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RESEARCH ARTICLE

FUNGI: MOSQUITO LARVICIDE

*Majumder, D.R., Khan, S., Sharif, S. and Shaikh, Z.

Department of Microbiology, Abeda Inamdar Senior College, Pune, India

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ABSTRACT

The use of Entomopathogenic fungi (Zygomycetes,Ascomcetes and Basidiomycetes) against mosquito larvae is one of the best environment friendly ways to eradicate Arthropods that cause a menace in the society. While most of us turn to chemical insecticides to destroy mosquitoes, these chemical agents have been known to cause allergic reactions in some and are simply harmful to others. Thus the use of Entomopathogenic fungi as a biological control agent should be brought in to use at a more commercial scale because of its target specific activity and as it is relatively safer than the commercially available synthetic biocontrol agents. This review elaborates on the mosquito larvicide activity of three phyla of fungi namely Zygomycota, Ascomycota and Basidiomycota. Mosquitoes not only cause irritable bites but are a major cause of spread of lethal diseases like Dengue, Chikungunya, Malaria, Filariasis, etc. In a developing country like India where at certain places there is low sanitation problem mosquito borne diseases are major threats. Almost 40 anopheline species have been reported for the cause of human malarial vector worldwide. (Mouatcho, 2010) The use of entomopathogenic fungi as mosquito larvicide would help prevent the spread at an early stage and would be a cost effective preventive measure too.

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INTRODUCTION

The discovery, in 1977, of the selective mosquito-pathogenic bacterium *Bacillus thuringiensis Berlinerisraelensis* (Bti) induced widespread interest in the search for other suitable biological control agents. In past few years interest in mosquito-killing fungi is evident, mainly due to continuous and increasing levels of insecticide resistance and increase in global risk of mosquito-borne diseases (Ernst-Jan Scholte *et al.* 2004). Global incidence of dengue has drastically risen in the last few years.

According to the World Health Organization (WHO), 390 million cases of dengue fever have been reported worldwide, and of the total number of cases, 96 million require medical treatment. The increase in number of cases of dengue in India was twice the original number from 2014 to 2015 and the worst affected city was Delhi with over 1800 cases of the fever. Apart from dengue other life threatening diseases that are a serious public health issues caused by mosquitoes include Malaria (1.3 – 3.0 million deaths) (Ohimain, 2017), Filariasis, Chikungunya (over 1 million), etc. The reasons behind this epidemic condition are depicted in the Figure1.

Corresponding autour: **Majumder, D.R.,**
Department of Microbiology, Abeda Inamdar Senior College, Pune, India.

Different Methods Used Previously For Control of Mosquito

Chemical Agents

Organophosphates, Di-chloro di-phenyl trichloroethane (DDT) are some of the chemical pesticides previously used for the control of mosquitoes. At present, use of chemical agents like (DDT) is banned because such agents cause widespread destruction of non-targeted organisms and leads to stress on environment. Pyrethroids, pyriproxifen (growth regulator) are now commonly used for controlling mosquito population but resistance has been developed against these growth regulators too. High cost is another drawback for chemical agents used in mosquito control. (Chandler *et al.* 2011; Benserradj and Mihoubi, 2014; Thiyagarajan *et al.* 2014; Lee *et al.* 2015 ;).

Different Biological Agents for Controlling Mosquito Population

Different bio control agents are depicted in Figure 2 and the drawbacks with their mode of action is given in Table 1

Advantages of Application of Entomopathogenic Fungi

•Fungus can be used as a potent commercial larvicide (insecticide) for mosquito larvae.

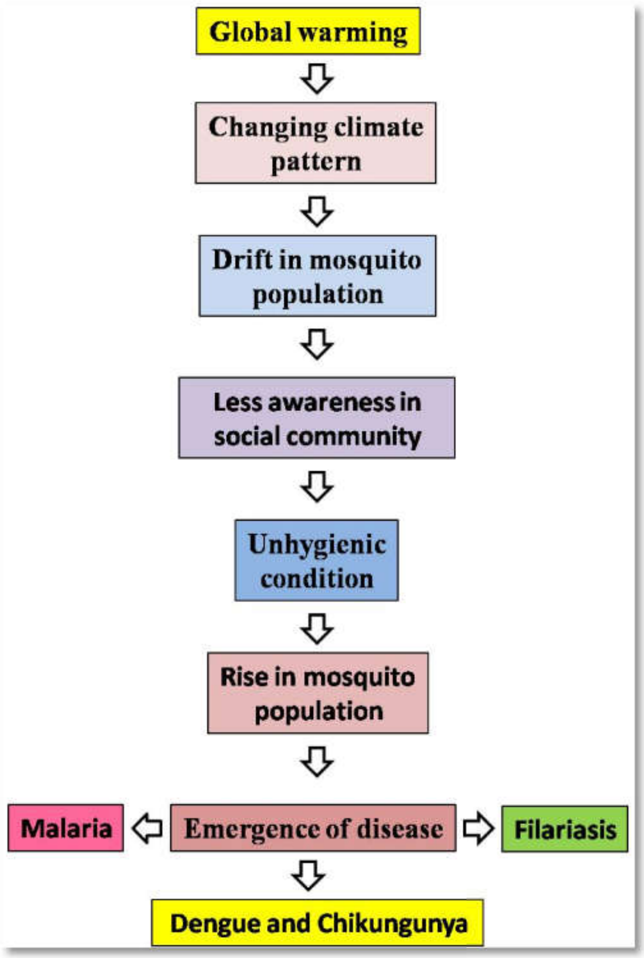


Fig. 1. Cause of Mosquito Borne Diseases (Epstein *et al.*, 1998; Kuhn *et al.*, 2003; Beck-Johnson *et al.*, 2013; Tyagi *et al.*, 2015; Dev *et al.*, 2015;Khan and Majumdar, 2016; Ohimain, 2017;)

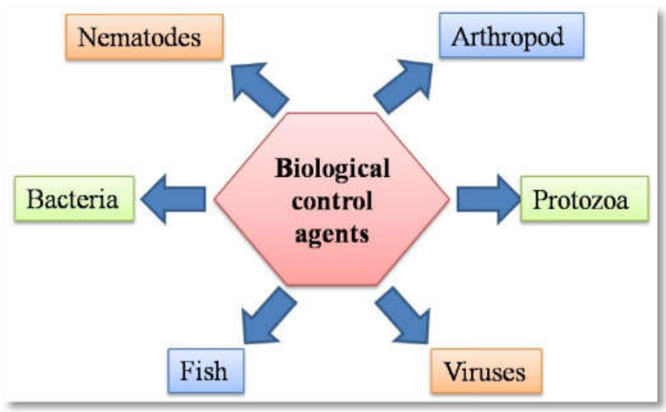


Fig. 2. Different Biological Agents for Controlling Mosquito Population (Shapiro *et al.*, 2005; Mouatcho, 2010;Ulrich 2016)

- Most commercially used mosquito larvicide come with a demerit of development of resistence against them while development of resistance against fungal pathogens have not been reported for insects yet. (Simon *et al*. 2005).
- Fungal larvicides are highly target specific.
- The fungal conidia/ fungal extracts can be mixed with adjuvants to increase their virulence factor (Gomes *et al*. 2015).
- Comparitvie studies of a commercially available larvicide (pylarvex) with fungal extracts (2 xathones and 1 anthraquinone) shows equal potentcy in their larvicidal activity (Josphat *et al*. 2010).
- Fungal larvicides come with a plus point that they are easily biodegradeable.
- They do not cause any damage to the ecosystem while eradicating the menace in their early stages of life.

This is well depicted in Figure 3

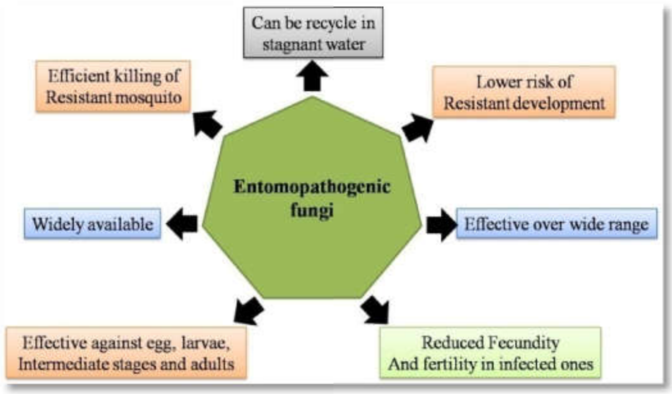


Fig. 3. Advantages of Entomopathogenic fungi (Andrade., 1993; Farenhorstand Knols, 2007; Mouatcho, 2010; Lee., 2015)

Types of fungi phyla used as Entomopathogenic

There are four groups of fungi viz. phylum zygomycetes, imperfect fungi, ascomycetes and basidiomycetes. This differentiation is based according to their sexual reproduction.(Figure 4 and Table 2)

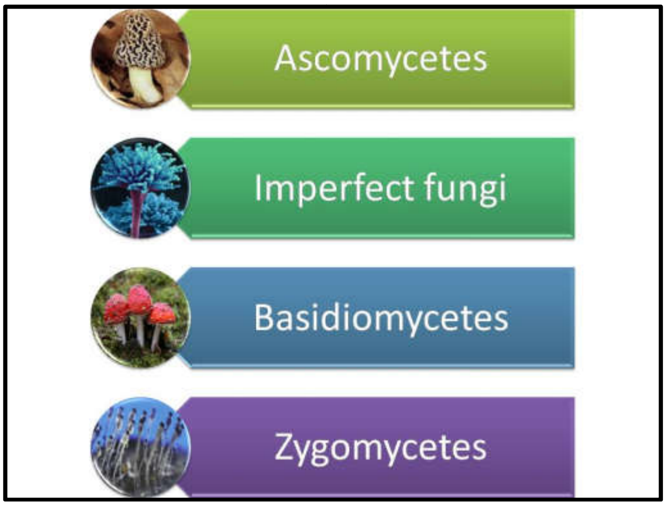


Fig.4. Types of Entomopathogenic Fungi (Carlile *et al.*, 2001)

Ascomycota: Most of the fungi known to us belong to the Phylum Ascomycota. Ascomycota, which is characterized by the formation of an ascus (plural, asci), a sac-like structure that contains haploid ascospores.

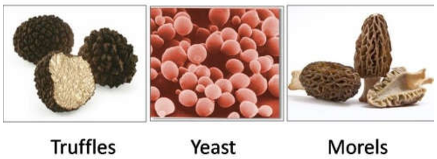
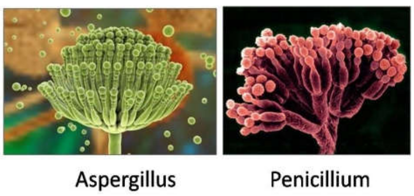
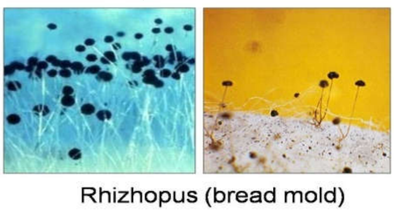
Imperfect fungi: Imperfect fungi are those that do not display a sexual phase. Deuteromycota is a polyphyletic group where many species are more familiar related to organisms in other phyla than to one another other; hence it cannot be called a true phylum and must therefore instead, be given the name form phylum.

Basidiomycetes: The class Basidiomycetes consists those members that produce their basidia and basidiospores on or in a basidiocarp. They concentrate growth primarily in the hyphae of a mushroom.

Table 1. Application of Specific Biological control agents (Shapiro *et al.,* 2005; Mouatcho, 2010)

No.	Biological control agent	Species	Mode of Action	Drawbacks
1.	Fish	*Aphanius discolour, Gambusia affinis.*	Feeds on Larvae Anophelines but not on Culicines.	-Not effective over wide range, -Selective in nature
2.	Arthropods	*Toxorhynchites brevipalpis*	Feeds on Larvae of *Aedes aegypti* in both natural and artificial environment.	-Temperature dependent, -Sufficient quantity of living prey required, -They exhibit cannibalism.
3.	Bacteria	*Bacillus thuringiensisvar. israelensis* *Bacillus sphaericus*	Ingestion of Larvicidal toxins,	-Ingestion is required, not a contact poison, -High cost production.
4.	Nematodes	Mermithidae family	Contact poison, releasing endotoxins in body cavities leading to death.	-Failure in production in Laboratory condition, -High cost production.
5.	Viruses	Mosquito iridescent viruses (iridoviruses, IRV), Cytopolyhedrosis viruses(reoviruses, CPV)	Virions infecting cytoplasm of epithelial cells of gastric ceca and posterior stomach causing chronic infection leading to death.	-Can be harmful to non targeted vertebrate and invertebrate. -Live host required for production
6.	Protozoa	Microsporidia of genus *Amblyospora, Nosema.*	Horizontal and transversal transmission of microsporidia from infected to uninfected host, killing their host by the chronic effects of parasitism	-Low level of infection, -Sensitive to UV light, -Intermediate host required.

Table 2. Characteristics of Different Entomopathogenic Fungi (Carlile *et al.,* 2001)

Phyla	Typical Example	Characteristics
Ascomycetes	Yeast, truffles, morels.  	Reproduce by sexual means; ascospores are formed inside a sac called ascus; asexual reproduction is also one of the methods to develop progeny.
Imperfect fungi	*Aspergillus, Penicillium.*  	Sexual reproduction has not been observed; most are thought to be ascomycetes who's capacity to reproduce sexually is gone astray.
Basidiomycetes	Mushrooms, toadstools, rusts.  	Reproduce by sexual means; basidiospores are borne on club-shaped structures called basidia; the terminal hyphal cell that develop spores is called a basidium; asexual reproduction occurs occasionally.
Zygomycetes	Rhizopus (black bread mold)  	Reproduce sexually and asexually; multinucleate hyphae lack septa, except for reproductive structures; fusion hyphae leads promptly to formation of a zygote, in which meiosis occurs just before it germinates.

Zygomycetes: Zygomycetes are the fast growing fungi characterized by old/primitive coenocytic (mostly aseptate) hyphae. Asexual spores consists of chlamydoconidia, conidia and sporangiospores packed in sporangia borne on simple or branched sporangiophores.

The Mosquito life cycle: Mosquitoes undergo a complete metamorphosis from egg to adult. They remain active as they transform from aquatic swimmers to terrestrial wing fliers. Mosquitoes are ectoparasites. They undergo the following stages in their life cycle (Figure 5)

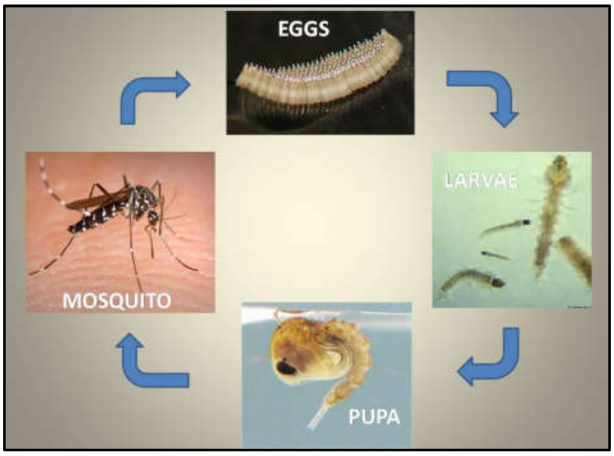


Fig. 5. Life cycle of Mosquito (Eckhoff, 2011; Le *et al.*, 2003)

Egg: After having her blood meal the female mosquito lays eggs one at a time or attached together in the form of "rafts" on the surface of water. The amount of eggs present in each raft consist hundreds of eggs. Water is the essential part of their habitat.

Larvae: The larva (e) lives in water and comes in the surface to breathe; they breathe through a breathing tube known as siphon. They feed on micro-organisms and organic matter present in water. Larvae shed (molt) their exoskeleton four times before they convert into a pupae undergoing the following larval stages viz; 1st instar, 2nd instar, 3rd instar and 4th instar larvae. Larvae are also known as wigglers.

Pupa: After the larvae have accomplished their fourth instar stage they become pupae. This is the stage in which they undergo metamorphosis and break open to become an adult mosquito. This is a non-feeding stage and the pupae appear like commas.

Adult: The adult mosquito eventually works its way out of the pupal case. As soon as after emerging it floats on the water surface once the wings and body gets hardened, it starts its life journey. The male usually emerges before the female. Most females die before they acquire their second blood meal but some may blood feed two or three times. Those females that obtain more than two different host blood meals transmit diseases since they have come in contact with several different hosts. The general mode of action of entomopathogenic fungi is shown in Figure 6

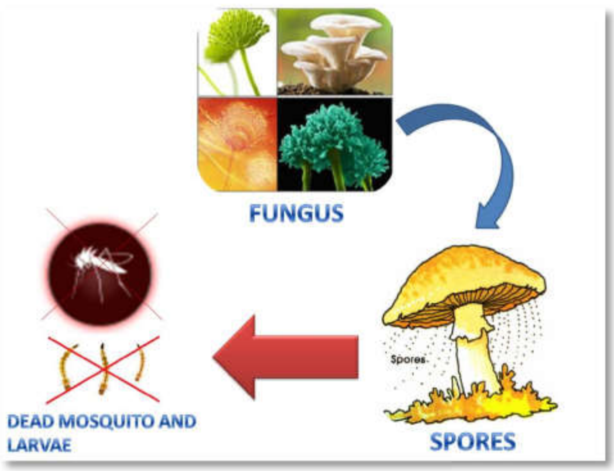


Fig.6. General Mode of action of Entomopathogenic Fungi (George *et al.*, 2013; Poopathi *et al.*, 2017)

Mode of action of fungus on larvae

The fungi act on the larva of mosquito in the following sequence (Figure 7)

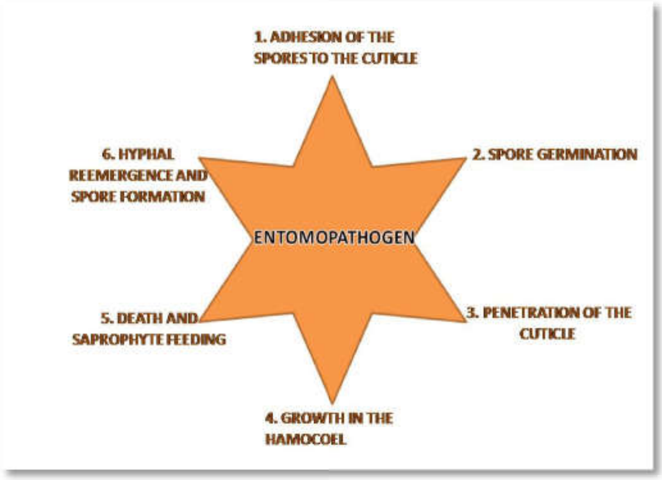


Fig. 7. Mode of Action of Entomopathogenic Fungi (Benserradj and Mihoubi, 2014; Lee *et al.*, 2015; Ravindran *et al.*, 2015)

Histopathological evidence

Normal and fungus affected gut lining of mosquito larvae are depicted in Figure 8.

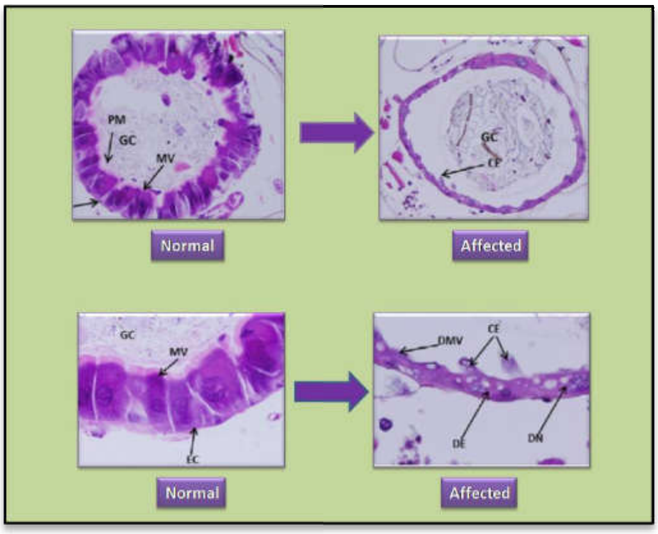


Fig. 8. Normal And Fungus Affected Gut Lining Of Mosquito Larvae (Abutaha *et al.*, 2015)

Cuticle: Microscopic evaluations reveal fungal spores attached to the cuticle of the dead larvae treated with fungus. Germinating spores and mycelia are also seen attached (Fawrou *et al*. 2009).

Abdomen: histological studies of the dead larvae reveal the invasion of spores into the gastric caeca & Invasion of conidia into the gut rupturing and disintegrating cells of gut. Germinating conidia observed at the tips of the abdomen (Seye *et al*. 2009).Certain abdominal epithelial cells showed accumulation granules in some areas of cytoplasm and faint and/or no nuclei. Certain other cells showed complete lack of cytoplasmic borders while some showed cytoplasmic vacuolization (Abutaha *et al*. 2015)

Table 3. Mode of action of different fungus acting on cuticle and abdomen of Mosquito. (Seye *et al.*, 2009; Darbro *et al.*, 2011; Benserradj and Mihoubi, 2014; Lee *et al.*, 2015; Narladkar *et al.*, 2015; Ravindran *et al.*, 2015.)

Part affected	Name of fungus	What acts as a slayer?
Cuticle	*Aspergillus clavatus. Metarhizium anisopliae, Beauveria bassiana Verticillium lecanii*	Chitinolytic enzymes. Subtilisin protease & destruxins, adhesins (Mad1 & Mad2)
Abdomen	*Cochliobolus spicifer*	Aliphatic compounds; Lipophilic components such as greases, macromolecules terpenoids, fatty acids and their esters.

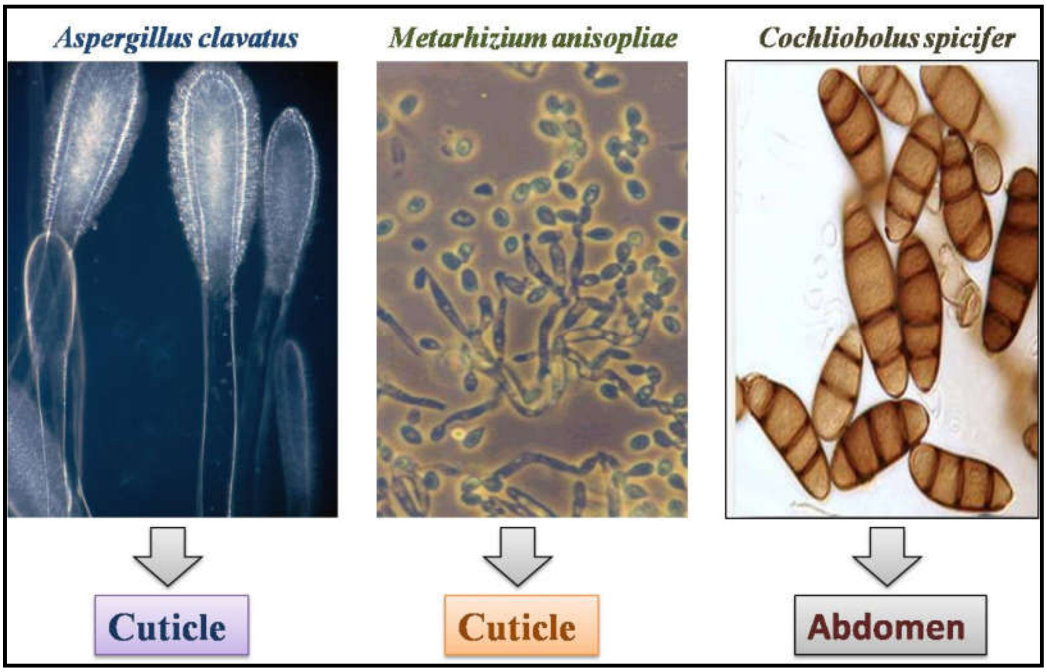


Fig.9. Morphology Of Different Entomopathogenic Fungi At Different Host Site (Hibbett *et al.*, 2007)

Respiratory siphon or alimentary canal: They are also the possible routes of invasion by fungus to cause the mortality of larvae (Seye*et al*. 2009). These are the main sites of infection when treated with fungal formulations (Bukhari *et al*. 2011). The attachment and growth of the fungus into the perispiracular valves of the siphon tube causes the blockage of air intake through respiration thus leading to the death of the larvae (Butt *et al*. 2013). The list of fungal species acting on cuticle and abdomen of Mosquito along with the bioactive component effective in killing the mosquito larvae is given in Table 3. Morphology of different entomopathogenic fungi at different host site from table 3 is seen in Figure 9

Future prospects

In some studies it is concluded that *Metarhizium anisopliae* have shown synergistic effect with Neem oil (Sun neem) in controlling the mosquito population by infecting adult stage of *Anopheles gambiae* and *Culex quinquefasciatus* more efficiently. (Seye *et al.* 2012; Gomes *et al.* 2015) Development of more such synergistic formulation having great potential in controlling the mosquito population should be prepared with proper dosage concentration and study of proper contact time should be analyzed.

Conclusion

In this world where pollution is a recurrent problematic issue, the use of chemical larvicide would multiply the problem. Therefore use of fungus as a larvicide (to mosquito) would help contribute to a better pollution free world.

The fungus is a better alternative to chemical larvicide as they do not generate resistance in the larvae and thus neutralise the threat posed by mosquito by preventing the spread of many deadly diseases by arresting the growth of mosquito at an early stage (larval stage).

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