Available online at www.worldnewsnaturalsciences.com

World News of Natural Sciences

An International Scientific Journal

WNOFNS 28 (2020) 34-50 EISSN 2543-5426

Potential biological control agents against mosquito vector in the case of larvae stage: A review

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ABSTRACT

Malaria is a major public health problem in most tropical and subtropical regions, including in our country, Ethiopia. A report published in 2010 stated that more than 1.2 million global malaria deaths due to malaria occurred in the one year alone. This figure includes both children and adults. Malaria disease is transferred from an infected person to an uninfected one by the bite of the female Anopheles mosquito. Herein, *Plasmodium falciparum, P. vivax, P. malariae*, *P. ovale* and *P. knowlesi* are the most important malaria causing parasites. Malaria disease does not have any effective treatment in the form of vaccines or drugs, so vector control is the only possible ways of prevention. Mosquito control using pesticides is presently the most widely used method for disease control. However, insecticide resistance has enabled pest resurgence, and the insecticides themselves have negative effects on human health, the environment and non-target organisms. In order to avoid these problems, biological control methods are proposed to control mosquito vectors. Biological control is an ecologically safe and effective means of minimizing pests and pest damage by means of using natural enemies. This review article offers an over view of the most favorable biological control methods for malaria extermination, such as larvivorous fish, entomopathogenic fungi, bacteria, viruses, nematodes and *toxorhynchites* larva. Here, we will discuss the current literature regarding biological control agents against the mosquito vector, and in doing so, will bring to light the importance of biological control in countering malaria. Finally, we will discuss the advantages and disadvantages of biological control methods, as compared with other methods commonly used to control malaria.

***Keywords*:** Agents, biological control, larvicides, larvivorous fish, malaria, mosquito vector

(Received 12 November 2019; Accepted 29 November 2019; Date of Publication 01 December 2019)

1. INTRODUCTION

Malaria is one of the most important vector transmitted diseases in tropical and subtropical areas of the world (Asia., 2007), including regions within our country, Ethiopia. The report of 2010 shows that, globally, more than 1.2 million deaths were attributed to malaria in the one year alone (including both children and adults) (Murray *et al.*, 2012). Malaria is transmitted from an infected person to a healthy one by the bite of the female Anopheles mosquito (Mullen and Durden, 2009). The most common malaria-causing parasites are *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. A fifth species, *P. knowlesi,* was recently discovered and infects mainly monkeys, but can also sometimes cause malaria in human beings (Kamareddine, 2012; Mullen and Durden, 2009). Malaria disease has no effective treatment in the form of vaccines or drugs, so vector control is the possible ways of prevention (Benelli *et al.*, 2016).

There are over 4500 species of mosquitoes in the world. These are grouped under 34 genera within the Culicidae family (Chandra *et al.*, 2013). The most common vector species are part of the genera Anopheles, Culex, Aedes, Psorophora, Mansonia, Haemagogus and Sabethes (Mullen and Durden, 2009). Mosquito vectors are a serious problem to community health as they are the vector for several dangerous ailments that potentially can affect more than 2 billion individuals living within the tropics (Odalo *et al.*, 2005). In addition to malaria, mosquitoes are responsible for the transmission of pathogens causing some of the most life affecting human diseases, such as yellow fever, dengue fever, chikungunya, filariasis and encephalitis (Bence, 1988; Benelli *et al.*, 2016; Collins and Blackwell, 2000; Ghosh *et al.*, 2005; Sarwar, 2015).

Mosquito control using chemical insecticides and personal prevention from mosquito bites are presently the most widely used methods for controlling this disease. However, chemical resistance has risen, hence, pest resurgence has occurred. Moreover, the insecticides have negative effects on human health, the environment and non-target organisms (Moraga *et al.*, 2006). In order to avoid these problems, biological control is proposed to control mosquito vectors.

Biological control is an ecologically safe and effective means of minimizing pests and pest damage by the use of natural enemies (Timmins, 1988). Almost all pests have their own natural enemies and suitable managements of natural enemies can successfully control several pests (Bence, 1988; Ghosh *et al.*, 2005; Mahar and Ridgway, 1993; Sarwar, 2015; Timmins, 1988). Biological control can be effective economically, and it should be the most commonly used control method to prevent malarial diseases (Mahar and Ridgway, 1993). Even though biological control cannot manage all malarial outbreaks, it should be the basis of an approach called integrated pest management, which means co-practicing several different pest control methods together. There are many different kinds of naturally occurring predators, parasites and pathogens of insect vectors such as the malarial mosquito. These include fishes, viruses, nematodes, fungi and bacteria. These differ in their mode of infection, site of replication, and mechanisms of pathogenicity (Porter *et al.*, 1993). Of the last, there are several different biological control approaches: killing the vector, changing vector behavior to increase self-mortality, and producing vectors that are either infertile or cannot transmit disease (Benelli *et al.*, 2016). Furthermore, these diverse biological control approaches affect different stages of vector growth (Benelli *et al.*, 2016). In the following sections, this review will focus upon the role of biological control agents against mosquito vectors by means of using different biological approaches.

2. HISTORY OF BIOLOGICAL CONTROL

The history of biological control has been expressed several times but it is not confined specifically to only one country. Biological control – the use of natural enemies and pathogens - has developed slowly from limited programs, to many programs through time. This means that in the ancient times, only limited species were used to control certain insect pests, but nowadays, there are many organisms that are used as bio-control for different pests, including mosquito vectors (Vail *et al.*, 2001).

The documented history of biological control dates back beyond 4000 years through ancient Egyptian records showing cats as being useful agents for rodent management. On the other hand, the idea that insects could be deliberately used to control other insects is also an ancient event. The first information of this practice seems to indicate that the practice was initiated by the Chinese, and, amazingly, involved the use of ant predators to destroy certain insect pests of citrus plants. Certainly, this agricultural practice has been applied through ages, continuing even into recent times in the Orient, where citrus growers keep and sometimes even purchase colonies of the predatory ant, *Oecophylla smaragdina* to colonize orange trees to limit the number of leaf eating insects (Bosch *et al.*, 1982).

While the deliberate control of insect and weed pests by biological agents has only become an effective method in pest management since around 1990, there are ancestor historical events that reveal the evolution of some of the essential ideas in the expansion of biological control. Indeed, many of these events indicate an amazing and perceptive insight into the workings of Nature. Without these early 19^{th} century findings and conceptualizations, modern environmental science (to which biological control has made substantial applications), would very likely have been much delayed. These findings and concepts speak about natural balance, natural checks to out of control population, natural control of numbers, symbiosis among diverse species (predominantly those of plants, animals, and their natural enemies), and the roles that natural enemies play in the determination of abundance (Bosch *et al.*, 1982; Emden, 2004). In this situation, natural enemies are organisms that kill or decrease the reproductive potential of another organism, such as predators and parasitiods (Poopathi, 2012; Porter *et al.*, 1993).

The history of applied biological control to a large degree shows our increasing understanding of ecology. Again, we can turn back the clock to the late 19th century when the idea and concepts underlying biological control contributed in important ways to the developing theories and principles of ecology and reinforced the practical preparation of biological control of pests. This is not surprising, since biological control is in its essence on ecological miracle, and its practice is an example of applied ecology (Bosch *et al.*, 1982).

3. BIOLOGICAL CONTROL AGENTS

Biological control methods have shown to play a big role in reducing the mosquito population. One method that has recently come to be used is vector control. There are three broad strategies to biological control: classical biological control (importation of natural enemies), augmentative biological control (augmentation of natural enemies) and conservation biological control (conservation of natural enemies) (Mahar and Ridgway, 1993). In this review I will discuss the role of biological agents such as larvivorous fish, bacteria, viruses, *toxorhynchites*, nematodes and entomopathogenic fungi for controlling mosquito vectors (Table 1).

Table 1. Mechanisms of action, modes of application, and several limitations of biological control.

Biological Control Agents	Mode of action and its effect	Mode of application	Limitations	
Larvivorous fish strains • Gambusia affinis • *Esomus dandricus,* • *Rasbora daniconius,* • *Trichogaster sp.* • *Aphanius dispar* • *Cyprinus carpio* • *Ctenopharyngodon idella* • *Clarias fuscus* • *Tilapia cyprinids* • *Oreochromis niloticus* • *Poecilia reticulate* • *Fundulus* species • *Nothobranchius sp* • *Cynolebias sp.*	• Reducing larval density by feeding	• At larval stages • At low doses • In restricted open field system away from applied fertilizers and pesticides	• Great variability at the level of efficacy • Negatively affects the native fauna when introduced in many habitats • Inappropriate for controlling mosquitoes in small water containers and in pools and puddles that rapidly dry out	• (Al-Akel and Suliman, 2011; Arijo *et al.*, 2017; Bence, 1988; Chandra *et al.*, 2008; Kamareddine, 2012; Mullen and Durden, 2009; Singaravelu *et al.*, 1997)
Bacterial strains • *Bacillus thuringiensis* • *Bacillus sphaericus* • *Streptomyces avermitilis*	• Spray and ingestion • Suppress late instars and outgrowing pupae • Destroy larval stomach by endotoxin protein production • Rapidly colonize the male reproductive system and female eggs of many mosquito vectors	• At larval stages • At large scales • Through vertical transmission from mother to offspring	• Bti has no reproduction to recycle the bacteria • Resistance to Culex species • Most of these studies are only experimentally approached	• (Ingabire *et al.*, 2017; Poopathi, 2012; Ramírez-Lepe and Ramírez-Suero, 2012)
Virus strains baculoviruses polyhedrosis virus densoviruses iridoviruses	Oral or anal transmitted Alter the ability of the mosquito to host the malaria parasite Transduce certain anti-Plasmodium	At both larval and adult stages In the micro-environment of the host	Only limited numbers of studies address the effect of viruses on malaria vector control No methods exist for growing viruses on artificial media	(Becnel, 2006; Becnel and White, 2007; Pirali-Kheirabadi, 2012a)

	genes or specific toxins in mosquito cells Reduce mosquito longevity	Through vertical transmission among mosquito generations		
***Toxorhynchites* strain** • *Toxorhynchites splendens*	By feeding reducing the larvae	Mainly at larval stages	Slow action	(Collins and Blackwell, 2000; Goettle and Adler, 2005; Pantuwatana *et al.*, 1979)
Nematode strains • *Allantonematidae*, • *Diplogasteridae*, • *Heterorhabditidae*, • *Mermithidae*, • *Neotylenchidae*, • *Rhabditidae*, • *Sphaerulariidae*, • *Steinernematidae* • *Tetradonematidae*	• Infect by infiltration of the cuticle and parasitism • Interfere in the mosquito reproductive behavior causing biological castration • Reduce mosquito populations • Decrease the rates of malaria transmission	• Mainly at larval stages	• Little is known about the parasitic effects of nematodes at the adult stages of mosquitoes • Limited number of species are become effective	• (Petersen, 1985; Pirali-Kheirabadi, 2012a; Platzer, 1981)
Entomopathogenic fungi strains • *Lagenidium*, • *Coelomomyces* • *Culicinomyces* • *Microsporidia* • *Metarhizium* • *Isaria* • *Lecanicillium*	• Upon direct contact with the mosquito cuticle • Slow killing • Affect the mosquito feeding, behavior and fitness conditions • Elevate the mosquito immune response and promote the production of secondary metabolites	• Indoor attracting odor traps • On indoor house surfaces • On cotton pieces hanging from the ceiling, bed nets and curtains	• Rapid fungal infections is required shortly after the mosquito picks up the malaria parasite	• (Andreadis, 2007; Darbro *et al.*, 2011; Evans *et al.*, 2018; McCoy *et al.*, 1988)

3. 1. Larvivorous fishes

Starting from around 1937, fishes have been used for controlling the larvae stage of mosquito. Release of native larvivorous fish into a lake/pond is one of the cheapest methods of vector control strategy, and brings about long-term reduction of mosquito vectors (Das *et al.*, 2018). This is because the introduction of an auto-reproducing predator into the ecology may give sustained biological control of pest populations. However, biological control of mosquito larvae using larvivorous fish becomes feasible and effective only when breeding areas are relatively few or are easily identified and treated (Chandra *et al.*, 2008). In addition to this, integrated methods of biological control should be carried out in order to reach the best targets of mosquito control (Al-Akel and Suliman, 2011).

Diverse type of fishes have been employed, While the usage of native fish is found to be more suitable in biological control (Chandra *et al.*, 2008; Howard et al., 2007), among the most widely used biological control agents are the mosquito fish (*Gambusia affinis*) (Mullen and Durden, 2009; Sarwar, 2015; Walton, 2007; Wickramasinghe and Costa, 1986). This fish is adapted to live in warm water and was originally native to the southern USA and northern Mexico. Later it has been introduced into over 60 countries, including the Pacific islands, Europe, the Middle East, India, South Asia and Africa, in efforts to reduce mosquito larvae (Mullen and Durden, 2009; Bence, 1988). Previous study conducted in India has indicated that mosquito fish are the greatest predator of the larvae of *Aedes aegypti* and *Anopheles stephensi*. This predatory efficiency focuses mainly on third instar larvae and in times of enhanced larval density (Arijo *et al*., 2017; Sinaravelu *et al*. 1997).

A study done on the larvicidal efficacy of four indigenous fish and one exotic fish has revealed that all the fishes have larvicidal potential, albeit with variations in their feeding effectiveness. The sequence of predation effectiveness noted in this study was, in terms of effectiveness from greatest to least, *Gambusia affinis* (exotic) and followed by *Esomus dandricus*, *Rasbora daniconius*, *Trichogaster fasciata* and *Trichogaster lalia* (Bano and Serajuddin, 2017). Another study conducted under laboratory conditions indicates that *Aphanius* is more effective than *Gambusia* in preying upon the third, fourth instars and pupal stage of mosquitoes. However, the opposite was true for the first two instars. On the other hand, *Aphanius* consumed more 2nd instar larvae in the natural habitat - particularly when many fishes were able to penetrate into shallow water (Homski *et al.*, 1994). The study also collected and identified 58 larvivorous fish species, but only 22 species of larvivorous fishes were considered to be effective larvicidal agents (Rao, 2014). Of importance is that killifish (*Aphanius dipar*) can reproduce both naturally and artificially to maintain a fish stock in order to protect local communities from several mosquito causing diseases such as malaria, dengue, encephalitis and many others (Al-Akel and Suliman, 2011).

Another widely used fish is the South American guppy (*Poecilia reticulata*). This is not as voracious as mosquito fish, but can be better adapted to water bodies subject to organic pollution. Moreover, it is more heat tolerant than *affinis* (Mullen and Durden, 2009). There are many other fish that can used to eat mosquito larvae, including carp (e.g. *Cyprinus carpio* and *Ctenopharyngodon idella*), and edible catfish (*Clarias fuscus*). Both can be introduced to water storage tanks to control *Aedes aegypti* (Mullen and Durden, 2009). Another study revealed that, several other edible fish have great potential to be used as larvivorous predators of mosquito (Arijo *et al.*, 2017). Indeed, in addition to mosquito control, some larvivorous fishes such as *Oreochromis niloticus* (formerly *Tilapia nilotica*) are commonly farmed for eating in the western Kenyan highlands. However, elsewhere, the direct introductions of tilapia (*Tilapia cyprinids*) into the ecosystem also has had devastating consequence in biological control (Howard *et al.*, 2007).

Previous research has indicated that immature to adult stages of black molly show good efficiency towards mosquito larvae, and, therefore, this fish can be used successfully to control the mosquito larvae of all species (Sumithra *et al.*, 2014). In experiments, *Trichogaster trichopteros* was the only black molly species in which both sexes fed completely on all of the present larvae. On the other hand, the male *Poecilia reticulate* showed a strong capacity for larvae feeding when compared with the female of the same species (Cavalcanti *et al.*, 2007). Most recently in southern Iran, 3 species of larvivorous fish (*Gambusia holbrooki*, *Aphanius dispar* and *Aphanius* sp) were identified as effective mosquito control agents (Shahi *et al.*, 2015). Predatory fish, such as *Aphanius dispar* and *Fundulus* species occur in saline waters and can be introduced into saltwater habitats.

Fish are, however, are considered inappropriate for controlling mosquitoes in small water containers and in pools and puddles that rapidly dry out. Still, some fish, such as species of *Nothobranchius* and *Cynolebias*, otherwise known as instant or annual fish, have drought resistant eggs, and these, are more appropriate for introducing into small temporary habitats that repeatedly dry out (Mullen and Durden, 2009).

Biological control of mosquito larvae by using larvivorous fish has shown many advantages over chemicals, but exotic mosquito fish may have negative effects on other native fishes and destroy local habitats. Such destructive fish, which have sometimes devastated indigenous species, should not be introduced into new areas (Mullen and Durden, 2009). Furthermore, these upon introduction, may reduce the number of other key aquatic invertebrate animals, such as predatory insects and zooplankton (Bence, 1988). Therefore, eco-friendly larvivorous fish that afflict less harm to the environment and local fish fauna are often more appropriate for biological control of mosquito larvae.

3. 2. Bacteria

Mosquitocidal bacteria are ecologically friendly alternatives to chemical insecticides for controlling mosquitoes, and, therefore, there have been great worldwide efforts to identify novel mosquitocidal bacteria within the natural environment (Poopathi *et al.*, 2014). *Bacillus. thuringiensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) are examples of mosquitocidal bacteria, and both have been employed as broad-spectrum biolarvicides under many conditions, with little or no ecological ill-influence, when taking into account ecological concerns such as safety for humans and other non-target organisms, reductions of pesticide residues in the aquatic ecology, improved activity of other natural enemies and improved biodiversity in aquatic environments. Both these *Bacillus* species are more like a microbial insecticides than true biological (living) agents that recycle and maintain themselves in the environment (Mullen and Durden, 2009).

Bacillus thuringiensis (*Bti*) preparation is the most commonly used microbial insecticides in worldwide (Ramírez-Lepe and Ramírez-Suero, 2012). It is unquestionably a most effective pathogen, as it can be easily mass produced, is toxicologically safe to humans and wildlife (the high-perceived safety and effectiveness of *Bti* was noted even at the very start of its use in intervention (Ingabire *et al.*, 2017)), and is more or less specific in killing mosquito larvae or the larvae of out of control species (Poopathi, 2012). After *Bti* is ingested, mortality is caused by an endotoxin that resulting a stomach poison, which is released from crystal proteins in the bacterial spores.

There are an enormous amount of studies on the effectiveness of *Bti* treatment in terms of mosquito abundance, but the results have varied (Land and Miljand, 2014). It is commonly formulated as a slow release agent that floats on the water surface and can give control for up to a month. Indeed, the Swedish government has undertaken several studies to assess the various effects of using *VectoBac G* to control the number of mosquitoes in waterlogged areas. *VectoBac G* is a granular formulation of the bacterium *Bacillus thuringiensis subsp. israelensis* (*Bti*) (Ramírez-Lepe and Ramírez-Suero, 2012). *Bti* is also formulated as a powder that is combined with water and sprayed around larval habitats. Of note, there is no reproduction of the bacteria, thus, in this form, there must be repeated sprayings (as with chemical larvicides).

The bacterium, *Streptomyces avermitilis*, produces toxins called avermectins, which are extremely effective in controlling different invertebrates from the classes Insecta, Arachinida and Nematode (Pirali-Kheirabadi, 2012b). Similarly, *Bacillus sphaericus* can also be formulated much as *Bti* is and it kills mosquito larvae in in the same way, but varies in some conditions as it can be recycled in larval habitats. Furthermore, this species is also more effective when used within organically polluted water and it is particularly effective against *Culex* species (Mullen and Durden, 2009).

It must be noted that resistance to both *Bti* and *Bs* has been recorded in laboratory colonies of a few mosquito species, especially *Culex quinquefasciatus*. Yet, although some field populations of *Culex pipiens* were seen as being resistant to *Bti*, no resistance was observed in *Aedes vexans* despite having been exposed to *Bti* for more than 25 years. Thus, *Bti* and *Bs* continue to be commonly used. In modern times, genetic engineering techniques, such as the development of recombinant strains of the two bacteria, seem to have enhanced their larvicidal functions. In addition to this, the genes responsible for production of the poisonous endotoxin have been transferred to other bacteria for the development of very effective and new bacterial strains that can be employed in mosquito vector control (Mullen and Durden, 2009).

3. 3. Virus

There are so many types of viral pathogens that cause disease in mosquitoes. These commonly belong to four major groups (Huang *et al.*, 2017), and include: the baculoviruses (Baculoviridae: Nucleopolyhedrovirus), cytoplasmic polyhedrosis viruses (Reoviridae: Cyprovirus), densoviruses (Parvoviridae: Brevidensovirus) and the iridoviruses (lridoviridae: Chloriridovirus) (Becnel and White, 2007; Federici, 1995). More than several tens of thousands of entomopathogenic viruses that are active against insect pests have been described, but still only very few are commercially accessible. Viruses either do not play great role in reducing parasites populations or else our knowledge is too limited to determine their true effects (Pirali-Kheirabadi, 2012b).

The main groups of pathogenic viruses in mosquitoes are divided into occluded (baculovirus and cyproviruses) and non-occluded (densovirus and iridoviruses) viruses. Baculoviruses, densoviruses and iridoviruses are DNA viruses, while cyproviruses are the major groups of RNA virus that are reported to affect mosquitoes (Becnel, 2006). Research done on mosquito pathogenic viruses has been reduced due to the incapability of transmit them to the larval mosquito host, but recently there have been great developments in the ability to transmit mosquito baculoviruses and cypoviruses with the finding that transmission is mediated by divalent cations (Becnel, 2006). Oral transmissions of both baculoviruses and cyproviruses to mosquito larvae are increased by the presence of magnesium and inhibited by calcium ions.

3. 4. *Toxorhynchites*

The larvae of *toxorhynchites* mosquito feed on other mosquito species and aquatic organisms that inhabit both natural and artificial containers. Because this habitat is the foundation of several medically important species of mosquitoes (Focks, 2007), *Toxorhynchites* species mosquito have been accepted as a potential biocontrol agents of vector species of mosquito under diverse situations (Collins and Blackwell, 2000). There have been many attempts to use them for this purpose since the beginning of the 19th century, although initially with relatively low levels of success. This has been attributed to a lack of knowledge of the general biology of *Toxorhynchites* mosquitoes (Collins and Blackwell, 2000). Mosquito in the genus *Toxorhynchites* fed on larvae stages of other mosquito species and often turn cannibalistic. They may eat as many as 400 larval mosquitoes during their larval growth - especially when released into small containers (Goettle and Adler, 2005). The combination of carnivorous larvae and harmless adults is very attractive in biological control, though shortages of research have not shown continuous larval management with this predator. Still, successful biological control has been reported using *Toxorhynchites* species in Japan, Southeast Asia, the Caribbean and the United States (Goettle and Adler, 2005).

Of the *Toxorhynchites*, *T. splendens* was indicated as being one of the most important species for mosquito control and has been used as integrated system in the biocontrol of mosquitoes. The ability of *Toxorhynchites splendens* larvae to consume *A. aegypti* larvae has been determined to be in the quantity of 20-25 larvae/day. For mosquito control purposes, the larvae of this species have been released into the water containers or breeding sites of *A. aegypti*, *A. albopictus* and *Culex quinquefasciatus*. In such situations, larvae were eradicated totally or almost completely within 3-4 days (Pantuwatana *et al.*, 1979).

3. 5. Nematodes

Nematodes are grouped under obligate or facultative parasites of insects (Pirali-Kheirabadi, 2012b). The Phylum nematode has five orders with 14 families of obligate parasites, but only the mermithidae have been found in natural populations of mosquitoes (Platzer, 1981). Some of these nematodes are of significantly interest because of their potential as biological control agents. In addition to Mermithidae, eight important nematode families such as Allantonematidae, Diplogasteridae, Heterorhabditidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinernematidae and Tetradonematidae include species that attack, kill, and sterilize insects, or alter host growth (Petersen, 1985). The mermithids are a larger and widely used species of nematodes for managing mosquito larvae. They are obligate parasites of arthropods, mainly insects, but have also been found in spiders, crustaceans, earthworms, leeches, and mollusks. They are usually specific to a single species or one or two families of insects and are commonly fatal to their hosts. Mermithids are principally attractive because they provide little or no environmental hazard, they offer no threat from competitive displacement of other desirable organisms because of their lifecycle, and the potential exists for inundative release to give high initial host reduction or inoculative releases to establish the nematode and give partial control for an indefinite period. Some species of mermithids were identified that control different species of mosquito larvae (Petersen, 1985). Mermithid nematodes have been documented from at least 63 species of mosquitoes worldwide, but till now they have received in little consideration. Such nematodes are major candidates as biological control agents because they affect particular growth stages of the host, are host specific; produce high levels of parasitism, kill the hosts, are easily handled, have a high reproductive potential, are free swimming and can be distributed easily in the infective stage to control mosquitoes. However, only one species of mermithid has been successfully mass cultured to date (Petersen, 1973). The species infects its host by infiltration of the cuticle, invasion through spiracles or anus or after ingestion by the host insect (Pirali-Kheirabadi, 2012b).

3. 6. Entomopathogenic fungi

Fungal pathogens affecting arthropods are found everywhere in tropical forests and are key components in the natural balance of arthropod populations. They can develop a range of specialized spore forms, as well as produce a variety of peculiar behaviors in their hosts, in order to increase infection (Evans *et al.*, 2018). Fungal diseases in insects are common and prevalent and can destroy mosquito vectors in an amazing manner. Nearly all insect orders are vulnerable to fungal diseases, including dipterans. Fungal pathogens such as *Lagenidium*, *Coelomomyces* and *Culicinomyces* commonly affect mosquito vectors, and have been studied broadly. However, many other fungi species also infect and kill mosquitoes at the larval or adult stage (Scholte *et al.*, 2004).

Several fungal pathogens have been found attacking and manipulating *A. Aegypti* in Africa forests and that these could be employed for an economic, environmentally safe and long-term solution to the flavivirus pandemics in the Americas (Evans *et al.*, 2018). The entomopathogenic fungi, *Metarhizium anisopliae* has confirmed in its feeding efficiency against mosquito species in the laboratory. Moreover, the virulence of *Metarhizium anisopliae* was tested against fourth instar larvae of *Culex pipiens* using five types of fungal concentrations. The results showed that the mortality of mosquito larvae treated with the different fungal concentration differ from 4 to 96%. Therefore, this study concluded that larvae mortality rate increased with increasing conidia concentration. Moreover, this study indicates that *Metarhizium anisopliae* has the potential to be a biological control agent for *Culex pipiens* and it is appropriate candidate for further study and development (Benserradj and Mihoubi, 2014).

Entomopathogenic fungus (*Beauveria bassiana*) may reduce disease transmission by decreasing mosquito vector endurance, although many isolates have not been tested for virulence against mosquitoes. There were 93 isolates of entomopathogenic fungi representing six species (*B. bassiana, M. anisopliae, Isaria fumosorosea, I. farinosa, I. flavovirescens, and Lecanicillium* spp.) that are considered as potential biological control agents of *Aedes aegypti* (Darbro *et al.*, 2011). The phylum chytridiomycota contains several entomopathogenic fungi species, but includes two genera (*Coelomomyces* and *coelomycidium* that are known to destroy the larvae of haematophagous diptera and which have been studied for the biological control of mosquitoes and black flies (Tanada and Kaya, 1993). The most commonly studied genera of entomophthoraleans fungi related to pest control contains *Conidiobolus*, *Entomophthora*, *Erynia* and *Neozygites*. In addition, *Basidiomycota* has a limited number of entomopathogens (McCoy *et al.*, 1988). Furthermore, the mitosporic fungi has in its ranks, many species of the most widely used entomopathogens, and members of the mitosporic entomopathogens are most commonly used biological insect vector control agents (Pirali-Kheirabadi, 2012b).

Microsporidia are one of the biggest and most diverse groups of parasitic fungi connected with mosquito species in the natural world. Indeed, it is quite likely that all mosquitoes serve as hosts for one or more *microsporidia* parasites. They are exclusive parasites of other eukaryotes and possess a unique and highly specialized mechanism for invading host cells by infectious spores (Andreadis, 2007).

4. ADVANTAGE AND DISADVANTAGES OF BIOLOGICAL CONTROL

4. 1. Advantages

Biological control has many advantages as a pest control method, particularly when compared with chemical insecticides. One of the most important benefits is that biological control is an environmental friendly method and does not introduce pollutants into the environment (Kok and Kok, 1999).

The other great advantage of this method is its selectivity. By this way, there is a restricted danger of damage to non-target species. Tebit (2017) underline that biological control does not create new problems, unlike conventional pesticides. According to Emden (2004), side effects can be totally excluded, as they have been very rare in the history of biological control (Emden, 2004). Selectivity is the most important factor regarding the balance of agricultural ecosystems because great damage to non-target species can lead to the restrictions in the population of natural enemies (Kok and Kok, 1999).

An additional advantage of biological control method is the ability to self-perpetuate. Biological control agents (BCAs) will increase in number and spread, because BCAs are self-propagating and dispersing. This is quite important regarding the economic feasibility of biological control (Reichelderfer, 1981).

Another advantage of the biological control method is that the pest is unable (or is very slow) to develop resistance (Tebit, 2017). However, it is probably possible for a target pest to develop mechanisms of defense against attack by a natural enemy. For example, we could imagine that effective control of a pest by a natural enemy could cause strong selection on the pest to develop mechanisms of escape or tolerance to attacks by the control agent, breaking down the biocontrol system (Holt and Hochberg, 1997). So far, this has not been too evident especially in relation to macro-controls such as larvivorous fish.

Furthermore, biological control can be cost effective. Its effectiveness is based on self-perpetuation and self-propagation as mentioned earlier. Therefore, if we establish a control agent in a specific area, it will reduce the target pest to an acceptable threshold for quite long time (Kok and Kok, 1999). In addition, a small number of biocontrol agents can grow to very high densities and provide continuous control of a pest over a large area. When the cost of deployment of BCAs is considered, biological control is generally less expensive than chemical control. The financial benefit of biological control is greatest in cases when there is no other option, such as inaccessible areas (Reichelderfer, 1981).

4. 2. Disadvantages

Biological control is usually more difficult to implement and maintain than insecticidal methods. The most important disadvantage of this method is the risk related with the income stability. In addition, BCAs are more susceptible to environmental conditions than chemical control. This consequently causes fluctuations to pest populations.

The other major problem is its incompatibility with conventional pesticides. As Emden (2004) mentions, biological control limits the subsequent use of pesticides, "where biological control agents are being used against one pest, it is clearly difficult to continue using insecticides against other pests on the same crop or other disease vectors in the same area. This may make the use of biological control impossible.

An addition to this, biological control has slow action. It does not lead to rapid control. It takes some days, or more often weeks, before mosquito populations are substantially reduced in size (Mullen and Durden, 2009). Furthermore, biological control is sometimes unpredictable, because natural enemies are significantly dependent on environmental conditions (Emden, 2004). The deployment of BCAs in a new environment requires a lot of research in order to succeed the desirable results because of climatic constraints.

Furthermore, the extermination of pests is not included in the aims of biological control. As Tebit (2017) points out, in general, it is accepted that the aim is to depress the pest population below the Economic Injury Level (EIL). Therefore, BCA is used to control pests in fresh fruits and vegetable, but the incomplete pest control is extremely undesirable. In this case, a minimum damage of product appearance is unacceptable by growers (Reichelderfer, 1981).

Selectivity, which was mentioned earlier as advantage, however, could also be possibly a disadvantage. Since BCA is a specific enemy to a single species, unaffected pests could cause damage (Reichelderfer, 1981). Moreover, biological control is difficult and sometimes expensive to develop in the field because it requires high qualified scientific staff (Tebit, 2017).

Variability in production batches is also additional a significant problem. This happens because the application of appropriate rearing procedures and the production of high quality BCAs increases the cost production of natural enemies. For this reason, quantity companies do often not apply measures in mass rearing and consequently production of good quality natural enemies may be difficult (Lenteren, 2003).

As earlier mentioned, biological control, method is environmentally safe. However, there are any risks associated with imports and releases of exotic natural enemies. As Kok and Kok (1999) note, biological control is most suited for exotic pest that are not closely related to indigenous beneficial species. On the other hand, it is unlikely for introduced predators that prey exclusively on mosquito larvae and pupae to eat harmless or even beneficial insects.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Generally, several approaches have been used in malaria control. These approaches either abort the development of the plasmodium parasite within the mosquito body, or suppress the mosquito vector itself. However, several factors such as depending on chemical vector control strategies, limited availability of resource and infrastructure, and poor management plans lead to a decrease in the effectiveness of malaria control at the vector levels.

In addition to this, mosquito control using chemical insecticide fails due to environmental differences and variations in the behavioral features of several mosquito species, such as the development of insecticide resistance among mosquito strains and pest resurgence. Due to such reasons, the need of developing different vector control strategies increases. This leads to applying biological control of mosquito vectors because such an approach has few and minor side effects.

Even though biological control is more difficult to implement and maintain, it has many advantages as a pest management strategy, particularly when compared with chemical insecticides. One of the most important benefits is that biological control is an environmental friendly method and does not introduce pollutants into the environment. Therefore, further studies are needed to search for potential biological agents that reduce the disadvantages of biological control and improve its advantage.

The reviewer proposes the following necessary points that will reduce the disadvantage and increase the advantages of biological control:

- Use biological control agents that are easily adaptable, constantly reproducing and feeding continuously no matter the local environmental conditions.
- Select and use biological agents that are adapted to conventional pesticides in order to avoid the incompatibility problems.
- Rear in large quantity and release the effective biological control agents in bulk to increase the speeds of killing power or reduce the mosquito vector.

- Use indigenous species as biological control agents to avoid the destruction of other beneficial organisms due to the introduction of exotic species.

Acknowledgments

I am very grateful to Engida Hagos who helped me by giving valuable comments and suggestions to this review paper. Without his contribution, this review would have been impossible. Furthermore, I would like to extend my gratitude to my friends who give me constructive comments so as to finish this article.

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