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New prospective on fungal pathogens for mosquitoes and vectors control technology

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Abstract The development of mosquito vector control technology with fungi has a new generation. However, fungi have numerous benefits over the microbes for preparation in insecticides as mosquito have a vigorous lethal of existence in fungal products. The fungal spores, metabolites, protein, toxins, enzymes, and nanoparticles have been shown significant efficacies against adults and its developmental stages of mosquitoes. The developments and formulations have used of new technology that produce new larvicides and adulticides. The marketable perfection of entomopathogenic fungi for mosquito control has been delayed by unacceptable action comparative to chemical compound. However, the new technologies are urgent need for their separation and preservation postponements the significance. Recently several chemical based strategies have shown favourable results in the field. This article propose new prospective on fungal infection used for mosquitoes control present to future based alternative.

Keywords Mosquito control, Fungi, Adulticides, Larvicides, Vector control technology

Introduction

The mosquitoes are medically important pathogens. They are parasites for viruses, bacteria, protozoans, and nematodes and others. They cause serious diseases such as malaria, dengue, yellow fever, Chikungunya fever, and filariasis. Due to their blood sucking behaviour mosquitoes are able to acquire the pathogens or parasites from one vertebrate host and pass them to another (Becker et al., 2010). The disease and death have affected by vector borne diseases. In recent years, vector-borne diseases have emerged as a serious public health problem in countries of the South-East Asia Region, including India. Many of these, particularly dengue fever, Japanese Encephalitis (JE) and malaria now occur in epidemic form almost on an annual basis causing considerable morbidity and mortality. Dengue is spreading rapidly to newer areas, with outbreaks occurring more frequently and explosively. Chikungunya has re-emerged in India after a gap of more than three decades affecting many states. Outbreaks have also been reported from Sri Lanka, Mauritius, the Reunion Island, and Maldives.

The risk factors, which play a key role in the spread and transmission of dengue and Chikungunya, include globalization, unplanned and uncontrolled urbanization, developmental activities, poor environmental sanitation, and human behaviour relating to water collection, lifestyles, widespread travel and human migration, both within the country and across borders. These are causes for much concern and highlight the need to comprehensively address the challenges faced in combating vector-borne diseases in the country. The recent outbreaks of Dengue and Chikungunya have been widely reported by and discussed both in the electronic and print media (WHO, 2011). Malaria is transmitted to humans by the bite of infected female mosquitoes of more than thirty Anopheline species. An estimated 3.3 billion people were risk of malaria in 2010, although of all geographical regions, population living in Sub-Saharan Africa has the highest risk of acquiring malaria, in 2010 (WHO, 2011). Approximately 3.5 billion people live in dengue endemic countries which are located in the tropical and subtropical regions of the world (WHO, 2011). Lymphatic filariasis,

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commonly known as elephantiasis, is a neglected tropical disease. The infection occurs when filarial parasites are transmitted to humans through *Culex quinquefasciatus*. More than 1.3 billion people in eighty one countries worldwide are threatened by lymphatic filariasis (WHO, 2011).

Today, we depend almost entirely on synthetic chemical insecticides for protection against mosquitoes. The appearance of insecticide resistance and adverse ecological effects has dismissed our confidence in conventional chemical methods despite their striking success in past decades. The procedures were regularly based on evidence about the distinct preferences of different vector species for breeding habitats. The information for vectors disease was used to through ecological methods to selected field conditions. There is evidence that environmental management had a clear impact on disease. However, elimination of disease was never on the agenda. The advent of DDT and other organochlorine pesticides during the 1940s changed this situation. The spraying the indoor surfaces of community and housings extremely reduced the numbers of mosquitoes. Similarly, chemical based insecticides have control the normal survival of vectors to of the stage at the infections. Malaria is eliminated from a number of countries. Moreover, the increased resistance of vectors to insecticides have resulted in failure to eliminate vectors and vector borne diseases. The vector control on insecticides meant that environmental management and other alternative methods can be exploited. Biological larvicides, adulticides other than DDT were developed, the most recent class being the pyrethroids, developed in the 1980s, and commonly used for mosquito control.

Fungal species belonging to the genera *Coelomomyces*, *Culicinomyces*, *Beauveria*, *Metarhizium*, *Lagenidium*, and *Entomophthora* have been considered when studying the role of fungus in vector disease control (Kamareddine, 2012). The ninety genera and more than seven hundred species of fungi are insect pathogens. These are distributed in virtually every major fungal taxonomic group except the higher bacidomycetes (Roberts and Humber, 1981). Their mode of action against mosquitoes involves attachment of the spores to the cuticle followed by germination cuticle penetration, and internal dissemination throughout the mosquito. In this process which may involve the production of secondary metabolites, the internal organs of the mosquito larvae are eventually degraded. The environmental factors such as ultraviolet light, temperature, and humidity can influence the effectiveness of fungal entomopathogens under field conditions (Shaalan et al., 2005). Moreover, the terrestrial fungi have been reported as pathogens or parasites of humans, animals, and plants endophytes, as symbionts of arthropods and root of plants and components of soil microbiota and others (Alexopoulous et al., 1996; Watanabe, 2010). The development of fungal entomopathogens as effective control requires knowledge of bioassay methods, as well as production, formulation and application methodologies. Moreover, five hundred fungi are commonly related with insects, some cause serious disease in their hosts, few have been used commercially as control agents. Fungi infect a border range of insects than do other microorganisms, and infections of lepidopterans (moth and butterflies), homopterans (aphid and scale insects), hymenopterans (bees and wasps) coleopterans (beetle), and dipterans (flies and mosquitoes) are quite common. In fact some fungi have very broad host ranges that include most of those insect groups. The previous worker has improved worldwide on vectors of malaria. The chemical treated nets have used for mosquito control as achieved significant coverage in a number of African countries, leading to substantial reductions in the prevalence of malaria. These countries were extremely endemic. Apart from the Entomopathogenic fungi have novel properties for control of malaria, filaria and dengue vectors (Abdul-Ghani et al., 2012; Singh and Prakash, 2010a; 2012a; 2012b; Scholte et al., 2003a). These significant characteristics have increased interest with continued effort, for the mosquito control.

1 Fungal infections Pathogenicity and Virulence

The entomopathogenic fungi have been successfully used for control mosquito to adults and larvae (Figure 1; Table 1). Several fungal species have been tested, especially for the control of mosquito larvae. In

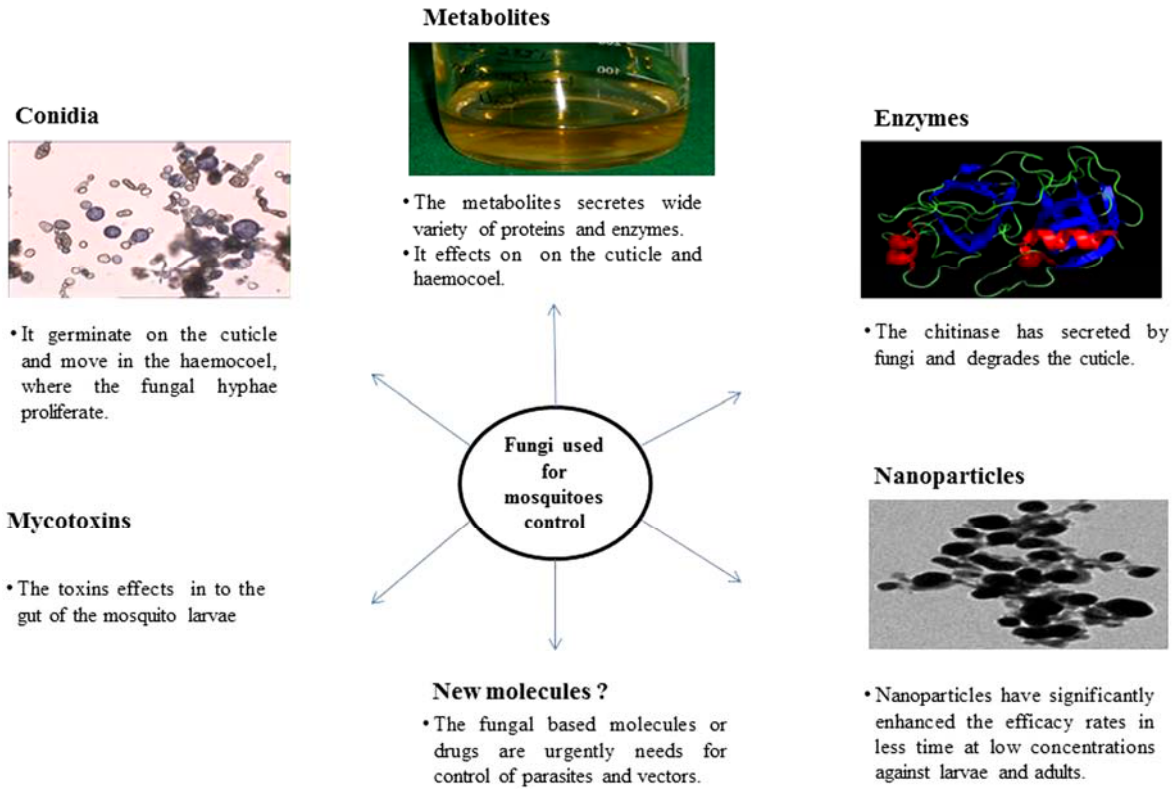

Figure 1 Fungal products tested for mosquitoes control with new perspectives in laboratories and field conditions

contrast to bacteria, fungi are adulticidal agents that could be developed for domestic use to reduce vector densities and impair their vectorial capacity. However, field investigations to determine deployability and feasibility are needed to demonstrate utility for malaria control within the context of IVM strategies ((Abdul-Ghani et al., 2012). In the infectious causes, the fungi have not need host ingestion, and external interaction to the mosquito cuticle. This is the method of initiation of infection. This cannot directly used in the community and field conditions. Recently, chemicals has used as insecticide delivering strategies. The conidia has used in outdoor attracting odor traps, on indoor house surfaces, on cotton pieces hanging from ceilings, bed nets, and curtains, and can persist for a couple of months on many of these surfaces (Kamareddine, 2012). The pathogenicity has defined as the ability to cause disease (qualitative measure) while virulence is the degree of pathogenicity (quantitative measure) (Watson and Brandly, 1949). Due to the increasing global interest in reducing environmental pollution with chemical pesticides, there have been several promising developments in fungus-based insect control, particularly since the 1990s. The molecular techniques have enabled the identification of isolates and virulence factors (Ansari et al., 2004; St. Leger et al., 1996).

Beauveria bassiana when used as a conidial dust more effectively kills larvae than adult mosquitoes (Clark et al., 1968). All tested *Anopheles* and *Culex* larvae are susceptible to the fungus while *Aedes* larvae are not (Clark et al., 1968). In addition, the fungus *Trichophyton ajelloi* is highly toxic against *An. stephensi* and *Cx. quinquefasciatus* in the laboratory, with the third-stage larvae of the former being the most susceptible (Mohanty and Prakash, 2000). The conidia of *Chrysosporium lobatum* cause high mortality of *An. stephensi* larvae in the laboratory, particularly those of the third instar (Mohanty and Prakash, 2002; Scholte et al., 2003a; 2003b) were the first to use the dry conidia of the entomopathogenic fungus *Metarhizium anisopliae* against adult *An. gambiae sensu stricto* and *Cx. quinquefasciatus* in the laboratory. It has been confirmed that the conidia of the fungus are extremely

Table 1 Fungal species and their products tested against mosquito vectors in laboratory and field

S.N	Fungal species tested	Fungal product	Mosquito species	Mosquito stage
1	*Metarhizium anisopliae*	Conidia, metabolites	*Anopheles gambiae s.s, A. funestus*, *A. stephensi*, *Culex quinquefasciatus*,	Adults, larvae (Farenhorest et al., 2009)
2	*Beauveria bassiana*	Conidia, metabolites	*A. gambiae s.s*	
3	*Isaria fumosurosea, I. farinosoa, I. flavovirescens, Lacanicillium* spp.	Spores	*Aedes aegypti*	Adults (Darbro et al., 2009)
4	*Leptogonia sp.*	Spores	*A. albopictus*, *M.titillans*, *M. dyari*	Larvae (Fukuda et al., 1997; Lord and Fukuda, 1990)
5	*Leptolegnia chapmanii*	Spores	*A. aegypti*, *A. albimanus*, *A. quqadrimaculatus*, *C. quinquefasciatus*, *Oc. taeniorhynchus*, *Oc. triseriatus*	Larvae (Lord and Fukuda, 1990)
6	*Leptolegnia caudate*	Spores	*A. culicifacies*	Larvae (Bisht et al., 1996)
7	*Pythium carolinianum*	Spores	*A. albopictus C. quinquefasciatus*	(Su et al., 2001)
8	*Lagenidium giganteum*	Spores, metabolites	*A. aegypti*, *A. gambiae*, *A. freeborni*, *A. quadrimaculutus*, *C. pipiens, A. stephensi*	Larvae (Rueda et al., 1990; Golkar et al., 1993; Kerwin et al., 1994; Rueda et al., 1991; Patel et al., 1990; Orduz and Axtell, 1991; Woodring et al., 1995; Singh and Prakash, 2010a)
9	*Coelomomyces angolensis*	Spores	*C. guiarti*	Larvae (Ribeiro, 1992)
10	*Coelomomyces indicus*	Spores	*A. indifenitus, A. stephensi, A. vagus*	Larvae (Whisler et al., 1999)
11	*Coelomomyces irani*	Spores	*A. maculipennis*	Larvae (Weiser et al., 1991a)
12	*Coelomomyces numularius*	Spores	*A. squamosus*	Larvae (Ribeiro and Da Cunha Ramos, 2000)
13	*Coelomomyces pentangulatus*	Spores	*C. erraticus*	Larvae (Ribeiro and Da Cunha Ramos, 2000)
14	*C. psorophorae var. tasmaniensis*	Spores	*Oc. Australis Op. fuscus*	Larvae (Buchanan and Pillai, 1990)
15	*C. stegomyiae var. stegomyiae*	Spores	*A. aegypti, A. albopictus*	Larvae, Adults (Shoulkamy et al., 1997; Lucarotti and Shoulkamy, 2000)
16	*Entomophthora destruens*	Spores	*C. pipiens*	Adults (Cuebas-Incle, 1992)
17	*Entomophthoraceae*	Spores	*C. pipiens*	Adults (Cuebas-Incle, 1992)
18	*Fusarium culmorum*	Spores	*C. pipiens*	Pupae (Ram and Mzy, 1995)
19	*Fusarium pallidoroseum*	Spores	*A. stephensi*	Larvae (Ravindranath, 1991)
20	*Fusarium semitectum*	Spores	*A. stephensi*	Larvae (Sur et al., 1999)
21	*Geotrichum candidum*	Spores	*A. pionysis, A. stephensi*	Larvae (Sur et al., 1999)
22	*Paecilomyces lilacinus*	Spores	*A. aegypti*	Larvae (Agarwala et al., 1999)
23	*Tolypocladium cylindrosporum*	Spores	*Oc. triseriatus*	Larvae (Nadeau and Boisvert, 1994)
24	*Trichophyton ajelloi*	Spores	*A. stephensi, C. quinquefasciatus*	Larvae (Mohanty and Prakash, 2000)
25	*Verticillium lecanii*	Metabolites Nanoparticles	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Larvae, adults (Soni and Prakash, 2010; 2012a, Singh and Prakash 2012b)
26	*Chrysosporium keretinophillum*	Metabolites Nanoparticles	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Larvae, adults (Soni and Prakash, 2010; 2012a)
27	*Aspergillus niger*	Metabolites Nanoparticles	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Larvae, adults (Soni and Prakash, 2012b)
28	*Fusarium oxysporum*	Metabolites Nanoparticles	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Larvae, adults (Soni and Prakash, 2010; 2012a)
29	*Culicinomyces clavisporus*	Metabolites	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Adults (Singh and Prakash 2012)
30	*Trichophyton ajelloi*	Metabolites	*A. stephensi*, *A. aegypti C. quinquefasciatus*	Adults (Singh and Prakash, 2012a)

pathogenic to both species with significantly earlier death among infected compared to uninfected control mosquitoes (Scholte et al., 2003a; 2003b). Thereafter, the auto dissemination of *M. anisopliae* among *An. gambiae* s.s. mosquitoes during mating activity has been found to be possible under laboratory conditions This horizontal transfer of fungal inoculum between mosquitoes during copulation might contribute to the spread of the fungus within target mosquito populations in the field (Scholte et al., 2004b). This is an advantage over synthetic insecticides as it spreads the mosquitocidal agents within mosquito populations. In contrast, synthetic adulticides are prone to the vertical transmission of resistance among mosquitoes. The *M. anisopliae* and *B. bassiana* are effective against *A. gambiae* s.s. with persistence at low concentrations and short exposure times (Mnyone et al., 2009a; 2009b). Germination of their spores takes place on the insect cuticle followed by penetration of the insect to grow in the hemolymph killing the mosquito within 7–14 days, depending on dose, formulation and fungal strain (Scholte et al., 2004a; 2005). The infectivity of their conidia persists up to 28 days after application irrespective of their concentration (Mnyone et al., 2009a; 2009b). In contrast, Darbro and Thomas (2009) showed that the persistence of *B. bassiana* is better than that of *M. anisopliae* with maintenance of about 50% viability 14 weeks after application compared to no longer than a week for all *M. anisopliae* isolates. In addition, both species are highly effective in reducing larval survival and adult emergence of *An. stephensi* and *An. gambiae* s.s. (Bukhari et al., 2010). Luz et al. (2010) showed how *Lecanicillium muscarium* isolated from a dead culicid mosquito is pathogenic to adults of *A. aegypti*, *A. arabiensis* and *C. quinquefasciatus* under laboratory conditions demonstrating how naturally occurring fungal pathogens of culicids might have potential for mosquito control. *Aspergillus clavatus* isolated from an African locust causes >95% mortality after 24 h against *An. gambiae*, *An. aegypti*, and *Cx. quinquefasciatus* larvae (Seye et al., 2009). The effectiveness and deployability of such fungi under field conditions have yet to be explored. Moreover, advances in spore formulations have improved fungal effectiveness under low humidity conditions and UV exposure (Kassa et al., 2004; de Faria and Wraight, 2007; Alves et al., 1998) and increased potential deployment options (Bateman and Alves, 2000). The development of solid-state mass-production systems has made large spore quantities available for field trials (Jenkins et al., 1998; Feng et al., 1994; Ypsilos and Magan, 2005). The advances in quality control, such as optimizations on the substrate, incubation temperature, harvest time and storage conditions (Jenkins and Grzywacz, 2000), have enabled the production of fungus products with standardized quality (Roberts and St. Leger, 2004).

The potential of fungi to kill Anophelines and reduce malaria transmission (Scholte et al., 2005; Blanford et al., 2005; Read et al., 2009) has resulted in a growing interest to develop practical and sustainable mosquito vector control methods based on these biological control agents that can be integrated into the existing arsenal of malaria control tools (Knols and Thomas, 2006; Thomas and Read, 2007). There are multiple methods available for infecting target insects with fungal spores. Dry conidia have been shown to be effective in infecting mosquitoes in the laboratory (Scholte et al., 2003a) but as they become air-borne when handled, the exact exposure dose cannot be determined. Use of fungal suspensions allows for accurate quantifications of spore concentration with microscopy counts and is considered to be more feasible for large scale experiments and field implementation. The *Trichophyton ajelloi*, *Chrysosporium tropicum*, *C. lobatum*, *L. giganteum* a fungal pathogen of *An. stephensi* and *Cx. quinquefasciatus* caused high mortality (Mohanty and Prakash 2000; Priyanka and Prakash, 2001; 2003). Metabolites of *L. giganteum* found significant pathogenic after filtration by Column chromatography (Vyas et al., 2006; Vyas and Prakash, 2007) Efficacy of culture filtrates of five strains of *M. anisopliae* isolated from insects were evaluated against *An. stephensi* and *Cx. quinquefasciatus*. The culture filtrates released from the strains of *M. anisopliae* in the YpSs and chitin broths were filtered and used for the bioassays after a growth of 7days (Mohanty and Prakash, 2008). Eleven fungal species in three genera were isolated from the soil at Agra, India by the feather-baiting technique. Out of the

eleven species, *C. lobatum* a deuteromycetous (Moniliales: Moniliaceae) and change of culture media produced significant pathogenicity of *C. quinquefasciatus* Say (Diptera: Culicidae) larvae under laboratory conditions (Mohanty and Prakash, 2008; 2009). The *Chrysosporium* and *Trichophyton* spp. were more pathogenic on *Cx. Quinquefasciatus* larvae than *Aspergillus* and *Penicillium*. The highest mortality was observed in the larvae of *Cx. Quinquefasciatus* when exposed to *T. ajelloi*. The density of fungal conidia was greatest on the ventral brush, palmate hair and anal region of the mosquito larvae after exposing for 72 hours (Mohanty and Prakash, 2010). The isolate and identified natural entomopathogenic fungi from female *Cx. quinquefasciatus* have been tested their adulticidal activity. All the female *C. quinquefasciatus* were killed within 4 days of exposure to *F. pallidoroseum* at a concentration of 1.11□×□1010 conidia per m^2. Significant difference of longevity was observed between the *F. pallidoroseum* treated *C. quinquefasciatus* and control mosquitoes. The LT_{50} of *F. pallidoroseum* was 2.08 days for 4hrs exposure to *C. quinquefasciatus*. Results from this study have confirmed that *F. pallidoroseum* can one of the alternative biological control agents of adult mosquitoes (Mohanty and Prakash, 2008). Moreover, the culture filtrates of *A. niger*, *C. clavisporus*, *L. giganteum*, *T. ajelloi*, *F. oxysporum* have found significant pathogenic against adult mosquitoes. When this culture filtrates have purified with chromatography found more pathogenic in short time (Singh and Prakash, 2010a; 2010b; 2011; 2012a; 2012b; 2012c; 2012d). Moreover, the current research needs to focus on developing a mycoinsecticide against adult mosquitoes. Fungal spores can be deployed against these flying insects by applying them on surfaces with which they make contact. A range of *M. anisopliae* and *B. bassiana* isolates have been only shown successful in infecting and killing *Anopheles*, *Aedes* and *Culex* mosquitoes when applied on several different substrates, (Scholte et al., 2003a; Blanford et al., 2005; Scholte et al., 2005; 2007). Depending on the dose and virulence of the isolate, hyphomycetes can kill mosquitoes within several days, mostly between 4 and 14 days (Scholte et al., 2003b; Bell et al., 2009; Mnyone et al., 2009a, b).

2 The Combination of Entomopathogenic Fungus with Insecticides

The compatibility of the pyrethroid insecticide permethrin and two insect-pathogenic fungi, *B. bassiana* and *M. anisopliae* for use in integrated mosquito control was assessed using a range of fungus-insecticide combinations against a laboratory colony and field population of resistant (*kdr*) *An. gambiae* s.s. mosquitoes from West Africa. The mosquito population was highly resistant to permethrin but susceptible to *B. bassiana* and *M. anisopliae* infection. Combinations of insecticide and fungus showed synergistic effects on mosquito survival. Fungal infection increased permethrin induced mortality rates in wild mosquitoes and reciprocally, exposure to permethrin increased subsequent fungal impact in both colonies. Simultaneous co-exposure induced the highest mrtality; up to 70.3 ± 2% within 4 days for a combined *Beauveria* and permethrin exposure. The observed synergism in efficacy shows the potential for integrated fungus-insecticide control measures to dramatically reduce malaria transmission and enable vector control in areas where insecticide resistance has rendered pyrethroids essentially ineffective. Similarly the *B. bassiana* and *M. anisopliae* could be further tested against the vectors of dengue and filaria endemic regions. By quantifying the impact of the combined use of fungal biopesticide and ITN interventions on malaria transmission and prevalence, the model indicates that these interventions combined may considerably improve malaria control even in situations each single intervention would have a relatively low impact. Modelling is no substitute for field studies, and attempts to make generalizations about vector biology need to be cautiously interpreted (Klowden, 2007). Recent vector control initiatives encourage the development of models that have the capacity to use field data to guide decision making (WHO, 2004). The combining fungal biopesticides and insecticide treated bed nets reveals that the biological mechanisms relevant to vectorial capacity. It can be built into existing continuous-time, population-level frameworks to allow direct parameterization from field and laboratory. This is a means by which models can increase their applicability

to integrated vector management strategies (Hancock, 2009). Moreover, Paula et al. (2011) have reported first time that a combination of an insecticide and an entomopathogenic fungus has been tested against *A. aegypti*. Firstly, the study showed the potential of insecticides insecticide Imidacloprid (IMI) as an alternative to the currently employed pyrethroid adulticides. This can be an alternative to applications of high concentrations of chemical insecticides, we suggest that adult *Ae*. *aegypti* could be controlled by surface application of Entomopathogenic fungi and that the efficiency of these fungi could be increased by combining the fungi with ultra-low concentrations of insecticides, resulting in higher mortality following relatively short exposure times.

3 Fungal Infection Counters Insecticides Resistance Species

Several studies show high levels of insecticide resistance in various parts of the world. The entomopathogenic fungi have significant option to malaria vector control. Farenhorst et. al (2009) have found that the insecticide resistant *Anopheles* mosquitoes remain susceptible to infection with the fungus *Beauveria bassiana*. The four different mosquito strains with high resistance levels against pyrethroids, organochlorines, were equally susceptible to *B. bassiana* infection as their baseline counterparts, showing significantly reduced mosquito survival. Moreover, this fungal infection reduced the expression of resistance to the key public health insecticides permethrin and DDT. Generally, the substantial decreases in mosquito survival and insecticide resistance levels induced by fungal infection support the potential use of fungal biopesticides against mosquito vectors in areas where insecticide resistance levels are increasing, potentially adding new product options to the very limited selection of chemicals currently available. Moreover, with fungal infection reducing the expression of permethrin and DDT resistance, developing ‘‘combination treatments’’ may enhance the efficacy and effective lifespan of key of larvicides, adulticides where the resistance has reached high levels.

The entomopathogenic fungus has been shown to reduce blood feeding of wild mosquitoes. This behaviour modification indicates that *B. bassiana* could potentially be a new mosquito control tool effective at reducing disease transmission, although further field work in areas with filariasis transmission should be carried out to verify this. In addition, work targeting malaria vector mosquitoes should be carried out to see if these mosquitoes manifest the same behaviour modification after infection with *B. bassiana* conidia (Howard et al., 2010). These fungi have been shown to be lethal to both insecticide-susceptible and insecticide-resistant mosquitoes under laboratory conditions. The goal of this study was to see whether entomopathogenic fungi could be used to infect insecticide resistant malaria vectors under field conditions, and to see whether the virulence and viability of the fungal conidia decreased after exposure to ambient African field conditions (Howard et al., 2011). Blanford et al. (2011) have demonstrated the transient exposure to clay tiles sprayed with a candidate biopesticide comprising spores of a natural isolate of *B. bassiana*, could reduce malaria transmission potential to zero within a feeding cycle. The effect resulted from a combination of high mortality and rapid fungal-induced reduction in feeding and flight capacity. Additionally, multiple insecticide-resistant lines from three key African malaria vector species were completely susceptible to fungus. Thus, fungal biopesticides can block transmission on a par with chemical insecticides, and can achieve this where chemical insecticides have little impact. This study can be support broadening the current vector control paradigm beyond fast acting chemical toxins. Farenhorst et al. (2011) have used the fungal spores dissolved in Shellsol and sprayed on small-meshed cotton eave curtain nets would be the most promising option for field implementation. The Biological control with fungus-impregnated eave curtains could provide a means to target host-seeking mosquitoes upon house entry, and has potential for use in integrated vector management strategies, in combination with chemical vector control measures, to supplement malaria control in areas with high levels of insecticide resistance. Further, Lynch et al. (2012) have been proved the fungal biopesticides that generate high rates of mortality at around the time mosquitoes first become able to transmit the malaria

parasite offer potential for large reductions in transmission while imposing low fitness costs. The best combinations of control and resistance management are generally accessed at high levels of coverage. Strains which have high virulence in malaria-infected mosquitoes but lower virulence in malaria-free mosquitoes offer the ultimate benefit in terms of minimizing selection pressure whilst maximizing impact on transmission. Exploiting this phenotype should be a target for product development. For indoor residual spray programmes, biopesticides may offer substantial advantages over the widely used pyrethroid-based insecticides. Not only do fungal biopesticides provide substantial resistance management gains in the long term, they may also provide greater reductions in transmission before resistance has evolved. This is because fungal spores do not have contact irritancy, reducing the chances that a blood-fed mosquito can survive an encounter and thus live long enough to transmit malaria. Delayed-action products, such as fungal biopesticides, have the potential to achieve reductions in transmission comparable with those achieved with existing instant-kill insecticides, and to sustain this control for substantially longer once resistant alleles arise. Given the current insecticide resistance crisis, efforts should continue to fully explore the operational feasibility of this alternative approach.

4 The Transgenic Fungi

Many laboratory groups are now developing transgenic fungi for better mosquito borne disease control. Such approaches are thought to be highly effective, very specific, exert negligible negative environmental impacts, and have relatively minimal effects on the parental wild-type mosquito strains (Fang et al., 2011). Recently, it was shown that infecting mosquitoes with genetically engineered *Metarhizium*, designed to produce antimalarial peptides, blocked the transmission of the malaria parasite from its vector. This approach overcomes the necessity of rapid field applied fungal infection shortly after the mosquito picks up the malaria parasite, and prevents any possibility of developing fungal resistant mosquito strains, since transgenic fungi only kill adult mosquitoes (Fang et al., 2011). Yet, the use of genetically engineered fungus compared to field applied fungal biopesticides is still not favored. Many argue that such strategies exert high fitness costs on the transgenic organism, are practically more complicated, and comparatively difficult to handle as field released pathogens (Fang et al., 2011). In some cases, relying on anti-malarial factors might result, in the long term, in malaria parasite resistance, regardless of the fact that some fungal strains, like *Metarhizium* for example, could express multiple transgenes with different modes of action (Fang et al., 2011). The *M. anisopliae* infects mosquitoes through the cuticle and proliferates in the hemolymph. To allow *M. anisopliae* to combat malaria in mosquitoes with advanced malaria infections. They produced recombinant strains expressing molecules that target sporozoites as they travel through the hemolymph to the salivary glands. Eleven days after a Plasmodium infected blood meal, mosquitoes were treated with *M. anisopliae* expressing salivary gland and midgut peptide 1 (SM1), which blocks attachment of sporozoites to salivary glands; a single-chain antibody that agglutinates sporozoites; or scorpine, which is an antimicrobial toxin. These reduced sporozoite counts by 71%, 85%, and 90%, respectively. *M. anisopliae* expressing scorpine and an (SM1)8: scorpine fusion protein reduced sporozoite counts by 98%, suggesting that *Metarhizium* mediated inhibition of Plasmodium development could be a powerful weapon for combating malaria.

5 Fungal Metabolites

The fungi produce chemical compounds that are consider being essential for normal growth and development, such as amino acids, nucleotides, proteins, and carbohydrates. These are referred to as primary metabolites. Any other compound that is not essential for growth and development is referred to as secondary metabolites. Some fungal secondary metabolites have medicinal properties in humans (penicillin, cephalosporin, statins). While others known as mycotoxins, are toxic (ergot, alkaloid, aflatoxins, ochrotoxins) (Keller et al., 2005). Even though fungal entomopathogens produce many secondary metabolites, for the most part, the role of metabolite in pathogenesis remains unclear (Molnar et

al., 2010). The detection of secondary metabolites in culture does not necessarily imply that it is being produced in the insect or that is plays role in the pathogenicity. The enzymes involved in pathogenesis of insects are generally grouped in to proteases and peptidases, chitinases and lipases (Khachatourians and Qazi, 2008). The enzymes involved in pathogenesis of insects are generally grouped in to proteases and peptidases, chitinases and lipases.

5.1 Proteases and Peptidases

Insect cuticle mainly is composed of chitin and protein; hence proteases and peptidases of EPF are important for the degradation of the insect cuticle, saprophytic growth of the fungi, activation of the prophenol oxidase in the hemolymph, and they act as virulence factor. The fungi from which protein degrading enzymes proteases, collagenases, and chymolea-stases have been identified and characterized are *A. aleyrodis*, *B. bassiana*, *B. brongniartii*, *E. coronata*, *Erynia*spp., *L. giganteum*, *Nomuraea rileyi*, *M. anisopliae*and *V. lecanii* (Charnley and St Leger 1991; Khachatourians, 1991; 1996; Sheng et al., 2006).

5.2 Chitinases

The insect cuticle which the fungus breaches is mainly constituted by chitin fibrils embedded in a protein matrix the quantity and type of proteins varying between insect species, tissue and growth stages (Andersen, 1974). The major component of insect cuticle is chitin, therefore both endo and exo-chitinases play critical roles in the cleavage of *N*-Acetylglucosamine (NAGA) polymer of the insect cuticle into smaller units or monomers. Khachatourians (1991) demonstrated that the extracellular chitinases are virulence determinant factors. Chitinolytic enzymes (*N*-acetyl-β-D-glucosa-minidases and endochitinases were present in the broth culture supplemented with insect cuticles from *M. anisopliae*, *M. flavoviride*, and *B. bassiana* (St Leger et al., 1996). The chitinase from *M. anisopliae*consists of acidic (pI 4.8) proteins with molecular masses 43.5 kDa and 45 kDa. The identified N-terminalsequences of both bands were similar to an endochi-tinase from *Trichoderma harzianum*. Valadares-Inglis and Peberdy (1997) located chitinolytic enzymes in enzymatically produced protoplasts and whole cells (mycelia) of *M. anisopliae*. No significant induction was observed from mycelia, yet protoplasts were found to induce these enzymes significantly. The majority of chitinolytic activity was cell-bound in both whole cells and protoplast preparations, and the activity was mainly located in the membrane fraction. Kang et al. (1998, 1999) reported a chitinase with molecular mass of 60 kD from *M. anisopliae* grown in a medium containing chitin as the sole carbon source with an optimum pH of 5.0, which is different from the chitinases values previously reported by St Leger et al. (1996) for endo-chitinases of 33.0, 43.5, and 45 kDa and exo-chitinases of 110 kDa. Screen et al. (2001) cloned the chitinase gene (*Chit1*) from *M. anisopliae* sf. *Acridum* ARSEF strain 324 and *M. anisopliae*s ARSEF strain 2575 (*Chit1*) using the pro-moter of *Aspergillus* (gpd) for constitutive expression. The A 42 kD chitinase of *M. anisopliae* was expressed and characterized in *Escherichia coli* by Baratto et al. (2006) using a bacteriophage T7-based promoter expression vector. Baratto et al. (2006) performed transcriptional analysis of the chitinase *chi2* gene of *M. anisopliae* var. showed that it has 1542 bp encoding for a deduced 419 amino acids. Nahar et al. (2004) reported that the extracellular constitutive chitin deacetylase (CDA) secreted by *M. anisopliae* converts chitin (a β-1, 4-linked N-acetyl-glucosamine polymer) into its deacetylated form chitosan (a glucosamine polymer). This CDA was not inhibited by solubilized melanin. Fang et al. (2005) purified an endochitinase from liquid cultures of *B. bassiana* supplemented with chitin. Bbchit1 was 33 kD (pI 5.4) and the encoding gene, *Bbchit1*, and its upstream regulatory sequences were cloned based on N-terminal amino acid sequence. *Bbchit1*contains no introns and it is present as a single copy in the *B. bassiana* genome. The amino acid sequence of Bbchit1 is similar to that of the endochitinase of *Streptomyces avermitilis*, *S. coelicolor* and *T. harzianum* (Chit36Y), but not to EPFs that reflect novel chitinases. Fang and co-worker (2005) constructed a *B. bassiana* transformants (*gpd-Bbchit1*), which overproduced *Bbchit1*and had enhanced virulence.

6 New Nanoparticle as larvicides and adulticides

Nanoparticles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology (Willems and van den Wildenberg, 2005). In recent years, the biosynthetic method using plant extracts has received more attention than chemical and physical methods, and even than the use of microbes, for the nano-scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. Nanoparticles have attracted considerable attention owing to their various applications. The silver nanoparticles are reported to possess anti-bacterial (Sathish Kumar et al., 2009), antiviral (Rogers et al., 2008), anti-fungal activity (Panacek et al., 2009). Synthesis of nanoparticles using plants or microorganisms can potentially eliminate this problem by making the nanoparticles more bio compatible. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for low-cost, energy-efficient, and nontoxic production of metallic nanoparticles (Thakkar et al., 2010).

The filamentous fungus *Cochliobolus lunatus*, has been used as an effective reducing agent for the synthesis of silver nanoparticles. This biological reduction of metal would be boon for the development of clean, nontoxic, and environmentally acceptable metal nanoparticles, the formed silver nanoparticles are hydrophilic in nature, disperse uniformly in water, highly stable, and had significant mosquito larvicidal activity against *A. aegypti* and *A. stephensi* (Salunkhe et al., 2011). Similarly, recently in our laboratory *C. tropicum* has found a pathogenic fungus. It is known to be an effective mosquito control agent. We have synthesized the silver and gold nanoparticles using *C. tropicum*. The silver and gold nanoparticles have been tested as a larvicide against the *Ae. aegypti* larvae. The larvicidal efficacy was noted when performed against all instars of *Ae. aegypti* at six different concentrations, and significant results could be observed. The gold nanoparticles used as an efficacy enhancer have shown mortality at three times higher concentration than the silver nanoparticles. The larval mortality was observed after different time of exposures. The effect of silver nanoparticles synthesized with *C. keratinophilum*, *V. lecanii*, and *F. oxysporum* f.sp. pisi has been evaluated against the adult mosquito of filariasis vector *C. quinquefasciatus*. Moreover, when AuNPs synthesized by *A. niger* have found to be more effective against the *Cx. quinquefasciatus* larvae than the *An. stephensi* and *Ae. aegypti* larvae. All larval instars of *Cx. quinquefasciatus* showed 100% mortality after 48 hours of exposure to the AuNPs synthesized by *A. niger* (Soni and Prakash, 2012a; 2012b). More significantly, the use of nanomaterial products in various sectors of science including health increased during the last decade. The application of hydrophobic nanosilica at 112.5 ppm was found effective against mosquito species tested. The larvicidal effect of hydrophobic nanosilica on mosquito species tested was in the order of *An. Stephensi* > *Ae. Aegypti* > *Cx. quinquefasciatus*, and the pupicidal effect was in the order of *An. Stephensi* > *Cx. quinquefasciatus*> *Ae. aegypti*. This is the first report that demonstrated that nanoparticles particularly nanosilica could be used in mosquito vector control (Barik et al., 2012).

The stability of the silver nanoparticles can be attributed to the formation of silver electride that may form a thin layer on the aqueous surface of the reaction mixture. This silver electride possibly may convert the silver to nanosilver. The protein present further is believed to cap the silver nanoparticles formed, restricting the agglomeration of the particles and thus checking the size and shape. The exact mechanism of the formation of these nanoparticles in these biological media is unknown. Presumably biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles. It seems quite probable that the phenols play an important part in the reduction of ions to AgNPs and AuNPs as the concept of antioxidant action of phenol compounds is not new. Therefore, in combination with mosquito nets or other vector control measures, such fungal synthesized AgNPs and AuNPs have significant impact on vectors of malaria

and filariasis and dengue. It can be potential candidates to be considered in integrated vector control programs. Fungal synthesized AgNPs and AuNPs are available and their application methods being simple and affordable can be useful in protecting from mosquitoes vectors.

7 Molecules for Mosquito Control technologies

Recently, Fan et al. (2012) have been agreed for exploiting host molecules to augment mycoinsecticides virulence. The pressing need exists for additional tools in insect control. Entomopathogenic fungi, such as *M. anisopliae* and *B. bassiana*, both US Environmental Protection Agency (EPA) approved biological control agents, offer an environmentally friendly alternative to chemical insecticides. One limitation to the use of entomopathogenic fungi is the relatively long time (6~12 days) it takes for the fungus to kill target insects. The major advantages of such a strategy are the following: first, the increase in virulence can be tailored to be host specific depending upon the host molecule (peptide) chosen; and second, the development of resistance can be minimized as the host peptide hormones regulate developmental processes that are species and tissue specific. Fan et al. (2012) have reasoned that interfering with key aspects of insect physiology through careful choice of species-specific host molecules has potential advantages over previous approaches in that a microbial pathogen of insects can be tailored to target specific insect species, thus avoiding nontarget effects. Furthermore, it might be more difficult for insects to develop resistance to such agents because the host molecules are critical for normal development. Important aspects of this idea remain to be fully verified, particularly with respect to issues concerning resistance development; however, our results demonstrate the feasibility of expressing host peptide molecules (hormones) in a fungal entomopathogen to increase its virulence. Fan et al. (2012) have examined two candidate host molecules representing a wide distribution of targets and effectors. Disruption of insect water balance by exploitation of insect diuretic hormones has long been suggested as possible means for insect control, however, effective means of delivering the peptide to insects has been lacking. This data show that fungal pathogens can serve as an efficient vehicle for exploiting these compounds. *Aea*-TMOF does not have vertebrate toxicity and has passed EPA/US Food and Drug Administration approval (Mnyone et al., 2010). The *Aea*-TMOF expressing *B. bassiana* strain was effective against adults and larvae, causing a decrease in fecundity and abnormal development, respectively. Whether these effects would meet the standard for commercial application is at present unknown. Further experiments examining impacts on feeding and disease transmission as well as using combinations of host molecules may lead to additional products with greater exploitability. The recent expression of a malarial sporozoite agglutinating antibody and antimicrobial toxin in the entomopathogenic fungus *M. anisopliae* has expanded the utility of fungal biological control in limiting the spread of diseases. In theory, the approach described in this report can be combined with the expression of such factors, leading to biopesticides with greater efficacy, specificity and safety. Even so, concerns regarding the field application and release of transgenic organisms and the constraints to adoption, whether economic or related to efficacy, permit further examination.

8 Conclusions

During the past decade, coverage the with vector control interventions increased sustainability in sub-Saharan Africa, with house hold ownership of at least one ITN reaching an estimated 53% by 2011 and remained at 53% in 2012. However, due to fewer deliveries of ITNs and increasing mosquito resistance to insecticides, recent success in malaria vector control may be jeopardized. World malaria report (WHO, 2012) recommends that in areas targeted for malaria vector control, all persons at risk should be protected by ITNs or IRS. The choice ITNs and IRS depends on number of entomological, epidemiological, and operational factors including seasonality of transmission, vector survival and behavior and insecticide susceptibility of anopheline vectors only. Now the new fungal vector control technology can be in mandate like ITN or IRS. Apart from that in our laboratory, Prakash and Priyanka (2007) have a Patent as novel larvicices or insecticide (1281/DEL/20061)

Chrysosporium tropicum Carmichael isolated from soil ecosystem and its chitinase with a combination of an algae and starch. The product formulation was used as dust and granular formulation that produce mortalities in all larval stages of selected mosquito species within 96 hrs. of exposure to the test concentrations in the laboratory. Formulation is prepared in capsules using different concentrations of culture filtrate of *C. tropicum* found to be effective in natural water also. The product formulation can provide an alternative and effective candidate to combat mosquito borne diseases such as malaria, filarial, and dengue. Moreover, *L. giganteum* produced Laginex affected on all mosquito larvae. This Laginex manufactured by AgraQuest, Inc. Colombia USA. The *B. bassiana*, *B. brongniartii*, *M. anisopliae*, and *V. lecanii* have used as biopesticides. Recently, Kamareddine et al. (2013) have produced trypsin modulating oostatic factor (TMOF) in an entomopathogenic fungus increases its virulence towards *An. gambiae*. The mycocontrol technology is encouraging for extensive future research. The possibility of using fungal based new molecules and new nanoparticles against mosquito larvae and adult mosquitoes has yet to be proven under field conditions. This needs further experimental and field trials. Further investigation need to be exerted to translate laboratory promising results of many of the microbial agents in the control of larvae and adults mosquito species into field control strategies. However, the implementation of laboratory generated data is may have need more support from environmental, and economical barriers. This new prospective image of the overall scene reviewed in the present review article may develop interested mosquito vector control technology.

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