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Review

Biological control agent for mosquito larvae: Review on the killifish, *Aphanius dispar dispar* (Rüppel, 1829)

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This review attempts to give an account on the recent advances on the killifish *Aphanius dispar dispar* as a biological control agent for mosquito larvae. Thirty six (36) articles of literature (scientific papers, technical and workshop reports) on this subject covering the period between 1980 and 2009 were reviewed. The larviciding process by using chemicals to control mosquitoes in the past resulted in a very harmful effects on the environment (bioaccumulation of DDT), resistance of mosquito vectors, destruction of non-target organisms and human health hazards. Biological control of mosquito larvae by using fish has shown many advantages over chemicals, but exogenous fishes such as *Gambusia affinis* may have negative effects on fishes and destroy the local habitat. Eco-friendly indigenous larvivorous fish with less harm to the environment and the local fish fauna is suitable for biological control of mosquito larvae. Furthermore, *A. dispar* is capable of natural and artificial reproduction to maintain a fish stock in order to eliminate mosquito larvae and protect people from many mosquito borne diseases such as malaria, dengue, rift valley fever (RVF), encephalitis and many others (Suliman, 2010). Hence, the indigenous killifish, *A. dispar* is found to play this role effectively and efficiently. Problems associated with its artificial breeding and fungal infection of its eggs can be further investigated. In addition to this, integrated methods of biological control should be carried out in order to reach the best targets of mosquito control.

Key words: Biological control, mosquito larvae, indigenous fish, *Aphanius dispar.*

INTRODUCTION

Different methods of mosquito larvae control are used throughout history, and they include both chemical and biological methods. Chemicals can affect non target populations and mosquito develop resistance against them (Kumar and Hwang, 2006; Thavaselvam, et al. 1993). The biological methods involve the uses of natural enemies and biotoxins to era-dicate mosquito vectors. Biological control includes larvivorous fish, invertebrate predators, nematodes, bacteria, fungal pathogens and plant (Azolla). The most successful method for mosquito control include the fish, bacterial pathogens, *Bacillus thuringiensis ispacelensis* (Bti) and *Bacillus sphaericus* (Bs) that attack the larval stages of the mosquito (Walker, 2002; Lacey and Lacey, 1990; Das and Amalraj, 1997). Chemicals (insecticides) were intensively used in the decade following world war II and generated a wide spread of enthusiasm against major scourges of man-kind, such as yellow fever and malaria. The World Health Organization (WHO) initiated global malaria eradication programs and succeeded in eliminating malaria from 36 countries, primarily by spraying inside human habitat with relatively inexpensive residual insecticide, DDT. Novak and Lampman (2001) gave a summary on the annual number of malaria cases in India that has been reduced from 75 million to 150,000 and deaths from 750,000 to 1500 during the period, 1952 to 1966, largely due to the use of organochloride insec-ticide. Governments around the world were forced to revaluate vector and pest control techniques. In addition to insecticide, there was great decline in beneficial insect species, outbreaks of secondary pets, contamination of the environment and food stuffs, and bioaccumulation of pesticide residues in non-target organisms, including human. Furthermore, in some countries, pathogens started to develop resistance to antimalarias (Novok and lampman, 2001).

Control of mosquito larvae started long time ago; so

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mosquito larvae habitat should be changed in order to reduce or eliminate malaria transmission which is known as the environmental management (Walker, 2002; WHO, 1980, 1982). Environmental management involves an attempt to extend and intensify natural factors which limit vector breeding, survival and contact with man, and it has been categorized into three categories:

Environmental modification

Environmental modification includes the physical modification of mosquito breeding areas in order to change vector habitat. Habitat modification may be done through drainage, leveling and filling (WHO, 1982). Draining may be accomplished by creation of ditches or drains to allow for water movement and to carry used water away from mosquito breeding sites. Standing water can also be moved by making channels. Mosquito breeding sites are reduced by filling small ponds or depresssions of water collection, changing banks of water impoundments, and straitening stream and river banks to reduce vegetations and vector populations (Thavasagayam, 1985). The careful design and regular maintenance are important components of environmental modification (Walker, 2002).

Environmental manipulation

Environmental manipulation can be achieved through temporary changes of the aquatic environment to reduce mosquito breeding and vector population. Activities of the environmental manipulation include changing reservoir water levels, flushing streams or canals, providing intermittent irrigation to agricultural fields such as rice fields, flooding or temporary dewatering of natural wetlands. Planting some water intensive tree species such as *Eucalyptus robusta* may help in reduction of standing water level in marshy lands (WHO, 1982). Other methods of environmental manipulation may be carried through planting of shade trees near potential mosquito larval habits to reduce vector abundance (Walker, 2002).

Modification of human habitats or behavior

Mosquito's population can be reduced through changes of placement and structure of human habitats and behavior (Rozendaal, 1997; Ault, 1994; WHO, 1982). Locating houses away from mosquito breeding sites (1.5 to 2 km) may help in the reduction of malaria incidences (WHO, 1982). The best housing sites are those located on well drained, high grounds and upwind of potential mosquito breeding sites (Rozendaal, 1997; Walker, 2002).

Vector management and reduction of malaria may be achieved through larvicides. Larviciding by treating breeding sites can be achieved either by using fishes to feed on mosquito larvae (biological control) or using chemicals to kill mosquito larvae. However, this lies within the second category "the environmental manipulation" (Walker, 2002).

Mosquito fish, *Gambusia affinis*, a larvivorous fish, is being introduced for biological control of mosquito larvae all over the world. Many other fishes are also used, but unfortunately, exotic fishes are considered to be the main threat to biodiversity reduction after habitat degradation. *G. affinis* is resistant to harsh environment with high fecundity, and life bearer that may give the fish an advantage over the local egg laying fishes (Cazorla, 2006). According to Oomen et al. (1990), *G. affinis* cannot penetrate dense masses of aquatic vegetations unless the vegetation density is reduced. El Safi (1985a, b) recommended the use of both *G affinis* and *Uveochromis niloticus* for the control of mosquito larvae in Gezeir irrigation canals effectively in that *O. niloticus* fed on aquatic weeds and *G. affinis* on the mosquito larvae. In addition to that *G. affinis* has some adverse effects on natural predators of mosquitoes and feed on fish broods and compete with the local fish in food sources.

The importance of this review is the risk that is encountered when chemicals and exogenous fishes are used in biological control of mosquito larvae after the discovery of the several disadvantages of using chemicals which cause a drastic effect on the environment and mosquito vectors. Various studies show that exotic fish species have some adverse effects on biodiversity reduction in freshwater ecosystems, economic loss, extinction of native species, as well as the adverse effects on biotic homogenization by several mechanisms (Cazorla, 2006). So, the application of a native larvivorous fish might be a good solution for these problems, together with an integrated program to eliminate mosqueto larvae.

This review present analyzing literature on *A. dispar* as a biological control agent in different parts of the world with special reference to Saudi Arabia and Gulf region.

The reviewed literature has been gathered for the period of 1980 to 2009. Thirty six (36) articles of the reviewed literature including published papers, workshops and technical reports were examined to address the subject under investigation.

The objectives of this review were to evaluate the indigenous killifish, *A. dispar* as an eco-friendly biological control agent for mosquito larvae and to examine its potentiality to be used for the biological control of mosquito larvae in the Kingdom of Saudi Arabia and the region as a whole.

MOSQUITO BORNE DISEASES

Mosquitoes transmit a large number of diseases such as

Malaria, yellow fever, dengue, filariasis and many encephalitis (WHO, 1989; Medical Entomology, 2009). Mosquito transmitted diseases are very important for human health as many people fall vulnerable to mosquito transmitted diseases every year and a large number of them dies annually. According to Wikipedia (2009), malaria (vector mosquito) infects 500 million people (severely ill) every year and more than one million die. Dengue fever (main vector *Aedes Aegypti*) infects 50 million people annually with 25,000 death cases. Yellow fever (principal vector, *Aedes simsoni*) infects 200,000 every year and 30.000 die. Some minor mosquito transmitted diseases are: Japanese encephalitis virus (main vector; *Culex tritaeiorhynchus*), West Nile virus (vector; *Culex* sp) and Rift valley fever (RVF) (*Aedes* and *Culex*). Table 1 shows some of the important mosquito transmitted diseases, their pathogens, vectors and disease reservoir

THE CHARACTERISTICS OF *A. DISPAR*

Eco-biological characteristics

A. dispar is a euryhaline larvivorous fish found in a wide range of salinities, "from fresh water to 500% (175 ppt) in the dead sea springs" (Plaut, 2000). *A. dispar* is found in oasis pools with hypersaline to freshwater. It can breed throughout the year with a peak in the period between April and June and well adapted to environmental conditions (Haas, 1982; Alkahem and Behnke, 1983; Krupp, 1983, 1988). Al-Akel et al. (1987) reported that the indigenous *A. dispar* has a high selective feeding capability for food in fresh water environment of Saudi Arabia, but they did not report any mosquito larvae in their guts which indicated the high efficiency of the fish in eliminating mosquito larvae from the studied area. Attaur-Rahim (1981) reported a larvivorous fish "*A. dispar* (Rüppel)" as indigenous and common omnivorous fish in Riyadh area that eats mosquito larvae in an unusual water storage tank. According to EPAA (2003), *A. dispar* is widely distributed along the east cost of the Mediterranean, coastal region of Bahrain, Djibouti, Egypt, Islamic republic of Iran, Iraq, Northern Somalia, Sudan and the Arabian Peninsula (Oman, Saudi Arabia, Yemen), up to Pakistan and the west coast of India where, historically, it is well known for its larvivorous efficiency

Biological control

Treatment of adult female mosquitoes with insecticides was considered to be the best way to control the vector of malaria, but this method ignores a fundamental biological difference between mosquito adults and larval stages that precede them, while adults are highly mobile, the larvae are confined within relatively small aquatic habitat and their biological control with the larvivorous fishes can be more easier (Killeen et al., 2002). The use of fish in mosquito control has been well known for more than 100 years and there are numerous reports of the effectiveness of various species, and some reports in their limitations. WHO (1981) lists 34 species, of these, 8 are used directly and 25 are promising in mosquito control. Those used directly are: *Tilapia zilli*, *Oreochromis mossambicus*, *Carassius auratus*, *A. dispar*, *Gambusia affinis* and *Poeclia reticulate*. Petr (2000) reported that the use of larvivorous fish for vector control is simple, inexpensive and should be considered as a component of integrated strategies. The use of native fish should be given preference to avoid possible undesired implications of introduction of new fish species. It is recommended to use more than one biological control agent, for example, a larvivorous fish together with a phytophagous fish, or two species of larvivorous fish (surface and middle-level feeders) (WHO, 1982). The use of biological control allows substantial cuts in chemical treatments, inspection efforts and control cost required to meet mosquito control target. Management in this case is clearly superior economically as well as preferable from an ecological perspective (Lichtenberg, 1987).

A. dispar is an indigenous dwarf and efficient larvivorous fish in Saudi Arabia and the Gulf region. It possesses all the required characteristics of a good agent for mosquito larval control and has been recommended for that purpose by many authors.

SUCCESSFUL TRIALS OF *A. DISPAR*

According to Ghosh and Dash (2007), the use of a larvivorous fish in malaria control is not new and has been shown to be effective and suitable in many circumstances. Walker (2002) summarized factors affecting the efficiency and success of a larvivorous fish to eliminate mosquito larvae using the following points: The suitability of the fish to the water bodies where the fish can breed successfully especially the native fishes do not require any modification of breeding sites, and the ability of the fish to consume enough mosquito larvae where there is no aquatic vegetations to interfere with the fish feeding and to give a refuge for mosquito larvae. Restocking of fish is important, because some loss may happen in normal breeding sites in urban areas there is need to restock the fish periodically to maintain sufficient population, to eliminate mosquito larvae (Fletcher, 1992; Walker, 2002; Suliman, 2010).

A. dispar was used for controlling mosquito larvae in different parts of the world. Fletcher et al. (1992) reported that *A. dispar* was found to reduce mosquito larval population to 1.6% as compared to the control which was 34%. Feachem (2009) reported that UAE succeeded in malaria elimination in their program in the period between

Table 1. Mosquito borne diseases.

Disease	Pathogen	Mosquito vector	Reservoir
Malaria	*Plasmodium falcipanum* *Plasmodium malariae* *Plasmodium ovale* *Plasmodium vivax*	*Anopheles* mosquito	Simians (monkeys and ape)
Lymphatic flariasis	*Brugia malayi* *Brugia timori* *Wuchereria bancrofti*	*Anopheles*, *Aedes*, *Culex* and *Monsonia*	Domestic cats, wild carnivores such as macaca and presbytis
Yellow fever virus	Yellow fever virus (*Flviviridae*)	Genus *Aedes*	Monkeys and the basic reservoir is the mosquito vector
Dengue and dengue hemorrhagic fever	Arvovirus (*Flavoviridae*)	Genus *Aedes*	Not identified, but probably man and monkeys
Japanese encephelitis	Japanese encephelitis virus	Genus *Culex*	Probably birds, night heron, egrets and domestic pigs

Adapted from Wikipedia (2009).

1960s and 1997. Two local larvivorous fishes were used in this program in mosquito breeding sites and these were *Tilapia and A. dispar*. Now, the few malaria cases were only imported from other countries such as Bangladesh, India, Pakistan and Sudan, but without transmission of malaria infections. The WHO (2003) reported that *A. dispar* has sharply reduced the anopheles larval density in Afghanistan, and successfully controlled mosquito larvae around the city capital of Djibouti. According to them, Oman also has a vast experience in the use of the indigenous larvivorous, *A. dispar* for malaria control. *A. dispar* were found to sufficiently control *Anopheles culicifacies adanensis* breeding in well and containers in an urban area of Ethiopia (Walker, 2002).

As a result of the several problems associated with the introduction of *G . affinis* to other areas, Oomen (1990) suggested the use of a native *A. dispar* to control mosquito larvae in India, but he also mentioned the problem of restocking because of seasonal drying dishes. *A. dispar* is a well known larvivorous fish. It can be used for biological control of mosquito larvae in areas to which it has well adapted (Haas, 1982; Al-kahem and Behnke, 1983; Krupp, 1983, 1988). *A. dispar* as indigenous larvivorous fish has been recommended for use in biological control of *Anopheles stephensis* in Bahran (Tariq et al., 2009).

Concern about exotic fish species

Introduction of the exotic, voracious and aggressive *G. affinis* has actually led to the elimination of native fishes very significantly (Walker, 2002). Das and Amalraj (1997) have some reservations about biological control against malaria and they considered it to be more difficult to use than chemicals. They drew attention that sometimes agents can be effective in controlling vectors at laboratory conditions, but they may fail in the field. In addition to that they may also be specific in terms of type of mosquito to be controlled and the type of habitat for their performance. CAMP (2002) reported that *A. dispar*, the indigenous fish to the region, was a potential threat to other native species when introduced as a mosquito control agent outside its natural habitat. It is generally a surface feeder, but can be mid-water or bottom feeder and outcomepetes native species when there are no surface mosquito larvae available.

The intensive use of chemicals in the control of mosquito larvae in the past has resulted to insecticide resistance decline in beneficial insect species, outbreak of secondary pests, contamination of the environment and food stuffs, and bioaccumulation of pesticide residues in non-target organisms, including human (Novak and lampman, 2001).

CONCLUSION AND RECOMMENDATIONS

The use of chemicals in mosquito control appears to have many disadvantages. It is harmful to non target populations as well as the environment and it also causes resistant to mosquitoes which make their control to be more difficult in the future. There are a number of mosquito-borne diseases. The mosquito control process requires alternative simple and sustainable methods of control. Biological control has many advantages as compared to chemicals because it can be effective and safe to human and non-target populations, it has low cost of production and lower risk of resistance development (Yap, 1985). *G. affinis* seems to have some negative effects on local fish fauna and the environment, but *A. dispar* is considered by Frankel and Goren (1999) as an excellent agent for use as biological control of mosquito larvae, although, mass production of the fish for restocking has some obstacles such as cannibalistic behavior of adult fish on the eggs and small fish fry as well as fungal infection on the eggs during incubation, but such problems can be resolved through modifications of the breeding system design (Frenkel and Goren, 1999). Indigenous *A. dispar* can be very effective and efficient in mosquito control. Unlike exotic species, it will not cause any harm to other native fish, they breed naturally without need to modify the environment and maintain a fish stock capable of eliminating mosquito larvae. The biological control of mosquito larvae with indigenous larvivorous, *A. dispar* should be applied in integrated pest management program in order to make it feasibly economical and ecological.

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