et al*.,

2001; Wondji *et al*., 2009) and *An. arabiensis* (Abdalla *et al*., 2007). Resistance can be as a result of enhanced metabolic degradation of the insecticide by specific enzymes (Hunt *et al*., 2005).

Hunt *et al.*, (2011) confirmed that resistance to one member of a class of insecticides is good evidence that resistance to other chemicals in the same class will occur. The occurrence of insecticide resistance in insect disease causing vectors and even in agricultural pest species poses potential and actual hindrances to successful insect control (Corbel *et al*., 2007). Pates and Curtis, (2005) have shown that mosquitoes can select for certain behaviorally resistant traits, such as earlier mosquito feeding times and earlier exiting from houses with treated nets. Data clearly indicate that insecticide resistance is widespread and often at very high frequencies, usually sufficiently high to preclude the use of the few insecticides approved by WHO for malaria control (Hunt *et al*., 2011). These problems have increased interest in alternative and integrated implementation of vector control methods that include biological control.

There are at present no effective biological control tools for adult mosquitoes. Methodologies that target the adult stage of mosquitoes continue to depend exclusively on the use of chemical insecticides (Chandra *et al*., 2008), whose continual effectiveness is threatened by mosquitoes developing resistance to the synthetic insecticides (Ransom *et al*., 2009). Combined with safety and environmental concerns of insecticide use (Cohn *et al*., 2007; Roberts, 2001), there is need to develop novel malaria control strategies that can reliably and sustainably be used to complement or replace the existing control measures.

*2.2. Entomopathogenic fungi for control of malaria mosquitoes*

Entomopathogenic fungi are defined as those fungi which have been shown to be pathogens of insects or cause some level of harm within insects (Samson *et al*., 1988). The longevity of the adult mosquito has the greatest impact on the vectorial capacity, and transmission intensity of a mosquito population. This can be targeted using biocontrol agents, which ensure that mosquito resistance does not readily develop due to the slow speed of kill (Thomas and Read 2007a, Read *et al*., 2009). Biopesticides that can therefore be used to control mosquitoes and other'

insect pests include plants, fish, nematodes, viral, bacterial, protozoan and fungal pathogens (Scholte *et al*., 2004a). Of these, Hyphomycetous entomopathogenic fungi are the most well suited for development as biopesticides against vector mosquitoes (Thomas and Read, 2007; Read *et al*., 2009) and have been evaluated for mosquito control purposes.

*Classification of fungi*

Fungi pathogenic to insects are found in the following taxonomic groups: Chytridiomycota, Zygomycota, and Deuteromycetes (Hyphomycetes) (Kierk *et al*., 2001). Eighty-five genera and over 750 species of entomopathogenic fungi are known. Two genera, Beauveria and Metarhizium (which belong to the class Hyphomycetes in Deuteromycota) and 12 species of fungi are being used as ingredient for myco-insecticides or myco-acaricides (DeFaria and Wraight., 2007). The most used species are *M. anisopliae* and *B. bassiana*. These two species have a worldwide distribution, occurring naturally in soils (Scholte *et al*., 2004a).

*2.2.1. Safety and environmental effects of entomopathogenic fungi*

The application of fungal spores has raised concerns over the potential health risks especially when applied indoors (Kanzok and Jacobs-Lorena, 2006; Ward & Selgrade, 2005). At present, this risk is considered low due to the opportunistic nature of the pathogen, inability to survive at human body temperatures and formulation of conidia in mineral oil (such as Ondina, Shellsol and Enerpar currently known as Castrol WOM 14 oil. Oil may give increased spore contact and adhesion to an insect's cuticle (Lomer *et al*., 2001), Suspension of conidia in oil allows effective spore application in extremely arid conditions e.g. in the Sahel (Langewald *et al*., 1999) by providing essential moisture for the developing fungus (Lomer *et al*., 2001; Prior and Greathead, 1989). Use of oil also maintains viability of stored fungi above that of dry conidia (Batta, 2003) and at high temperatures (Thomas *et al*., 1995). Oil formulations may also improve spore survival by enhancing tolerance to UV irradiation (Alves *et al*., 1998). These may indicate that different oils can extract or dissolve different components from the cuticle to promote fungal growth, and this may be another consideration in formulation choice. The fungus could be an efficient alternative to use of chemical insecticides (Mora *et al*., 2017)

In laboratory studies, two Beauveria species (*B. bassiana* and *B. brongtiiartii*) were found in surface-drinking water in Norway but not in underground water (Hageskal *et al*., 2006). Airborne spores of common fungi such as Aspergillus species, Penicillium species and Fusarium species are abundant indoors and exposure to fungal spores is commonplace both in tropical and temperate climates (Simon-Nobbe *et al*., 2008). It is therefore thought that applications of Beuveria or Metarhizium spores indoors would not significantly increase exposure levels (Thomas *et al*., 2005). *Beauveria bassiana* was cultured from corneal scrapings from a male who had suffered abrasion to the eye (Sachs *et al*., 1985).

No adverse effects, infection or distress was reported following inhalation, oral or eye exposure, or following subcutaneous or intraperitoneal injections of rats, mice and rabbits with either dry spores or conidial suspensions of *M. anisopliae* or *B. bassiana* (e.g. Zimmermann, 2007). Within treated chicken houses in the USA, *B. bassiana* had no adverse effects on house fly pupal parasitoids nor coleopteran predators (Kaufman *et al*., 2005). Potential allergens have been identified in some fungi (Westwood *et al*., 2006) but there are no reports of asthmatic conditions amongst workers applying fungi (Roberts, 1977), nor any reports of harmful effects in those who have used *M. anisopliae* and *B. bassiana* for indoor pest control. Based on toxicity tests of *B. bassiana* strain GHA in various animals, this fungus poses no risk to humans and the use of this fungus does not result in toxin levels harmful to the environment (Strassser *et al*., 2000; US Environmental Protection Agency, 2006). *Beauveria bassiana* has been identified as a medically important fungal pathogen on recipients. For example, its extracts have anticoagulant and immune system modulating activities, which could provide beneficial physiological activities for humans (Yoon *et al*., 2003b). This fungus could also be used as an additive to wheat flour for preparation of bread and noodle (Yoon *et al*., 2003a). *Beauveria bassiana* itself or fungus diseased larvae of Bombyx mori have been used as medicine for hundreds of years in China.

**Advantages of using *B. bassiana* and *M. anisopliae* fungi**

These fungi are host specific, can be cost-effectively mass-produced even locally, they can readily live as saprophytes in nature, and do not require ingestion by the host (Michalakis and Renaud, 2005, Zimmermann 2007a, b). The effect of *B. bassiana* and *M. anisopliae* on

mosquitoes is not immediate; they infect and kill adult mosquitoes slowly through external contact (Blanford *et al*., 2005; Scholte *et al*., 2007). Compared to insecticides, fungi have low virulence as they kill an insect in 6-14 days after infection depending on the fungal species and isolate used. The slow killing mechanism of the fungus imposes a limited selection pressure on the mosquitoes, thus reducing the likelihood of anti-fungal resistance (Knols and Thomas 2006; Thomas and Read 2007a).

Fungal spores of *B. bassiana*, whose viability is high, are of good quality in terms of persistence and virulence compared to *M. anisopliae*. This makes it highly competitive and an attractive alternative (Mnyone *et al*., 2009; Howard *et al*., 2010). Results of experiments carried out in the laboratory using Anopheles mosquitoes show that an insecticide-resistant mosquito infected with *B. bassiana* and *M. anisopliae* becomes sensitive again to the insecticides (Farenhorst *et al*., 2009). Therefore, there is a synergistic effect in using both the fungi and indoor residual spraying (fIRS) with insecticides.

*2.2.2. The fungal infection process*

The host cuticle is the first line of defense against infection and has a central role in determining fungal infectivity. The entomopathogenic fungi infect mosquitoes through direct contact with the cuticle (Mora *et al*., 2017). The fungi penetrate thinner, non-sclerotised areas of the cuticle, like joints, between segments, or the mouthparts, and through tarsal contact (Vega *et al*., 2012). First, conidia (spores) adhere to the insect cuticle (see Figure 1) by use of adhesive mucus secreted as the conidium swells during the pre-germination period (Boucius and Pendland, 1998). Conidia then germinate on the insect cuticle (Khachatorians, 1991). Before penetration, germ tubes produce appressoria (penetration structure; see Figure 1) and infection pegs. The cuticle is multilayered and tough but fungus is able to surmount this obstacle by mechanical means and by action of cuticle-degrading enzymeswhich include proteases, chitinases and lipases (Monzón, 2001; Vega *et al*., 2012), aided by secretion of the organic acids, oxalic acid (Khachatourians, 1991). In order to overcome the insect's defense mechanisms, the fungus synthesizes proteases that degrade the humoral immune system and cyclic depsipeptides such as destruxins, that paralyses the insect (Pal *et al*., 2007)

Fungus grows by vegetative growth in the insect haemocoel. They absorb and deplete nutrients and the fat body and finally release toxins which lead to destruction of insect cells (Clarkson and Chamley 1996). The blastospores (yeast-like cells as shown in Figure 1) are responsible for circulation within the insect haemolymph and toxin production (Shah and Pell, 2003). Granulocyte numbers dramatically reduce three days after fungal challenge (Hajek and St. Leger, 1994). Infected mosquitoes become unable to feed and move hence die before they have a chance to mature and pass on the malaria parasite (Pal *et al*., 2017). After the insect's death and under favourable humidity and temperature, the fungus emerges from the dead host and sporulation or conidiogenesis occurs on the outside of the cadaver (Shah and Pell, 2003;Srivastara *et al*., 2009).

Fungus that grows on the outside of the insect produces aerial conidial spores can be harvested and recycled (Eyal *et al*., 1994).

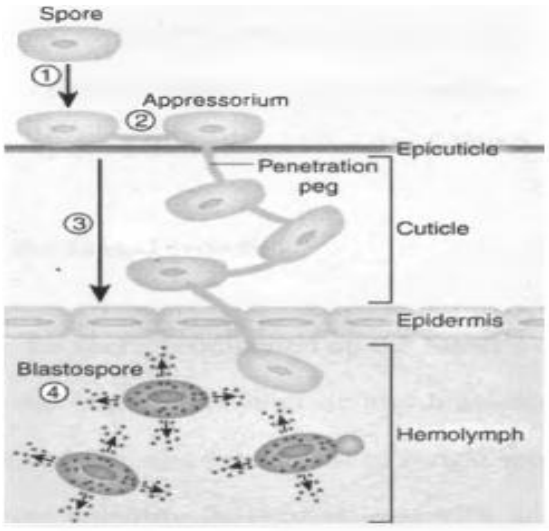


Figure 1: Mode of action of entomopathogenic fungi on contact with the arthropod host (source: Thomas and Read 2007b).

*2.2.3. Conditions favorable for fungal growth*

For microorganisms to propagate and survive in the environment, certain abiotic factors should be considered. The most important factors are temperature and humidity or moisturecontent. Each of these factors is as discussed below:

*2.3.1 Effect of temperature on fungal growth*

This influences germination, growth and viability of the fungus on and in the host insect and in the environment. High temperatures may inactivate an entomopathogen before contact with the insect or may reduce or accelerate growth within an insect. In contrast, low temperatures may reduce or stop germination and growth, thus impair or prolong a successful infection. Therefore, the optimal growth temperature for most isolates is 27-28°C (Tefera and Pringle, 2003), although some exceptions of cold-resistant and heat- resistant isolates have been reported (Boucias and Pendland, 1998).

*2.3.2 Relative humidity for fungal growth*

High moisture is necessary for spore germination on the insect's cuticle and sporulation after death of a mosquito. On the 'other hand, high or low humidity in conjunction with high temperature may affect the viability and persistence of fungal spores. Generally, germination of conidia requires a relative humidity (RH) of at least 97% and they survive longest at a combination of moderate temperatures and high relative humidity (26°C-97% RH or 19°C-97% RH) or low temperatures and low relative humidity (4°C-0% RH). When relative humidity is high, conidia can be quite tolerant to high temperatures although its viability decreases rapidly when exposed to UV light (Hallsworth and Magan, 1999).

*2.2.4. Effects of fungi on adult mosquitoes*

The sub-lethal fungus infections might possibly reduce both the probability of a female mosquito engaging in host-seeking behaviour and blood feeding, and it might also reduce the probability of a gravid female searching for, locate, and reaching a suitable oviposition site. In the case of malaria epidemiology, a reduction in the number of bites per individual mosquito will reduce the risk of malaria transmission (Scholte *et al*., 2006). Fungi have a direct effect on adult mosquitoes

and development of Plasmodium in mosquitoes (Blanford *et al*. 2005). This was demonstrated in a laboratory experiment using mouse malaria as a model system. Blanford and colleagues observed that only 8% of mosquitoes infected with both the parasite and fungi contained transmissible parasite offspring 14 days after exposure to fungi, compared with 35% of mosquitoes infected with Plasmodium alone (Blanford *et al*. 2005). Combining the effects on mosquitoes and Plasmodium development, fungi could reduce malaria transmission by approximately 80-fold. The effect might even be greater, given that fungal infection also decreases the propensity of infected females to feed on blood (Blanford *et al*., 2005).

Laboratory trials (Farenhorst *et al*., 2008; Mnyone *et al*., 2009) and small scale field trials (Scholte *et al*., 2005; Lwetoijera *et al*., 2010) show that malaria vectors are susceptible to entomopathogenic fungal infection. Successful infection of adult mosquitoes with the entomopathogens depends on conidial formulation, the dose, exposure time and the fungal Strain used (Blanford *et al*., 2005; Thomas and Read, 2007). Previous studies have used many different combinations of formulations or substrate to demonstrate the effectiveness of the entomopathogenic fungus to infect and kill mosquitoes (Howard *et al*., 2010). However, not all of the application methods previously used can be deployed easily in the field. It is therefore important to test potential methods that can be used to disseminate fungus to large populations of wild mosquitoes in the field.

Laboratory experiments by Scholte *et al*., (2005) demonstrated the potential of oil-based formulations of fungal entomopathogens to reduce malaria transmission; this alters the survival or maturation of Plasmodium in mosquitoes. Oil-formulated conidia are more efficacious than water-formulated conidia. Therefore, conidia are formulated in oil in order to avoid the repelling effect of conidia towards adult mosquitoes (Mnyone *et al*., 2010), because oil offers better adhesion and spreading of the formulation on the insect cuticle. Furthermore, the oil film formed on the insect cuticle creates good conditions for fungal invasion and germination in the host (Luz and Batagin, 2005). Oil can also improve the tolerance of conidia to extreme temperatures. Another benefit of using oil is that it prevents conidia from free dispersion in the air and thus

reduces the probability of flying mosquitoes and humans getting in contact with it (Scholte *et al*. 2003; 2004a; Darbo and Thomas, 2009).

The propensity to select humans for blood feeding is arguably the most important component of mosquito vectorial capacity (Zwiebel and Takken, 2004) as it determines the oviposition, mating and blood-feeding success. It takes ten days for ingested gametocytes to develop into infective sporozoites, with estimated values of 3.4-5.8 for the average number of feeding cycles required for parasite development (Killeen *et al*., 2000). Results obtained by Scholte *et al*., (2006) indicate that if female malaria mosquitoes become infected with fungus early in-their lives, it becomes far less likely that they will transmit malaria.

A laboratory study in which infected female mosquitoes were blood fed by arm in small cups revealed that fungal infection reduces (but does not eliminate) feeding propensity and fecundity (Ffrench-Constantino *et al*., 2005). Reduction in feeding upon infection with fungus might be attributed to degradation of tissues (including the midgut) in combination with the production of secondary metabolites (Amiri *et al*., 1999; Scholte *et al*, 2006). If a mosquito survives certain time periods without blood feeding before it dies, it remains, in epidemiological sense, inactive as a vector (Hajek and Saint Leger., 1994; Scholte *et al*., 2006). In another laboratory study, adult female *An. gambiae* mosquitoes that were three days old after infection with fungus (*M. anisopliae*) showed a decrease in feeding propensity (Ondiaka *et al*., 2008). Elsewhere, reduced host-seeking behavior was observed in fungus-infected mosquitoes (Blanford *et al*., 2011). Such an impact on mosquito behavior will result in a reduction of female lifetime vectorial capacity and hence malaria transmission risk.

The primary effect of fungus in increasing mosquito mortality substantially reduces the probability of mosquitoes survival to any given feeding cycle. This therefore reduces the probability of a fungus-infected mosquito reaching the infectious state (Scholte *et al*., 2006). This was verified in a study by Blanford *et al*., (2005) in which mosquitoes infected with the fungus *B. bassiana*, next to having a high fungus-induced mortality, expressed a strong inhibitory effect on *P. chabaudi*. Scholte *et al*., (2006) observed an increase in mortality in *An.*

*gambiae* around the time of sporozoite maturation. In another laboratory study, mosquitoes were exposed to high conidial concentrations ($2x10^{10}$ and $4xl0^{10}$conidia/$m^2$) in a relatively short time (15-30 min). Results from this experiment showed 100% mortality of mosquitoes within 10 days (Farenhorst *et al*., 2008; Mnyone *et al*., 2009). The death of such vectors results to a reduction in their population (Mnyone *et al*., 2012).

Further laboratory experiments show that both *M. anisopliae* and *B. bassiana* could be used in resistance management and integrated vector management programmes to target insecticide-resistant and insecticide-susceptible malaria vectors (Howard *et al*., 2010; 2011). Insecticide-resistant mosquitoes being simultaneously bombarded with both fungus and an insecticide probably overwhelms the enzymes that normally make a mosquito resistant to the insecticide (Farenhorst *et al*., 2009), rendering a much lower risk of resistance development (Scholte *et al*., 2007). Another laboratory study shows that horizontal transfer of fungal inoculum between mosquitoes is possible during copulation and may contribute to spreading of the fungus within target mosquito populations in the field (Scholte *et al*., 2004). Lwetoijera *et al*., (2010) showed that effective use of odor-baited stations as a delivery method for the entomopathogenic fungus may be an option to target mosquitoes while they are outdoors. Biological control may therefore offer sustainable alternative tools for malaria control compared to use of chemical insecticides (Vázquez-Martínez *et al*., 2013). Further research should provides a useful and practical tool for applying fungi against wild adult malaria mosquitoes in the field. Additionally, information on how often surfaces need to be re-treated with fungus should be investigated. Such information may lead to reduction in malaria mosquito population, and eventual reduction in malaria transmission. The tools could be incorporated into integrated vector management programmes for control of malaria.

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