Saudi Journal of Biological Sciences 29 (2022) 1998–2006

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Review

Some biologically active microorganisms have the potential to suppress mosquito larvae (*Culex pipiens*, Diptera: Culicidae)

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ARTICLE INFO

Article history:
Received 20 October 2021
Revised 5 December 2021
Accepted 12 December 2021
Available online 16 December 2021

Keywords:
Biological control
Mosquito
Bacteria
Fungi
Viruses

ABSTRACT

Malaria is a disease caused by protozoan species of the genus *Plasmodium*. It is widespread and becoming a challenge in several African countries in the tropical and subtropical regions. In 2010, a report was published showing that over 1.2 million death cases were occurred globally due to malaria in just one year. The transmission of the disease from one person to another occurs via the bite of the *Anopheles* female. It is known that *Plasmodium ovale, P. vivax*, *P. malariae*, *P. falciparum,* and *P. knowlesi* are the highly infective malaria species. The problem of this disease is the absence of any effective medical treatment or vaccine, making the mosquito control is the only feasible way for disease prevention. Pesticides are currently the most widely used method for mosquito control, despite its well-known negative effects, including health hazards on human, the increasing insecticidal resistance, and the negative impact on the environment and beneficial organisms. Biological control (also called: biocontrol) of insects has been a promising method to overcome the negative effects of using chemical insecticides, as it depends on just using the natural enemies of pests to either minimize their populations or eradicate them. This article provides an overview of the recent and effective biological means to control malaria, such as bacteria, fungi, viruses, larvivorous fish, *toxorhynchites* larva and nematodes. In addition, the importance, advantages, and disadvantages of the biocontrol methods will be discussed in comparison with the traditionally used chemical methods of malaria control with special reference to nanotechnology as a novel method for insects' control.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.12.028

1319-562X/

Contents

1. Introduction

Malaria is the most prevailing mosquito-borne human disease in the tropical and subtropical regions on earth, with huge negative impacts on medical, economic, and social levels (Asia, 2007). It was reported in 2010 that in a global scale, there was more than 1.2 million death cases because of Malaria in only one year (which include both adults and kids) (Murray et al., 2012). Malaria transmission from infected individual to another healthy one is mediated by the bite of an Anopheles female (Mullen and Durden, 2002). Parasites that are very common in causing malaria are: *Plasmodium vivax, P. falciparum, P. ovale* and *P. malariae*. Recently, *P. knowlesi* was also discovered, which mainly infects monkeys, but sometimes can also infect human beings (Kamareddine, 2012; Mullen and Durden, 2002). This disease is notorious for the absence of any effective medical treatment or vaccine, which makes mosquito control the only feasible way of disease prevention (Benelli et al., 2016a, 2016b). Mosquito species (reaching over 4500) are grouped in 34 genera under the *Culicidae* family (Chandra et al., 2013). Species of *Anopheles*, *Aedes*, *Culex*, *Mansonia*, *Haemagogus*, *Psorophora* and *Sabethes*, are the most common vectors of malaria (Mullen and Durden, 2002). The problems of mosquitoes are not only limited to malaria transmission, since it also serves as vector for some of the most life-threatening human diseases that can infect more than two billion humans in tropical region (Odalo et al., 2005). Examples of those diseases are dengue fever, yellow fever, filariasis, chikungunya, and encephalitis (Bence, 1988; Benelli et al., 2016a, 2016b; Ghosh et al., 2005; Sarwar, 2015).

Currently, the most extensively used method in controlling mosquito is chemical pesticides (in addition to personal protection methods), despites its well-known negative effects. Most clearly, are the health hazards on human, the increasing insecticide resistance, and the negative impact on the ecological system and beneficial organisms (Moraga et al., 2006). Therefore, various studies demonstrated several Eco-friendly natural compounds which may have insecticidal activity among these compounds are bioactive peptides (Saad et al., 2020a, 2021a; El-Saadony et al., 2021a,b), polyphenolic extracts (Saad et al., 2020b, 2021b,c), essential oils (El-Tarabily et al., 2021; Abd El-Hack et al., 2021; Alagawany et al., 2021), and nanomaterials (Saad et al., 2021d; El-Ashry et al., 2022; El-Saadony et al., 2021c), additionally microbial control is a safe and an effective attitude in controlling heavy metals and insects (Desoky et al., 2020). These techniques were used to circumvent such problems, biological control (also known as: biocontrol) was introduced as an alternative in order to control mosquito vectors. Fruthermore, genetic engeneering can be used to develop the plants' autoresistance aganist insects (Saad et al., 2020). Biocontrol is an environmentally friendly and effective approach of controlling pests and their damages via the use of their natural enemies (Timmins, 1988). Most, if not all pests are known to have their own biological enemies, which can be exploited to control even more than one pest at once (Bence, 1988; Ghosh et al., 2005; Sarwar, 2015). Due to its economical efficiency and the health and environmental benefits, biocontrol should be the highest recommended method for malaria control (Mahar and Ridgway, 1993). Biocontrol can even work more efficiently if employed as part of a more comprehensive control management, using multiple methods of pest control in concert, a process known as "integrated pest management". Insects and mosquito natural enemies can be pathogens, parasites, or predators, which include fishes, nematodes, bacteria, fungi, and viruses. Those organisms have a wide variation in their infection mode, replication site, and pathogenicity mechanisms (Porter et al., 1993). There are several mechanisms for biocontrol pathogenicity, such as: vector killing, creating behavioral-based increasing self-mortality, creating infertile vectors or incapable of disease transmission (Benelli et al., 2016a, 2016b). Recently, nanotechnology tended to find new toles for insects' control for instance, external parasites of pigeons are a nuisance to birds and considered as a mean for diseases transmission either mechanically or biologically (Attia and Salem, 2021; Salem et al., 2022; Soliman et al., 2022) therefore, chitosan-silver nanocomposite was used for the control of experimentally infested pigeons with *Pseudolynchia canariensishas* (*P. canariensis*) and the used nanocomposite revealed a promising effect in the elimination of *P. canariensis* infestation in pigeons as well as, ameliorated the negative effects of insects infestation (Attia et al., 2022). Therefore, the use of these natural practices is safer, environmentally friendly, and more effective than chemical formulations and limits the spread of diseases (Swelum et al., 2020).

2. Biocontrol agents

Biocontrol has expanded slowly over time from a limited practice to a more effective and enlarged biocontrol methods. The main development has occurred in the number and varieties of the biocontrol agents used to fight different pests and mosquito vectors (Vail et al., 2001). Biocontrol methods have already played a major role in minimizing the population of mosquito, mainly through the control of vector. The following parts of this review will discuss the role of different biocontrol agents in killing mosquitoes using different mechanisms. different biological control methods are seen in Table 1 and Fig. 1.

2.1. Bacteria

On the international scale, there is a great and continuous efforts to identify new mosquitocidal strains of bacteria from the environment. That is because using bacteria as biocontrol agent has been proven as environmentally friendly method and is a promising alternative for controlling mosquitoes using chemical insecticides (Poopathi et al., 2014). Some of the prominent examples of mosquitocidal bacteria are *Bacillus thuringiensis* (*Bt*) and *Bacillus sphaericus* (*Bs*), as both have been used as biolarvicides with a wide range of insect hosts. Both species are working under variable conditions, with almost undetectable adverse ecological effects, such as human safety concerns, affecting non-target organisms. That makes them highly beneficial in reducing pesticide residues in the environment, and thus improving the activities of other natural enemies and enhancing the biodiversity in natural environments. Those two species are not only used as living biocontrol agents but are now mostly used as preparations of non-living microbial insecticide (in the form of toxin) (Mullen and Durden, 2002).

Globally, the most frequently used microbial insecticides are the preparations of *Bacillus thuringiensis* (*Bt*) (Ramírez-Lepe and Ramírez-Suero, 2012). It is by far, the most efficient pathogen, due to its ease of mass production, high safety level to human and environment (Ingabire et al., 2017), and its obvious variation in the host range specificity (for mosquito larvae) of its species (Poopathi, 2012).

Bacillus thuringiensis has a crystal endotoxin protein attached to the spore. This protein remains nontoxic until it reaches the insect's gut, where it is processed and becomes toxic due to high pH level in the insect gut. That same mechanism is their point of strength, when it comes to human safety aspects, since the pH of the human stomach is acidic, which is not suitable for converting the toxin to its toxic form (Reyaz et al., 2021). There is abundance of studies regarding the effects and benefits of using Bt as insecticidal, with wide variation in the level of effectiveness (Land and Miljand, 2014). The most common form of Bt is a slow-release preparation with a lasting effect for up to one month. Another form of Bt application is a soluble powder, applied to the larval area. After spraying the bacteria, it will not be able to reproduce, therefore, repeating the application is necessary. One of the other toxin-producing bacteria is *Streptomyces avermitilis*, which produces avermectins toxin, known to be highly effective against classes of Insecta, Nematode and Arachinida (Pirali-Kheirabadi, 2012). *Bacillus sphaericus* is like Bt in its killing mechanism, however its capable of multiplication in the larval environment. In addition, it is specifically effective in controlling *Culex mosquito* (Mullen and Durden, 2002). It is worth mentioning that, even though few species of mosquitoes (e.g, *Culex quinquefasciatus* in

Table 1
Different types of biological control of mosquito.

Biological control type	Examples	Mechanism of action	References
Predator fish	*Gambusia affinis*, *Aphanius dispar* *Cynolebias* species *Fundulus* species *Clarias fuscus* *Cyprinus carpio* *Nothobranchius* species *Poecilia reticulate* *Tilapia cyprinids*	They have capability of to feed on developmental stages of mosquito	(Chandra et al., 2008; Das and Prasad, 1991; Louca et al., 2009; Subramaniam et al., 2015, 2016; Van dam and Walton, 2007)
Amphibians	Tadpoles, Frogs, Toads	They have capability of to feed on developmental stages of mosquito	(Bowatte et al., 2013; Brodman and Dorton, 2006; Murugan et al., 2015)
Omnivorous copepods	*Cyclops vernalis*, *Megacyclops formosanus*, *Mesocyclops*	They have capability of to feed on developmental stages of mosquito	(Benelli et al., 2016a,b; Schaper, 1999; Vu et al., 1998)
Odonate young instars	*Libellula forensis*	They have capability of to feed on developmental stages of mosquito	Singh et al. (2003)
Water bugs	*Lethocerus americanus*	They have capability of to feed on developmental stages of mosquito	Bailey (1989), Shaalan et al. (2007)
Larvae of another mosquito species	*Toxorhynchites spp*	They have capability of to feed on developmental stages of mosquito	Steffan and Evenhuis (1981), Focks et al. (1985)
Bacteria	*Bacillus thuringiensis* *Streptomyces avermitilis* *Bacillus sphaericus*	Bacterial as biocontrol agent has been proven as environmentally friendly method and is a promising alternative for controlling mosquitoes using chemical insecticides	Ingabire et al. (2017), Kendie (2020)
Fungi	*Microsporidia* *Coelomomyces* *Metarhizium*	Fungi that are pathogenic to arthropods are widespread in the tropical area, and play a major role in the arthropod population balance	Evans et al. (2018)
Virus	*Densoviruses* *Baculoviruses* *Iridoviruses*	The number of entomopathogenic viruses that have known active insecticidal effects reach up to tens of thousands, despite the very limited number of its commercially available products, probably because of our limited knowledge or experiments testing the viral field effects	Becnel and White (2007)
Toxorhynchites	*Toxorhynchites splendens*	*Toxorhynchites* is a mosquito genus known for the capability of its larvae to feed on species of mosquito and other aquatic organisms living in either natural or artificial habitats	Focks (2007)
Nematode	*Rhabditidae* *Heterorhabditidae* *Steinernematidae*	Nematode having species that can parasite, kill, or affect host growth. The infection style of nematodes is either by entering during insect feeding, penetrating throughout the cuticle, or entering via anus or spiracles	Petersen (1985)

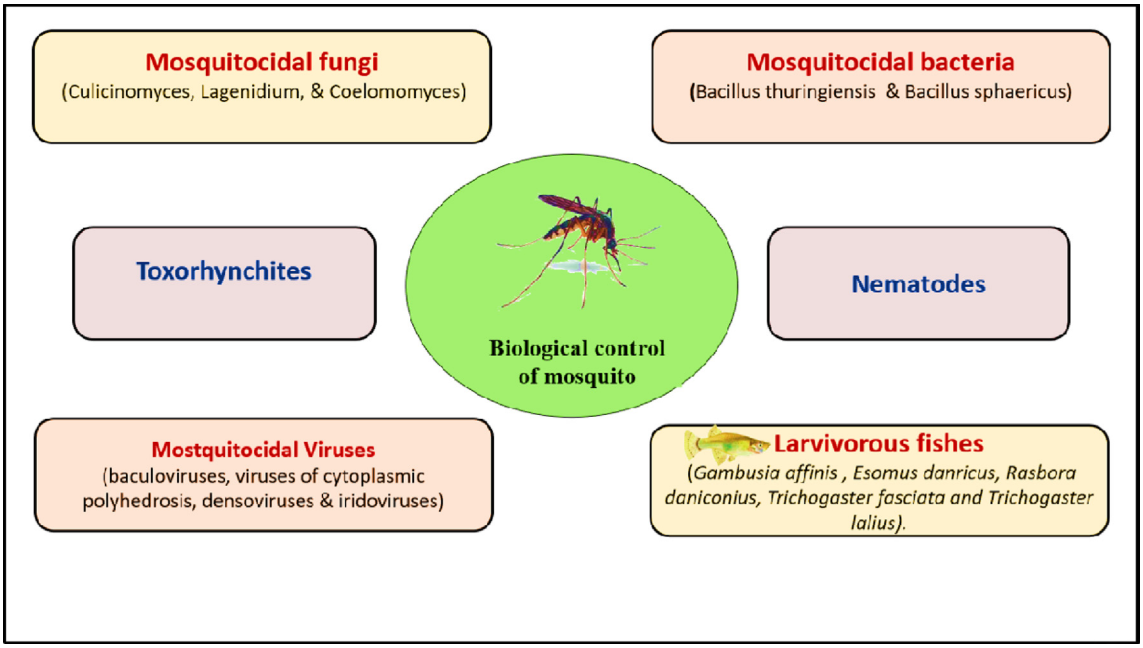

Fig. 1. Different methods for biological control of mosquito.

the laboratory and *Culex pipiens* in the field) have developed resistance against Bt and Bs, other species like *Aedes vexans* has never developed resistance for Bt even after 25 years of exposure. Therefore, Bt and Bs are still maintaining their reliability in the field of microbial insecticides.

Recently, genetic engineering technology has increased the potential of microbial insecticides, for example by recombining the important genes from the mentioned species, therefore, broadening their host range of insects. In other cases, endotoxin-producing genes can be transferred to other bacteria that are more adaptive to the environment, highly reproducible, or even transferring the genes to the target plant itself (Mullen and Durden, 2002).

2.2. Fungi

Fungi that are pathogenic to arthropods are widespread in the tropical area, and play a major role in the arthropod population balance. The variation of fungal spore forms, in addition to the different abnormal behaviors that they cause to the host, both increase the efficiency and rate of the infection (Evans et al., 2018). It is very common that insects are infected by fungi, which usually cause definite death. Almost all insects are susceptible to fungal infections, including mosquitoes. Many fungal species can infect and destroy mosquitoes at different stages, some of which are *Culicinomyces*, *Lagenidium*, and *Coelomomyces* which infect mosquito vectors, and have attracted a lot of researcher's attentions (Scholte et al., 2004). Several fungi are capable of attacking *A. aegypti* in some African regions, which can be used as economic, as well as environmentally friendly tools for controlling the pandemics of flavivirus in north and south America (Evans et al., 2018). *Metarhizium anisopliae*, an entomopathogenic fungus, has shown promising laboratory results in its infection of different mosquitoes. In addition, different concentrations of the fungus were tested for infectivity against *Culex pipiens* (fourth instar larvae), where the mosquito mortality rate reached up to 96% by increasing the concentration of fungal conidia. These results introduce *Metarhizium anisopliae* as a promising candidate for biocontrol of *Culex pipiens*, and thus, it deserves further studies and research focus (Benserradj and Mihoubi, 2014). *Beauveria bassiana* is another entomopathogenic fungus that was reported to decrease the endurance of mosquito vectors, and it required further virulence studies against mosquitoes. It has been reported that the isolate numbers of entomopathogenic fungi is 93, classified under six species. Those species: *Isaria flavovirescens*, *I. fumosorosea*, *I. farinosa, M. anisopliae, B. bassiana, and Lecanicillium* spp., can be a suitable potential source for biocontrol fungi against *Aedes aegypti* (Darbro et al., 2011). The genera of *Coelomycidium* and *Coelomomyces*, under the phylum Chytridiomycota, showed high mortality rate for haematophagous diptera, and had considerable attention as biocontrol agent against mosquitoes and black flies. It is worthy of note that the same phylum (Chytridiomycota) has several other entomopathogenic fungi that deserve attention (Tanada and Kaaya, 1993). The genera related to entomophthoraleans fungi that received most attention in the field of pest control are *Conidiobolus*, *Neozygites, Entomophthora*, and *Erynia*, while the least number of entomopathogenic fungi were reported in *Basidiomycota* (McCoy et al., 1988). On contrary, mitosporic fungi contains various prominent entomopathogenic species, and many of them are most frequently used as biocontrol agents for insect vectors (Pirali-Kheirabadi, 2012). Similarly, *Microsporidia* are known as one of the largest and highly versatile parasitic fungal group against mosquitoes, and it is highly possible that mosquitoes are considered hosts for at least one, if not more, parasitic *microsporidia*. This group also include many effective parasites for other eukaryotes and have a very specific system for penetrating host cells through their spores (Andreadis, 2007).

2.3. Virus

Mosquitoes are vulnerable for infection by too many viruses, which are mainly classified under four main groups (Huang et al., 2017), including: Baculoviruses, (genus: Nucleopolyhedrovirus, under family: Baculoviridae), viruses of cytoplasmic polyhedrosis (genus: Cyprovirus, under family: Reoviridae), densoviruses (genus: Brevidensovirus, under family: Parvoviridae), and iridoviruses (genus: Chloriridovirus, under family: Iridoviridae) (Becnel and White, 2007; Federici, 1995). The number of entomopathogenic viruses that have known active insecticidal effects reach up to tens of thousands, in spite of the very limited number of its commercially available products, probably because of our limited knowledge or experiments testing the viral field effects (Pirali-Kheirabadi, 2012). There are two major viral pathogenic groups that infect mosquitoes, namely: occluded (baculoviruses and cyproviruses) and non-occluded (densoviruses and iridoviruses). Those are also divided into DNA viruses (Iridoviruses, Baculoviruses, and Densoviruses), and RNA viruses (cyproviruses) (Becnel, 2006). The weak transmission efficiency of pathogenic viruses to the mosquito larvae is a major obstacle that hindered the advancement of using them in mosquito control. However, recent researches have shown that incorporation of divalent cations has greatly facilitated the transmission of cyproviruses and baculoviruses to mosquito larvae. The presence of magnesium ions with cyproviruses or baculoviruses in the larvae feeding, increases the viral infection rate substantially, while the presence of calcium ions inhibits the infection rate (Becnel, 2006).

2.4. Larvivorous fishes

Using fishes to control mosquito's larval stage have started in nearly 1937. Raising larvivorous fish in water is a highly economical method of mosquito control, and it also has a sustained long-term effect as it is a natural and auto-reproducing enemy (by means of predation) for insects (Das et al., 2018). This method, however, seems to be effective and practical when the areas of mosquito breeding are fairly few, and can be identified easily (Chandra et al., 2008). Moreover, to achieve the highest mosquito control efficiency, an integrated biocontrol method must be followed (Al-Akel and Suliman, 2011). While several types of fishes can be used, it is best to use the native fish, as it is highly sustainable in its environment (Chandra et al., 2008). *Gambusia affinis* is one of the most fishes being used widely as a biocontrol agent (Wickramasinghe and Costa, 1986; Walton, 2007; Sarwar, 2015). This fish was basically native to southern USA and northern Mexico and is capable of living in warm water. However, after only few years, it was used in almost 60 countries for the purpose of controlling mosquito larvae. Those countries are located in Europe, the Pacific islands, India, Middle East, Africa, and South Asia (Mullen and Durden, 2002). Earlier studies have proven that mosquito fishes are the greatest enemies of the *Anopheles stephensi* and *Aedes aegypti* larvae. Their predation efficacy focuses mostly on the 3rd instar larvae (Singaravelu et al., 1997; Arijo et al., 2017). Bano and Serajuddin (2017) studied five fish species to evaluate their larvicidal efficiency; four of which were indigenous, and one was foreign. They found that these fishes have considerable larvicidal effect, with some differences in the level of efficiency. The highest predation success was found in the exotic (*Gambusia affinis*), followed by *Esomus danricus*, *Rasbora daniconius*, *Trichogaster fasciata* and *Trichogaster lalius*. Different results were found from another laboratory study, where *Aphanius* was found to be a better mosquito predator than *Gambusia* for 3rd and 4th instars and mosquito pupal stage. Opposite results, however, were found for the 1st and 2nd instar larvae. In addition, when more *Aphanius* fish exist in a shallow water, they attack more larvae (2nd instar) in water environment (Homski et al., 1994). Another study focused on collecting and identifying larvivorous fish, found that the efficient larvicidal fishes were only 22 out of 58 screened fish species (Rao, 2014). *Aphanius dipar* (a killifish type) has the capacity to reproduce, either naturally or artificially, so that it can maintain a reasonable population, which can be a major control for the disease-causing mosquitoes (Al-Akel and Suliman, 2011). There are other fish species that are not as aggressive as mosquito fish, however, they can be suitable for water polluted with organic contaminants and is also more adapted for hot environment than *Gambusia affinis*. One widely used example is *Poecilia reticulate*, a South American guppy fish (Mullen and Durden, 2002). There are other fish species suitable for controlling *Aedes aegypti* in water tanks, where they feed on mosquito larvae, some of which are: *Ctenopharyngodon idella* and *Cyprinus carpio* (carp fishes), and *Clarias fuscus* (edible catfish) (Mullen and Durden, 2002). *Oreochromis niloticus* (previously named *Tilapia nilotica*) is an example of larvivorous fishes that is not only used for mosquito control, but is also being raised for eating in Egypt, Sudan and Kenyan highlands. Whereas, there was a major adverse effect on biological control that occurred after introducing other tilapia species (*Tilapia cyprinids*) into the water system (Howard et al., 2007). Other research has shown that black molly was proven successful as mosquito control agent for all species, since almost all its stages has shown substantial efficacy against mosquito larvae (Sumithra et al., 2014). Out of the different species of black molly, *Trichogaster trichopterus* was the only one where both male and female were able to feed on mosquito larvae. On contrary, the males of *Poecilia reticulate* were much more effective against larvae, unlike the females of that species (Cavalcanti et al., 2007). Other species were verified as effective agents for mosquito control in southern Iran, such as *Aphanius* sp., *Aphanius dispar* and *Gambusia holbrooki* (Shahi et al., 2015). Some larvivorous fishes (e.g, *Fundulus* sp., and *Aphanius dispar*) are inhabitants of saltwater, hence, they can be used for mosquito biocontrol in marine water ecosystems.

Despite all the positives of using fish as biocontrol agent, one downside is that they are not suitable for mosquito control in small water bodies or pools that may frequently dry out. Even though, there are some fish species such as *Cynolebias* and *Nothobranchius*, known as annual fish, since their eggs can resist drought. Such aspect makes them more suitable for the previously mentioned water containers (Mullen and Durden, 2002). It is obvious that there is a number of advantages for mosquito fish biocontrol over the traditional chemical mosquito control. However, still there is a problem with using exotic mosquito fish as they can harm the native fishes and local habitats, therefore, a great deal of precaution should be taken in this regard (Mullen and Durden, 2002). In addition, such exotic fishes may also harm and diminish some important aquatic invertebrates (e.g., other zooplanktons and predators) (Bence, 1988). Consequently, only environmentally friendly mosquito fishes that do not adversely affect the local habitat and fish fauna can be used for mosquito larvae biocontrol.

2.5. Toxorhynchites mosquito

Toxorhynchites is a mosquito genus known for the capability of its larvae to feed on species of mosquito and other aquatic organisms living in either natural or artificial habitats. Since mosquito species living in such habitats comprise most of the medically significant mosquitoes, *Toxorhynchites* mosquito has been considered as promising biological control agent for mosquitoes living in variable environments (Collins and Blackwell, 2000; Focks, 2007).

First trials to use *Toxorhynchites* were as early as the beginning of the 19th century, with no much success at the time (Collins and Blackwell, 2000). *Toxorhynchites* mosquitoes feed on other mosquito larvae with a number reaching up to 400 larvae throughout their larval stage, with higher numbers and efficiency in containers of a small size, and also feeding usually occurs as turn cannibalistic type (Goettle and Adler, 2005). When using carnivorous larvae in combination with harmless adults, more biocontrol efficiency is achieved, however, there is no enough research showing sustainable larval control using *Toxorhynchites*. Despite of that, *Toxorhynchites* was used as biological control agent successfully in countries like: United States, Japan, the Caribbean and Southeast Asia (Goettle and Adler, 2005). It was reported that *T. splendens* is one of the most successful species of *Toxorhynchites* biocontrol agents and was used as part of an integrated mosquito biocontrol system.

T. splendens can eat 20–25 larvae of *A. aegypti* daily. In order to test this species against *Culex quinquefasciatus*, *A. albopictus* and *A. aegypti* it was used in their water environment, where mosquitoes were almost totally destroyed in less than four days (Pantuwatana et al., 1979).

2.6. Nematodes

Nematodes are classified as parasitic insects that are either obligate or facultative in their feeding style. There are five orders under the Phylum nematode, and 14 obligative parasitic families under those orders. Out of those, the only family found in the mosquito natural habitat is Mermithidae (Platzer, 1981; Pirali-Kheirabadi, 2012). Several nematodes have been reported to be suitable candidates for biocontrol of insects. Beside Mermithidae, there are eight other families of nematode having species that can parasite, kill, or affect host growth. The infection style of nematodes is either by entering during insect feeding, penetrating throughout the cuticle, or entering via anus or spiracles (Pirali-Kheirabadi, 2012). Those families are: Allantonematidae, Diplogasteridae, Rhabditidae, Sphaerulariidae, Heterorhabditidae, Neotylenchidae, Steinernematidae and Tetradonematidae (Petersen, 1985). Mermithids has the largest nematode species used for controlling mosquito larvae. The main host of Mermithids is insects (feeding as obligate parasites), but still, they also attack spiders, crustaceans, mollusks, leeches, and earthworms. As they cause the infection, they usually kill the host, and they can be specific for single species or a hole family of insects. This family has gained much interest since they cause so little, if any ecological hazard, they are not threatened by other competitive beneficial organisms, and a long-term existence for its individuals in the target control site can be achieved based on the conditions of inoculation (Petersen, 1985). Even though Mermithids have been reported to infect more than 60 mosquito species, they still did not attract as much attention. These nematodes can be highly promising candidates as biocontrol agent since they are specific to their host, can kill the host at certain growth stage, can be handled easily, highly effective parasites, reproduce in a high rate, and are also swimming efficiently (Petersen, 1973).

3. Advantages of biocontrol

Biocontrol of pest insects has several advantages over the traditional chemical pest control methods. First and most important is the safety for the environment, since chemical pesticides are major pollutants (Kok and Kok, 1999). Second advantage is the selectivity of the pests, as the non-target insects will not be deteriorated, as is the case with the traditional chemical pesticides (Tebit, 2017). In fact, there was almost no side effects for using biological control throughout the past years (Emden 2004). The principal advantage is selectivity, as it sustains the balance between different living organisms in the agricultural environment, since any harm to the non-target organism can minimize the numbers of natural pest enemies (Kok and Kok, 1999). Another benefit for the biocontrol approach is the ability for self-propagation and spreading through the environment which is very important from the economic point of view (Reichelderfer, 1981). Additional benefit for the biocontrol approach is the difficulty, and probably incapability of the pest to develop biocontrol resistance (Tebit, 2017). On the other hand, the target insect might be able to develop a defense mechanism to escape the attack by the biocontrol agent. It might be possible that an effective biocontrol, may push the pest for a strong selection to develop a scape or tolerance mechanism against the attack of the biocontrol agent (Holt and Hochberg, 1997). Luckily, there is no evidence to support this scenario as of yet, especially for the large biocontrol agents such as mosquito fish. The advantage of the cost effectiveness is mainly due to its sustained propagation. Thus, if a biocontrol agent is established in a certain area, the population of the target pest would be minimized to a threshold of acceptable level for a long period of time (Kok and Kok, 1999). In addition to the previous advantages, a biocontrol agent can start with a small number and then grow to a fairly high population to develop an extended pest control over a wide distance. Taking into account the cost of preparing the biocontrol agent, it is gener-

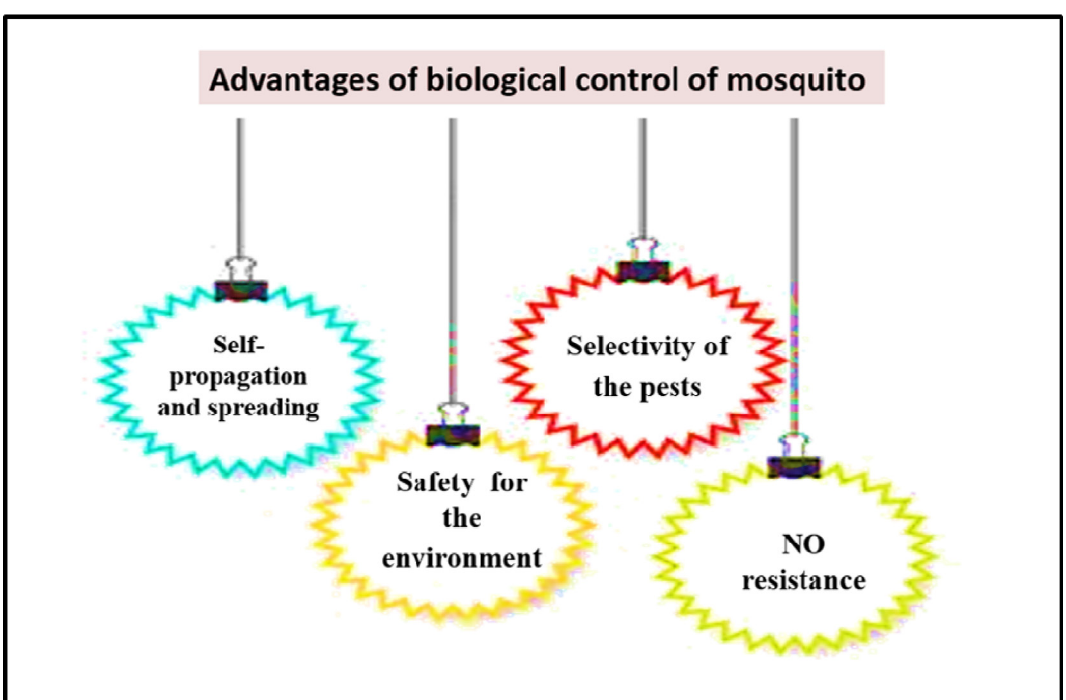

Fig. 2. Advantages of biological control pf mosquito.

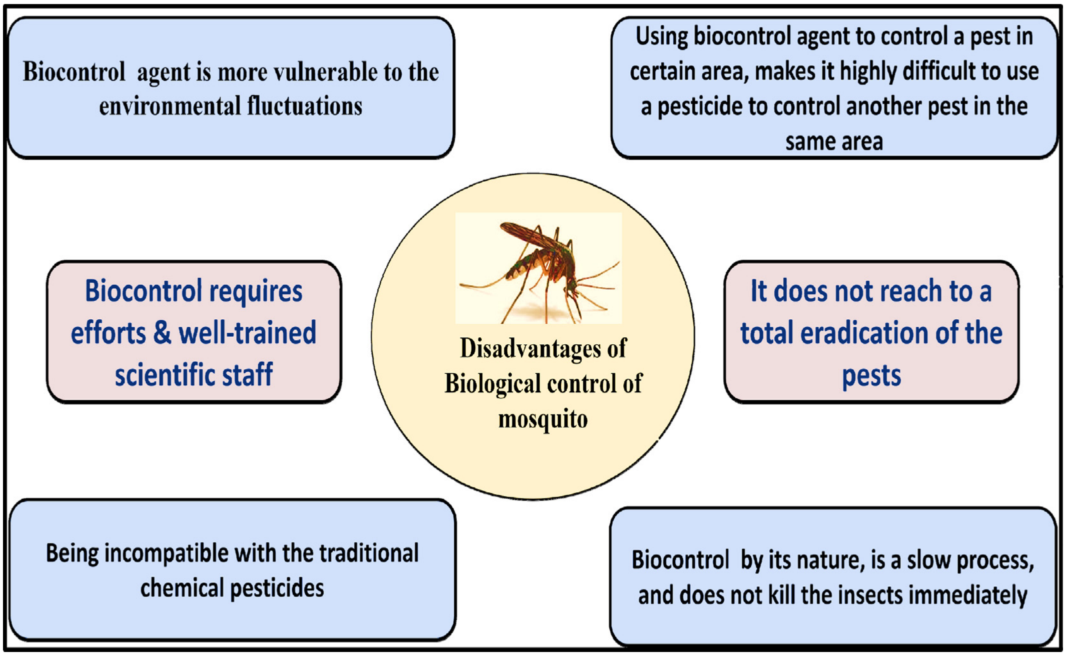

Fig. 3. Disadvantages of biological control of mosquito.

ally more economic than the cost of chemical pesticide production (Reichelderfer, 1981). Advantages of the usage of biological control of mosquito are summarized in Fig. 2.

4. Disadvantages of biocontrol

Disadvantages of the usage of biological control of mosquito are summarized in Fig. 3.

The major disadvantage of biological control approach is the possibility of income instability for the agriculture producer. Besides, the biocontrol agent is more vulnerable to the environmental fluctuations compared with the insecticidal methods, which in turn affects the efficacy of the method. Also, using chemical pesticides is obviously easier than the biocontrol approach. Being incompatible with the traditional chemical pesticides, is another main problem. Emden, (2004) stated that using biocontrol agent to control a pest in certain area, makes it highly difficult to use a pesticide to control another pest in the same area. In addition, biocontrol by its nature, is a slow process, and does not kill the insects immediately. It might take days, or more likely few weeks, in order to diminish mosquito populations to an acceptable level (Mullen and Durden, 2002). Moreover, natural competition is highly dependent on the environment, which makes it sometimes unpredictable (Emden, 2004). To get desirable results from using biocontrol approach in a new environment, one should apply much research because of the limitations in such environment. It is also known that it does not reach to a total eradication of the pests and is acceptable to minimize their numbers to the Economic Injury Level (EIL) (Tebit, 2017). Thus, biocontrol systems can be used with the vegetables and fruits, even though the incomplete pest extermination is highly undesirable, since the lowest level of damage of the product shape is undesirable by the agricultural producer (Reichelderfer, 1981). Also, one of the advantages that was stated before, which is selectivity, could probably be a disadvantage, as the biocontrol agent specificity for a single pest, could allow another pest to flourish and cause harm (Reichelderfer, 1981). In addition, biocontrol requires well-trained scientific staff, which would raise the cost of its production (Tebit, 2017). Another drawback is the inconsistency of production batches. This would be because applying high-quality measures for rearing biocontrol agent, raises the production cost. Such higher cost pushes some mass-production companies to ease on some production measures and sacrifice some level of quality (Lenteren, 2003). Biocontrol is most appropriate for using with exotic pest that is closely related to the native beneficial species (Kok and Kok, 1999).

5. Conclusions and future directions

The different approaches used for malaria control are either targeting the plasmodium development inside the mosquito or repress the mosquito as the vector for the plasmodium. Nonetheless, some factors related to the strategies of chemical control of vector, such as the incomplete infrastructure, lack of resources, and imperfect management plans, reduce the efficiency of malaria control. Besides, chemical control of mosquito fails because of the environmental variations, and the different behaviors of some mosquito species, for example, the building up of insecticidal resistance in some mosquito strains, and the subsequent pest re-emergence. The previously mentioned reasons made it necessary to develop different strategy for vector control. Biological control was a promising alternative, which has a minimal harmful side effect. Despite being relatively difficult to apply and maintain as a strategy for pest management, biocontrol approach has several advantages over the traditional chemical pesticide approach. The most outstanding advantage is the environmental safety, as it does not release any pollutants to the environment. Consequently, further research is required to discover more potential candidates as biocontrol agents to minimize the drawbacks of biocontrol and to further extend its advantages. The following targets can be suggested to minimize the disadvantages of biocontrol and to maximize the advantages. First, is to use indigenous biocontrol agents in order to avoid harming the native beneficial organisms. Second, is to use biocontrol agents that have efficient reproduction rate and high adaptation level to the surrounding environment. Third, is to rear and release large quantities of highly efficient biocontrol agents to achieve a high killing rate and mosquito reduction. Fourth, is to search for biocontrol agents that has tolerance to the traditional pesticides to allow for using both at the same time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors extend their appreciation to the deanship of Scientific Research at King Khalid University, Abha KSA for supporting this work under grant number (R.G.P.2/61/42).

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