Fungal entomopathogens for the control of adult mosquitoes: a look at the issues

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Malaria is arguably the most serious vector-borne disease worldwide. The alarming number of deaths caused by malaria is increasing, which is partly due to the increase in mosquito resistance to chemical insecticides. Entomopathogenic fungi are a promising new biological tool for controlling malaria transmission. A few years ago several research groups discovered independently that the hyphomycetous fungi *Metharizium anisopliae* and *Beauveria bassiana* are able to infect and kill adult *Anopheles* mosquitoes. Besides reducing longevity, these entomopathogenic fungi were shown to reduce the development of *Plasmodium* parasites in the mosquito and negatively affect its feeding propensity and fecundity. Through decreasing both mosquito longevity and the number of mosquito bites, the implementation of fungi as biological control agents can potentially have a large impact on malaria transmission. In this article, we review the status of this novel approach, critical scientific issues and the prospects and requirements for developing this strategy to operational levels in disease-endemic countries.

Keywords: Anopheles, fungi, malaria, vector control, Africa

Malaria is one of the major public health challenges undermining progress and contributing to poverty in the world's developing countries. The disease causes more than 300 million acute illnesses and at least 1 million deaths annually (WHO 2005). Around 90% of these deaths occur in Africa, mostly in young children and pregnant women (WHO 2003). Malaria also presents major obstacles to social and economic development. The World Bank has estimated the cost of malaria in Africa to be more than US$ 12 billion every year in lost GDP (WHO 2003).

As anti-malarial drug resistance is spreading in developing countries (Schellenberg *et al.* 2006) and vaccines are not yet available (Graves & Gelband 2006), the possibilities of treatment are severely limited, increasing the need of other preventive measures. Vector control methods aim to reduce malaria transmission by shortening or interrupting the lifespan of mosquitoes. Epidemiological models predict that the most effective way to reduce malaria transmission is to target adult female mosquitoes (McDonald 1957). All methods currently deployed in tropical regions against adult mosquito populations that transmit malaria are based on insecticide application. These methods include the use of indoor residual spraying (IRS) and insecticide-treated bednets (ITNs). However, DDT and pyrethroid resistance in *Anopheles* mosquitoes have become widespread in different regions of Africa (Bloland 2001) and represents a serious threat for successful and sustainable implementation of IRS and ITN programmes. Therefore, there is an increasing need to develop novel malaria control strategies that can complement or replace existing control methods.

ENTOMOPATHOGENIC FUNGI

Metarhizium anisopliae and *Beauveria bassiana* are entomopathogenic fungi that have already shown their use in the control of several agricultural insect pests (Arthurs & Thomas 2000). These Hyphomycetes can infect and kill insects without being ingested. The spores of the fungi, called conidia, attach to the insect's external tegument in a passive and non-specific way (McCoy *et al.* 1988) and subsequently germinate and penetrate the cuticle. Once in the hemocoel, the mycelium grows throughout the host, forming hyphal bodies called blastospores. The fungi produce destruxins causing paralysis (Goettel & Inglis 1997) and insects die between three and fourteen days after infection, depending on species, size and fungal isolate. Under suitable conditions hyphae can emerge from the cadaver and produce conidia on the exterior of the host which can be dispersed by wind or water. Both *M. anisopliae* and *B. bassiana* are ubiquitous worldwide and comprise a large number of different strains/isolates which differ in their geographical origin or host specificity. Using these endemic fungi as a biological control method within their natural environment will therefore impose limited risk of disrupting fragile ecological equilibria.

FUNGI AS VECTOR CONTROL AGENTS

Studies have shown that *M. anisopliae* and *B. bassiana* conidia are pathogenic to adult *Anopheles* mosquitoes and significantly reduce their longevity. In the laboratory, many isolates were shown to induce mosquito mortality of more than 80% within 14 days of infection (Blanford *et al.* 2005). A field experiment in Tanzania demonstrated that cloths impregnated with oil-based *M. anisopliae* conidia, could infect 23% of female *Anopheles gambiae* mosquitoes resting on the cloths, shortening the average mosquito life span by 4 to 6 days compared to controls (Scholte *et al.* 2005). As mosquitoes cannot transmit sporozoites until about two weeks after an infectious blood feed, a more rapid killing of the mosquito is not necessary for reducing malaria transmission (Blanford *et al.* 2005).

Epidemiological models emphasize that reducing mosquito longevity can have a large impact on malaria transmission. So far, field studies could only infect 23% of the mosquitoes resting on the cloths, though even at this moderate coverage entomological inoculation rate, models estimated the impact on malaria transmission to be substantial (McDonald 1957).

Besides reducing mosquito longevity, an infection with *B. bassiana* in mouse models showed to have a direct effect on the development of *Plasmodium* parasites in the mosquito (Blanford *et al.* 2005). A significant reduction of sporozoite-positive mosquitoes was found due to fungal infection. Only 8% of mosquitoes infected with both the parasite and fungi contained transmissible parasites 14 days after exposure to the fungi, compared with 35% infected with *Plasmodium* alone. Reducing the development of *Plasmodium* in mosquitoes can increase the effectiveness of fungal infections in terms of malaria control. It was calculated that the fungal effects on mosquito survival and *Plasmodium* development together can lead to an 80-fold reduction of malaria transmission (Blanford *et al.* 2005).

Furthermore, research showed that *An. gambiae* females infected with *M. anisopliae* took smaller blood meals and exhibited reduced appetite related to increasing fungal growth (Scholte *et al.* 2006). These pre-lethal effects are thought to be caused by the degradation of tissues in combination with the production of secondary metabolites by the fungus. A reduction of the mosquito's feeding propensity will decrease the number of mosquito bites and reduce the likelihood of pathogen transmission. Another secondary effect of *Metarhizium* infection on female *An. gambiae* is a reduction in fecundity (Scholte *et al.* 2006). This observed decrease in egg-laying capacity is most likely to be a direct effect of the reduced amount of blood ingested per blood meal. The effects of fungi on the mosquito's feeding behaviour and fecundity have not yet been included in epidemiological models though they could further increase the effectiveness of fungi as a vector control method.

Moreover, in the laboratory limited horizontal transfer of fungal conidia between mosquitoes has shown to be possible during copulation (Scholte *et al.* 2004) and may contribute to the spread of the fungus through the mosquito population, thereby increasing the infection rates and the effectiveness of these biological control agents.

Another effect of a fungal infection is that it may influence the mosquito's phenotypic susceptibility to chemical insecticides and may disproportionally kill insecticide-resistant mosquitoes. Laboratory studies have delivered promising results of *M. anisopliae* and *B. bassiana* against a multiple insecticide-resistant strain of *Anopheles stephensi*, fuelling the hope that fungal pathogens can be deployed to overcome problems in areas where pyrethroid resistance is widespread (Stevenson *et al.*, unpubl. data).

CRITICAL ISSUES

Environmental and health risks

Hyphomycetous fungi are known to be able to kill a wide range of insect species. However, the impact of this lack of specificity will be limited as survival of fungal spores in the environment is low due to exposure to UV radiation and high temperatures. Furthermore, it is likely that, used in an indoor environment, the fungal spores will affect mainly other nuisance insects or vectors of pathogens. The acceptability of potentially negative impacts of entomopathogenic fungi on non-target organisms should be determined from a cost-health benefit perspective and compared with environmental effects caused by currently used insecticide-based strategies (Knols & Thomas 2006).

Though *M. anisopliae* has demonstrated neither infectivity nor toxicity in mammals, laboratory experiments show that mice may develop allergic reactions when exposed to fungal antigens (Ward *et al.* 1998). Human infections with *M. anisopliae* are extremely rare and represent low-virulence opportunistic infections of a kind potentially caused by a large number of ordinary fungi from daily life. However, the risk of human exposure to fungal spores will be limited due to its application in oil formulations, which causes the spores to adhere to the substrate and therefore does not contribute substantially to spore load in the air.

Infectious diseases such as HIV are leaving an increasing number of people in malaria-endemic countries immuno-compromised which could result in increased susceptibility to fungal pathogens and impose additional health risks (Hutchinson & Cunnigham 2005). There is however inadequate knowledge on the infection mechanisms and potential risks of *Metarhizium* and *Beauveria* in immuno-compromised humans. HIV patients are only affected by organisms against which T-cell mediated interactions are protective of which only a few relatively virulent fungal pathogens are known to be handled by (Levitz 1992).

Resistance

Some insect groups, such as locusts, ants, and termites, can become resistant to *M. anisopliae* infection (Wilson *et al.* 2002, Lamberty *et al.* 2001). However, fungal resistance is not widespread among insects as fungi can use an array of weapons to attack the insect, such as chitinases, proteases and toxins (Hajek & St. Leger 1994). Furthermore, fungi have a lower virulence than insecticides, killing the insect in approximately 10 to 12 days depending on the isolate. As female mosquitoes can still have reproductive success, this slow killing mechanism of fungi will impose a limited selection pressure on the mosquitoes and reduce the likelihood of antifungal resistance (Knols & Thomas 2006). So far no resistance has been observed in *Anopheles* mosquito species and even in the unlikely event of antifungal responses, cross-resistance with chemical insecticides will be highly unlikely (Blanford *et al.* 2005). Therefore through combining fungal treatment with insecticides or by using a rotation schedule, the development of phenotypic resistance can be avoided.

Another form of mosquito resistance might be behavioural. A widespread indoor application of fungi could impose strong selection on mosquito behaviour such as outdoor resting and feeding behaviour or altered host preference. Selection for mosquitoes that avoid impregnated areas or traps might occur and could significantly impair the effectiveness of this biological control method. However, laboratory studies to date have shown that fungal conidia do not have a repellent effect on *Anopheles* mosquitoes (Scholte, unpublished data).

PRACTICAL ISSUES

The effectiveness of an entomopathogenic fungus as a biological control agent in malaria control depends on its ability to reduce malaria transmission. It is therefore necessary to ensure the highest possible rate of mosquito infection by these fungi in field settings. There are, however, complex relationships between the dose, infectivity, virulence, horizontal transmission and survival of fungi under field conditions, resulting in several practical difficulties related to their field application.

Choice of fungal isolate

A key factor in improving infection rates is the choice of fungal strain. Fungal isolates can differ in their effectiveness as a biological control agent through differences in infectivity, virulence and ability to alter parasite development in the mosquito. Another important aspect is the fungus' ability to influence the mosquito's host-seeking behaviour and reduce its feeding propensity, which might be strain or isolate-specific. More extensive screening of fungal isolates will allow for the choice of the most effective fungus in terms of in malaria control.

Persistence and formulation

The effectiveness of fungi in field settings depends highly on the persistence of fungal conidia in terms of viability/infectivity after application and storage. Field research performed with *Metarhizium* conidia in an oil-formulation on cotton sheets, showed a decrease in conidial viability to 63% in three weeks (Scholte *et al.* 2005). This would mean that surfaces or cloths would require frequent re-treatment, which might be impractical for wide-scale field implementation (Kanzok & Jacobs-Lorena 2006). Besides selecting more persistent fungal isolates an important means to increase conidial persistence is the choice of formulation (Daoust *et al.* 1983) which should take into account that there is a trade-off between spore accessibility and viability. Furthermore, the choice of growth media can have a significant effect on infectivity and persistence (Ibrahim *et al.* 2002). Since fungi are sensitive to high temperatures, optimising transport and storage conditions can further improve conidial persistence.

Delivery

An effective coverage, needed to achieve a sufficient level of fungal contamination of mosquitoes, is highly dependent on the choice of application method. The choice of delivery method should include several factors, such as the most effective conidial dosage, minimal application to reduce costs and health risks, maximum exposure and effect on horizontal transmission. There are several application methods possible, each differing in their efficacy, effectiveness, acceptability and overall costs.

Indoor partial surface application uses impregnated dark cloths on which mosquitoes tend to rest, increasing the chance of fungal contact (Scholte *et al.* 2005). Furthermore, it protects spores from exposure to sunlight, which is beneficial for fungal persistence. A full surface application, *i.e.* the spraying of the indoor walls would increase the probability of fungal infection. However, this would increase the exposure to humans and could lead to problems with acceptability.

Impregnating bednet fibres with fungal conidia is another option of indoor application. With this method the occupant will serve as an attractant for mosquitoes, increasing the chances of fungal contact and infection. However,

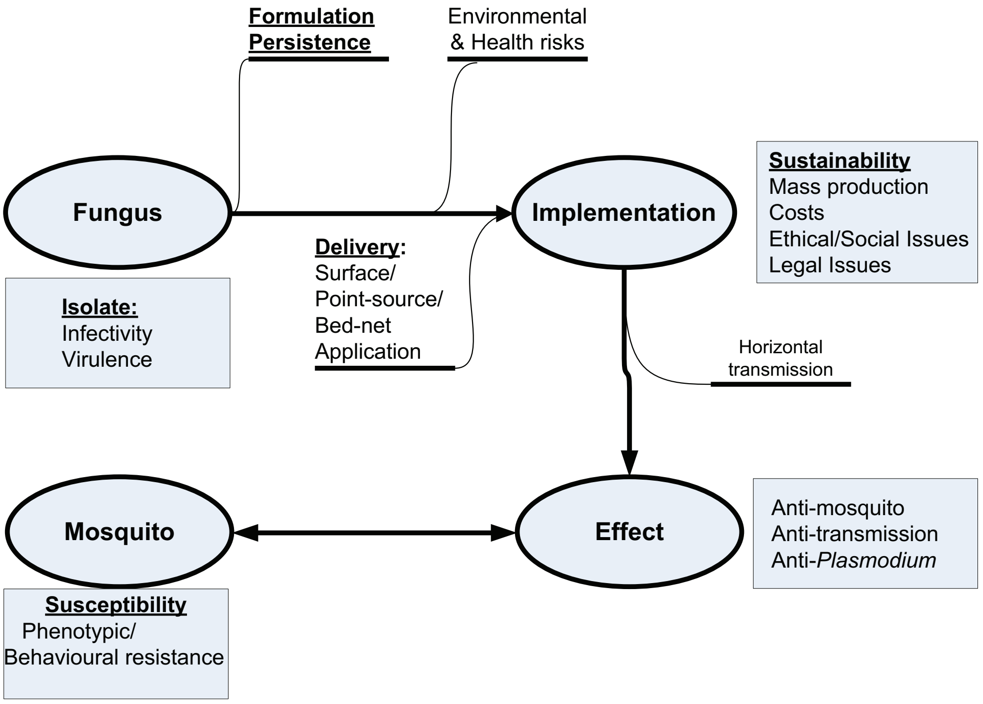

Figure 1. Overview of determinants affecting the effectiveness and sustainability of the implementing fungi as vector control agents in the field

increased exposure to fungal spores due to handling and close contact renders it less acceptable.

Applying the fungi in small and contained areas either indoors or outdoors could be beneficial for fungal persistence and result in a higher effectiveness, especially when cool and humid sites can be created. Furthermore, covering only small areas using the point-source method would minimize fungal application and limit human exposure, thereby increasing its acceptability.

Overall, the choice of application system depends on the effectiveness, acceptability and overall costs of each method. A full cost-benefit analysis of each application method is needed to determine which delivery mechanism will be optimal in field settings.

CONCLUSIONS AND FUTURE PERSPECTIVES

Current malaria control methods face major problems with drug and insecticide resistance, stressing the need to search for new alternative control methods. Entomopathogenic fungi are a promising new biological tool for controlling malaria transmission. Studies have shown that fungi can effectively infect and kill adult *Anopheles* mosquitoes. Field trials so far have shown a high efficacy even at modest coverage. Besides reducing longevity, the fungi also reduce the development of *Plasmodium* parasites in the mosquito and negatively affect its feeding propensity and fecundity. Moreover, the possible large effect of fungal infection on insecticide-resistant mosquitoes may further improve its effectiveness as a vector control method.

The feasibility and sustainability of the implementation of fungi as a vector control method in the field will depend on several factors, including the choice of fungal isolate and formulation (Fig. 1). Moreover, the choice of application and delivery method will highly influence the infection coverage and the effectiveness of fungi in the field. Ethical, social and legal issues are inextricably linked with the choice of application and field implementation of a vector control method and should be taken into consideration. Though negative effects on the environment and human health are considered to be relatively low (especially compared to insecticides) and resistance against the fungi is unlikely, more research on these subjects is necessary to improve its acceptability.

Entomopathogenic fungi should preferentially be applied in integrated vector management (IVM) strategies, in order to achieve the greatest disease control benefit in the most cost-effective manner and to limit any chance of resistance. One possibility would be to develop an integrated control programme without the use of chemicals, where the application of fungi against the adult mosquito vectors is combined with biological larval control and untreated bednets. Such a strategy could decrease malaria transmission to such levels that disease prevalence will be affected and reduce the emergence of serious levels of insecticide resistance. With substantial financial, political and local support for long-term and large-scale application, the use of fungi for control of adult mosquito vectors can be a valuable and viable aspect of IVM strategies currently being developed.

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