

REVIEW

Mosquito control: A review on the past, present and future strategies

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Summary: Nearly half a million deaths occur worldwide annually due to mosquito-borne diseases. Mosquito control has become the major strategy in controlling these diseases, especially in the absence of effective vaccines for disease prevention. At the beginning of the last century, mosquito control was mainly done by personal protection methods and larval control by application of petroleum oil and Paris green powder to water bodies. A breakthrough in mosquito control came in the 1940s with the introduction of synthetic neurotoxic insecticides which could suppress mosquito populations rapidly throughout the globe. However, a resurgence of populations with resistance to these insecticides was witnessed within a decade after their introduction. Environmental pollution caused by synthetic insecticides also became a major concern. Novel personal protection methods, community-level operations on source reduction, insect growth regulators and polystyrene beads for larval control, and biological control were introduced as alternatives. Biological control was mainly by larval predators such as fish, dragonfly nymphs, microcrustaceans and *Toxorhynchites* larvae; bacterial larvicides such as *Bti*; plant-based mosquitocides; and green-fabricated nanoparticles. However, even today, mosquito control programmes heavily depend on synthetic neurotoxic insecticides applied through insecticide residual spraying (IRS), fogging, larviciding and impregnated bed nets. Increased detoxification and target site insensitivity, developed as major insecticide resistance mechanisms, have been extensively studied in mosquitoes assisting proper management of available insecticides for which not many alternatives are available.

Despite all our efforts, an unprecedented global emergence of mosquito-borne diseases is evident demanding novel strategies for mosquito control. The introduction of transgenic strains of mosquitoes to suppress or replace mosquito

populations reducing disease transmission has become the latest effort. Population reduction has been achieved *via* releasing mosquitoes with a dominant lethal gene (RIDL) and by combining the conventional sterile insect technique (SIT) with *Wolbachia* mediated incompatible insect technique (IIT). Population replacement has been successful *via* releasing *Wolbachia* infected mosquitoes that are refractory to pathogen development and transmission. Advancement of gene- and allelic- drive systems will soon allow us to effectively spread refractory genes and insecticide susceptible alleles into mosquito populations overriding normal inheritance.

Keywords: Biological control, insect growth regulators, insecticides, mosquito control, mosquito-borne diseases, transgenic mosquitoes.

INTRODUCTION

Until the late 1880s, malaria was thought to be a disease caused by bad air coming from swamps and rivers (the term malaria derived from the Italian ‘mal-aria’ or ‘bad air’). Malaria parasite was identified by Sir Alphonse Laveran in 1880, and in 1897, Sir Ronald Ross found the malaria parasite in the gut of an anopheline mosquito revealing that it is a mosquito-borne disease. Towards the end of the nineteenth century, it was established that certain species of insects, other arthropods and freshwater snails act as vectors of some important diseases (Rozendaal, 1997). Since then, the control of vectors became the main strategy in the control of transmission of vector-borne diseases, especially in the absence of effective vaccines for the prevention of these diseases.

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Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually and a majority of these deaths occur due to mosquito-borne diseases such as malaria (causes more than 400,000 deaths every year) and dengue (more than 40,000 deaths annually) (WHO, 2020).

Mosquitoes belong to the Family Culicidae of the Order Diptera. The family consists of more than 3500 extant mosquito species which are distributed throughout tropical and temperate regions and beyond the arctic circle. Apart from being a biting nuisance, some mosquito species transmit deadly pathogens. Species that act as vectors of diseases are mainly included in the Genera *Anopheles*, *Culex*, *Aedes* and *Mansonia* (Harbach, 2013). Pathogens transmitted by mosquitoes include protozoans (e.g., malaria parasites), nematodes (e.g., filarial worms) and viruses (e.g., dengue, chikungunya, yellow fever, zika, Japanese encephalitis, West Nile fever, Rift Valley fever). Effective mosquito control interventions are vital for the suppression of mosquito-borne diseases. Given the diversity of mosquito species, their biology and the diseases they transmit, different intervention strategies have been implemented across many regions globally and the selection of appropriate interventions for a particular vector to be controlled is of paramount importance. These interventions can be operated at personal level, community level or at government level.

PERSONAL PROTECTION TO AVOID CONTACT WITH MOSQUITOES

Avoiding or not visiting risky places during peak mosquito biting hours is recommended as a strategy to control mosquito bites. Hour before sunrise and hour after sunset are the peak biting periods for most mosquito species. Although there are diurnal biting mosquito species like *Aedes* spp., many prefer nocturnal biting between sunset and sunrise. Steps should be taken to close the windows and doors which are open to outside, in the early evenings. Host-seeking is odour-driven, and finding a host depends on the quality of the odour plume and the obstacle free route towards the host. Therefore, proper house design is effective in preventing mosquito house entry. At the end of the nineteenth century, the Italian malariologist Angelo Celli recognized malaria as a disease of rural poverty and demonstrated the effectiveness of house modifications in reducing mosquito entry (Celli, 1900). Most malaria infections in sub-Saharan Africa are acquired indoors through the mosquitoes predominantly entering houses *via* open eaves. Closing the eaves has effectively reduced vector entry in thatched houses (Jatta *et al.*, 2018). Having a

ceiling with an appropriate material can also close the mosquito routes *via* eaves. The gaps in walls associated with mud and grass construction, instead of bricks, are a greater determinant of mosquito vector entry (Ippolito *et al.*, 2017). A significant protection to inhabitants can be given by making houses and shelters insect proof while allowing a good ventilation. Anti-mosquito screenings with cotton netting, metal/plastic screens or insecticide treated screens can be used for this purpose. Also, raised buildings have reduced malaria transmission in Africa. Since most malaria mosquitoes fly less than 1 m from the ground, raising buildings off the ground has prevented the entry of *Anopheles gambiae*, the principal African malaria vector. Mosquito house entry declined with increasing height, with a hut at 3 m reducing *An. gambiae* house entry by 84% when compared with huts on the ground (Carrasco-Tenezaca *et al.*, 2021).

In southwest Ethiopia, people and cattle live in proximity and the presence of calves within and close to human dwellings has acted to draw malaria mosquitoes toward the human occupants increasing the risk of malaria. Hence, deployment of cattle far from human residence has been recommended to reduce human exposure (Zeru *et al.*, 2020). Where malaria is transmitted by zoophilic vectors, two types of malaria control strategies have been proposed based on animals; using livestock to divert the vector (zooprophylaxis) or using livestock as baits to attract vectors to insecticide sources (insecticide-treated livestock). It has been recommended to use insecticide-treated livestock as an intervention in integrated control efforts for malaria and livestock diseases which are transmitted by moderately zoophilic vectors (Franco *et al.*, 2014). It is important to consider the direction of winds before constructing a house close to a large water body or a marshy land because the wind can directly bring in the adults emerging from the water body (Rozendaal, 1997). A diversion can be made by putting up an animal hut like cattle shed or a piggery in between the water body and the house to divert mosquitoes to animals. However, keeping piggeries near paddy fields in close association with human settlements worsened the transmission of Japanese encephalitis in Anuradhapura, Sri Lanka in the late 1980s, since the pig acts as the amplifying host for the virus (Peiris *et al.*, 1992).

Bed nets and indoor residual spraying (IRS) of insecticides have a high impact on reducing malaria transmission indoors. Long-lasting insecticide-treated nets (LLINs) have added advantages by having the repellent action. Because of this, mosquitoes do not enter through the holes or edges of the net and also they do not bite the net touching body parts from the outside through

the net material (Curtis, 1992; Rozendaal, 1997). In order to get protection from outdoor mosquito bites, various personal protection methods are available and these act by providing a physical barrier between the vector and host, or by repelling or killing the vector. Long-sleeved shirts and long trousers are worn to limit the area of exposed skin. Permethrin-treated clothing is protective against a wide range of mosquito species and has been successfully used by commercial company workers and militaries for decades (Banks *et al.*, 2014). Topical repellents, such as DEET (*N,N*-diethyl-*m*-toluamide), various derivatives of eucalyptus extracts [eg. parmenthane-3,8-diol (PMD)], can be used effectively in disease control. Volatile pyrethroid-based mosquito coils are commonly used to repel mosquitoes in indoors and portable mosquito coils have been highly recommended against outdoor biting mosquitoes (Tangena *et al.*, 2018). Ultrasonic sound waves in the range of 20–70 kHz which are well above the upper human audible limit produce enough stress on the mosquito nervous system to repel them. Experiments have shown that mosquitoes are repelled significantly by ultrasonic emission in the ranges of 40–55 kHz frequencies (Ikeri *et al.*, 2017).

SOURCE REDUCTION (PREVENTION OF MOSQUITO BREEDING)

The term source reduction refers to any measure that prevents the breeding of mosquitoes. Mosquito species differ in their preferences for breeding habitats. Thus, some species breed in clean water containers in and around houses, whereas others prefer polluted water in sanitation systems, man-made and natural habitats in rural areas, or even brackish water. It is important to study the exact nature of breeding habitats of a target species before implementing the intervention. Methods to control larvae include eliminating or changing the breeding places to make them unsuitable for the development of larvae, making breeding places inaccessible to adult mosquitoes, releasing fish or other predators that feed on larvae, and applying larvicides (Rozendaal, 1997).

Source reduction can be permanently achieved by altering or eliminating breeding places e.g., covering or screening water containers, draining ponds and marshes, filling in ditches and pools etc. This is known as 'Environmental Modification'. 'Environmental Manipulation' refers to semi-permanent measures e.g., cleaning up containers, clearing vegetation, flushing streams and repairing drains. In contrast to environmental modification, methods of environmental manipulation have to be repeated to remain effective e.g., water-level fluctuation, flushing (stream sluicing), changes in water

salinity, shading of stream banks (used against larvae of mosquito species such as *An. maculatus* and *An. minimus*), clearing of vegetation (against larvae that do not prefer direct sunlight e.g., *An. balabacensis*). Removal of water plants is used to control larvae and pupae of the vector of brugian filariasis *Mansonia* spp., that pierce submerged parts of water plants with their respiratory siphons to reach air spaces within the plant tissues for breathing (Chang, 2002). Also, clearing water plants can increase the exposure of mosquito larvae to larvivorous fish and, to water currents and wave movement that would flush them. Straightening and steepening of shorelines of streams prevent formation of small water pockets for breeding.

Aedes aegypti and *Ae. albopictus* prefer to lay eggs in discarded receptacles such as used tins, pots, cups, bottles, tyres and coconut husks etc. Promising results have been obtained through awareness programmes followed by garbage cleaning at community level to eradicate these breeding grounds. Badly designed drainage and sewage disposal systems, and water-holding vegetation such as ornamental plants and pineapple plantations can also provide breeding grounds, especially for *Aedes* mosquitoes. Good planning, maintenance and law enforcement can drastically reduce mosquito breeding in these places controlling the disease (Sim *et al.*, 2020). Chemical insecticides or other larvicidal agents such as oils or biological agents can also be applied to mosquito breeding sites, especially for places where habitat elimination may not be possible eg. pineapple vegetation, ornamental ponds.

Mosquitoes that breed in irrigation water can be controlled through careful water management systems. Tree species such as *Eucalyptus* that grow rapidly and have higher transpiration rates are used for drying marshy lands and other areas with high water tables to prevent mosquito breeding (Rozendaal, 1997). Expanded polystyrene beads that are non-toxic to humans and other organisms can be spread on the water as a measure to control mosquito larvae. The floating layer thus formed acts as a barrier between the water and air, and mosquito larvae die because they cannot reach the water surface to breathe. The beads do not decay and remain floating for years but are vulnerable to flushing. They have been successfully used to control mosquito breeding in abandoned gem pits in Sri Lanka and the expanded beads with a 2 mm diameter were the most effective for suffocating *Anopheles* larvae and pupae (Yapabandara & Curtis, 2002).

Towards the end of the 19th century, petroleum oil was heavily used to prevent mosquito breeding for

malaria control. Application of the oil to water bodies makes a thin oil layer over the water surface interfering with larval breathing. From 1921 to the 1940s, Paris green, a green powder of copper acetoarsenite, was also adopted for the same purpose. Powder particles float on the water surface kill the surface feeding anopheline larvae (Rozendaal, 1997). Since the introduction of organochlorines as larvicides in the 1940s, application of synthetic insecticides became a major method in mosquito larval control.

USE OF SYNTHETIC INSECTICIDES

Today, vector control programs largely depend on the use of synthetic insecticides, especially during disease outbreaks. There are several classes of vector control insecticides; organochlorines, organophosphates, carbamates, pyrethroids, bacterial larvicides, insect growth regulators and novel classes such as neonicotinoids, spinosyns and pyrroles. The first four classes listed are referred to as “conventional” insecticide classes. Most classes have broad-spectrum effects on other organisms while bacterial larvicides and insect growth regulators are more specific to targeted organisms (WHO, 2021).

Conventional insecticides

Commonly used synthetic insecticides can be divided into 4 major groups: Organochlorines, Organophosphates, Carbamates and Pyrethroids. The organochlorine DDT was first discovered in 1939 and introduced as an insecticide useful in the control of malaria, yellow fever and many other insect-borne diseases in the 1940s. Organophosphates came to known as neurotoxins in the 1930s but their synthesis in large quantities as pesticides came after World War II. The popularity of organophosphates increased due to environmental concerns and resistance development against organochlorines in the 1960s. Although the insecticidal activity of carbamates was discovered in 1931, large quantities were synthesized for vector control purposes in the late 1950s (Kuhr & Dorough, 1976). Pyrethroids are analogs of the pyrethrum extracted from *chrysanthemum* flowers that were long known to have insecticidal properties. The first-generation pyrethroids were developed in the 1960s (e.g. bioallethrin, tetramethrin, resmethrin) and the second-generation pyrethroids (e.g. permethrin, cypermethrin and deltamethrin) in the 1970s. Unlike first-generation compounds, second-generation ones are more resistant to degradation by light and air.

The target site of organophosphates and carbamates is the insect acetylcholinesterase which hydrolyses the neurotransmitter acetylcholine on the post-synaptic nerve membrane. Inhibition of acetylcholinesterase by the insecticides results in continuous stimulation of post-synaptic nerve membrane leading to the death of the insect. For pyrethroids and a group of organochlorines (DDT + its analogs) the target site is the voltage gated Na⁺ channel (VGSC) regulatory proteins of the nerve membrane. The binding of these insecticides prevents closing of VGSC hindering the active outside pumping of sodium ions. This leads to continuous firing of the nerves causing the ultimate coma and death of the insect. For the rest of the organochlorines (cyclodienes), the target site is γ -aminobutyric acid (GABA) receptors which regulate Cl⁻ conductance through the nerve membrane. Binding to GABA receptors will also cause continuous firing of the nervous system leading to the death of the insect (Karunaratne, 1998).

Pyrethroids are safer since they have low mammalian toxicity. Both organophosphates and carbamates have shorter residual life. Organochlorines have high persistence in the environment accumulating toxicity through the food chains. Although organochlorines are banned in several countries due to environmental concerns, they are still heavily in use especially in African countries. During the period 2010–2019, the annual global amount of insecticides used for disease vector control was 3314 metric tonnes (t) of organochlorines, 1625 t of organophosphates, 677 t of carbamates and 194 t of pyrethroids. These insecticides were mainly used for residual spraying, followed by space spraying, larviciding and treatment of nets (WHO, 2021).

Insect growth regulators

Insect growth regulators (IGRs) are a diverse group of chemical compounds that are highly active on pre-imaginal stages of insects and the mortality occurs many days after the treatment. This is indeed a desirable feature of a control agent because the larvae of mosquitoes are an important source of food for fish and wildlife (Mulla, 1995).

The first account on the potential use of IGRs in insect control was in 1956, when juvenile hormone (JH) was isolated from the abdominal crude extract of the moth *Hyalophora cecropia* (L.). Later, it was established that topical application of the hormone prevents insect metamorphosis (Tunaz & Uygun, 2004). According to their mode of action, IGRs can be divided into two groups; Chitin synthesis inhibitors (CSIs) and Insect

hormone analogs. Larvae treated with CSIs develop until molting but fail to ecdyse due to inhibition of the synthesis of the new cuticle. The second group includes substances that mimic the action of insect hormones. Molting hormone (ecdysone) is responsible for cellular programming and, together with juvenile hormone (JH) it initiates the molting process. When JH levels are high, the epidermis is programmed for a larval molt, otherwise, the epidermis is programmed for metamorphosis. Thus, application of JH analogs suppresses pupation (Tunaz & Uygun, 2004). During the period 2010–2019, the annual global amount (metric tonnes of the active ingredient - t) of IGR insecticides used for disease vector control were 0.1 t methoprene (JH analog), 0.6 t novaluron (CSI), 9.2 t pyriproxyfen (JH analog) and 9.9 t diflubenzeron (CSI) (WHO, 2021).

Neonicotinoids, pyrroles and spinosyns

Neonicotinoids were developed through the 1980s and 1990s and, similar to pyrethroids, have a lower mammalian toxicity. They selectively bind and interact with the insect nicotinic acetylcholine receptors (nAChRs) which are ligand-gated ion channels that mediate fast synaptic transmission in the insect nervous system. During the period 2010–2019, the annual global amount (active ingredient) of neonicotinoids used for disease vector control was 36 metric tonnes (WHO, 2021).

Spinosyns are a large family of unprecedented compounds produced in the fermentation of two species of *Saccharopolyspora*. They have a unique mode of action involving disruption of nicotinic acetylcholine receptors. When compared with many other insecticides, the spinosyns generally show greater selectivity towards target insects and lesser activity against many beneficial predators as well as mammals and other aquatic and avian animals (Kirst, 2010). Their insecticidal spectrum, unique mode of action and lower environmental effect make them useful new agents for modern vector control programs. The annual global amount (active ingredient) of spinosyns used for disease vector control during 2010–2019 was 16 metric tons (WHO, 2021).

Pyrroles (e.g., chlorfenapyr) are a broad-spectrum class of insecticides new to vector control. They are pro-insecticides that require initial activation by insect monooxygenases to produce the active compound. Unlike most of the other classes of insecticides, the site of action of pyrroles is not the insect nervous system and therefore shows no cross-resistance to mechanisms that confer resistance to standard neurotoxic insecticides.

They act at the cellular level and disrupt respiratory pathways and proton gradients through the uncoupling of oxidative phosphorylation in mitochondria (Oxborough *et al.*, 2015).

INSECTICIDE RESISTANCE IN MOSQUITOES

The development of resistance against commonly used insecticides is a major threat to mosquito control programmes in a situation where not many alternatives are available. Therefore, research on insecticide resistance, molecular mechanisms which underlie the resistance and rational resistance management are of paramount importance in controlling the development and spread of insecticide resistance in vector populations. Insects develop resistance against insecticides through two major mechanisms; Metabolic resistance (changes in insect enzyme systems for rapid detoxification of insecticides) and Target-site insensitivity (alterations of the target sites to prevent their binding to insecticides) (Figure 1). Reduced penetration (cuticular resistance), behavioural changes and increased excretion may also act as minor mechanisms (Karunaratne, 1998; Hemingway *et al.*, 2004; Karunaratne *et al.*, 2018).

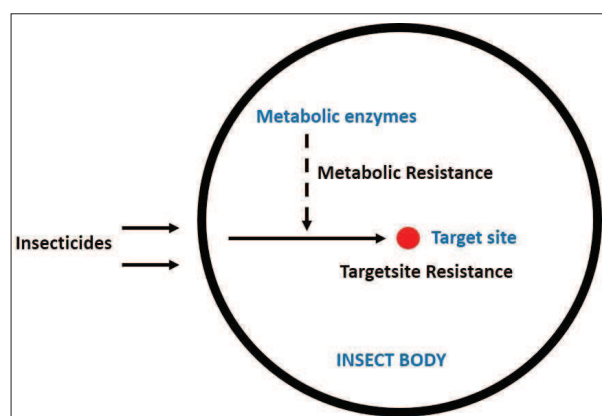


Figure 1: Mechanisms of insecticide resistance. Insecticides that enter the insect body can be detoxified by metabolic enzymes or can be prevented from binding to target sites due to structural alterations in target sites.

Three major groups of enzymes i.e., esterases (also known as carboxylesterases), glutathione S-transferases (GSTs) and monooxygenases (also known as mixed function oxidases or cytochrome P450s) are known to be involved in insecticide detoxification in the insect body. Enhancement of enzyme activity in resistant insects compared to susceptible insects can be due to

quantitative and/or qualitative changes of the enzymes in resistant insects. Increased amounts (quantitative changes) are produced in resistant insects as a result of gene amplification or increased upregulation of the gene. Increased catalytic centre activity (qualitative changes) is achieved through coding sequence mutations (Karunaratne *et al.*, 2018).

Quantitatively changed esterase-based resistance mechanism has been studied extensively at biochemical and molecular levels in *Culex quinquefasciatus* and *Cx tritaeniorhynchus* mosquitoes. Esterases act by rapid binding and slow turning over of the insecticide. They sequester rather than rapidly metabolize the insecticide. Therefore, in order to keep the system effective, large quantities are required. It was found that the esterase gene is amplified up to 80 copies in resistant mosquitoes for the increased production of esterases (Karunaratne *et al.*, 1993; 1998; Hemingway & Karunaratne, 1998). Increased esterase activity may not always be a result of increased quantity of the enzyme found in the resistant mosquito. A number of *Anopheles* species have a non-elevated esterase mechanism that confers resistance, specifically to malathion, through an increased rate of metabolism rather than through increased quantities. These esterases are called malathion carboxylesterases and have a higher catalytic center activity towards malathion than their susceptible counterparts (Karunaratne & Hemingway, 2001).

Resistance to insecticides can be provided by GSTs through several different pathways such as O-dealkylation or O-dearylation conjugation for organophosphates, dehydrochlorination and GSH conjugation for organochlorines, detoxification of lipid peroxidation products and passive sequestration for pyrethroids (Che-Mendoza *et al.*, 2009). Glutathione S-transferases are a diverse family of enzymes with more than 30 GST genes in mosquitoes. Additional diversity is contributed by alternative splicing to produce GSTs with differing substrate specificities (Ranson & Hemingway, 2005). In addition to conferring resistance *via* direct metabolism or sequestration of chemicals, GSTs also provide protection against oxidative stress-induced by insecticide exposure (Pavliidi *et al.*, 2018).

Cytochrome P450 monooxygenases are also a complex family of enzymes that bind to molecular oxygen and receive electrons from NADPH to introduce an oxygen molecule into the substrate. Despite their ability to detoxify most of the insecticides of all classes, they mediate bio-activation of organophosphates by converting the 'thionate' analog to more toxic 'oxon' analog (Karunaratne *et al.*, 2018).

Insects acquire target site insensitivity mainly through non-silent point mutations within structural genes. However, only a limited number of changes can decrease insecticide sensitivity without disrupting the normal physiological functions of the target site. Therefore, the number of possible amino acid substitutions is very limited. Hence, identical resistance-associated mutations are commonly found across highly diverged taxa (Karunaratne *et al.*, 2018). Out of the two *ace* genes which encode different acetylcholinesterase proteins in insects, several resistance-associated point mutations have been identified in *ace-1* gene in insect pests including mosquitoes (Guo *et al.*, 2017). 'knock-down resistance' or 'kdr' type gene mutations at *VGSC* gene prevent the target site binding to DDT and pyrethroids. More than thirty unique resistance associated mutations or combinations of mutations have been detected in pyrethroid and DDT resistant insect populations (Rinkevich *et al.*, 2013). 'Resistance to dieldrin' (a cyclodiene organochlorine insecticide) or 'Rdl' type alanine-to-serine (A296S) mutation is associated with GABA target site insensitivity in several species of dieldrin and fipronil in resistant mosquitoes (Yang *et al.*, 2017).

In order to identify detoxification genes associated with resistance to insecticides, microarrays containing unique oligonucleotide probes for these genes have been constructed for *Ae. aegypti* and their expression level in insecticide resistant and susceptible strains have been compared. This '*Ae. aegypti* Detox Chip' has facilitated implementation of insecticide resistance management strategies (Strode *et al.*, 2008). Increased activity of metabolic enzymes (esterases, GSTs and monooxygenases) can also be detected by conducting biochemical assays. Since acetylcholinesterase is an enzyme, its insensitivity to insecticides can also be tested biochemically by an inhibition assay (WHO, 1998). Various molecular techniques are now available to detect target site mutations involved in resistance (Karunaratne *et al.*, 2018).

BIOLOGICAL CONTROL

Because of the development of resistance and the environmental pollution caused by conventional insecticides, biological control has always been welcomed as an alternative. Scientists had paid attention to use the prey-predator relationship in the environment for effective mosquito control from the very beginning. For this purpose, natural enemies such as aquatic insects, micro-crustaceans, fish and amphibians that prey upon mosquito immature forms have been extensively tested.

Additionally, microorganisms and their derivatives including bacterial, and botanical larvicides have also been developed.

Use of natural enemies as biological control agents

Aquatic insect species of the Orders Odonata, Coleoptera, Diptera and Hemiptera have the ability to prey upon mosquito preimaginal stages. Water bugs and immature stages of odonates are considered as voracious feeders on mosquito larvae (Shalan & Canyon, 2009). In a study carried out in Sri Lanka, predatory efficacy of nymphs of five dragonfly species i.e., *Anax indicus*, *Gynacantha dravida*, *Orthetrum sabina sabina*, *Pantala flavescens* and *Tholymis tillarga* were tested against *Ae. aegypti* larvae and the highest predation rates were observed in *Anax indicus* followed by *Pantala flavescens*. The latter has been recommended as the best potential biological agent to control dengue vectors in the field considering its wider distribution and notable predation (Sammanmali *et al.*, 2018).

Mosquito larvae belong to the genus *Toxorhynchites* and, certain species of *Armigeres* and *Culex* prey upon other mosquito larvae (Surendran *et al.*, 2013; Chathuranga *et al.*, 2020; Donald *et al.*, 2020). Adult females of the genus *Toxorhynchites* are autogenous and do not require a blood meal for egg development. Although the prevalence of *Toxorhynchites* mosquitoes is mainly confined to forested areas in nature, they have been successfully reared in laboratories in large numbers and released to the environment to reduced dengue vector populations (Wijesinghe *et al.*, 2009). Laboratory experiments have shown that *Tx. splendens* has preferential predation against *Ae. aegypti* larvae compared to other mosquito vector species such as *Ae. albopictus* and *An. sinensis* (Zuharah *et al.*, 2015). However, *Toxorhynchites* as a biocontrol agent in the field has limitations due to its lower fecundity, prolonged larval development and the risk of cannibalism among own offspring (Donnald *et al.*, 2020).

Microcrustaceans such as copepods are predators of immature forms of mosquitoes. Predatory efficacy of cyclopoid copepods on larvae of vector mosquitoes has been investigated in detail (Roa *et al.*, 2002; Udayanga *et al.*, 2019). A study carried out in Sri Lanka tested the feeding efficacy of five copepods namely *Mesocyclops leuckarati*, *Mesocyclops scirassus*, *Cyclops vernalis*, *Cyclops varicans* and *Cyclops languides* on dengue vectors. The copepod *M. leuckarati* has shown the highest predatory efficacy for both *Ae. aegypti* and *Ae. albopictus* (Udayanga *et al.*, 2019). However, introduction of microcrustaceans to all possible *Aedes* breeding sites

has been a severe limitation for its application (Hales & Panhuis, 2005).

Use of larvivorous fish as a mosquito control strategy in different aquatic habitats has been documented from many countries. Even though predatory efficacy of several different fish species on mosquito larvae has been reported, species belonging to the genus *Gambusia* and *Poecilia* are widely used as biological control agents against mosquito larvae in the field. *Poecilia reticulata* (guppy) is widely distributed in tropics and has a very high reproductive rate. Because of their small size they can creep into micro-habitats created especially by the root systems of aquatic plants. Also, as surface feeders they have a high opportunity in capturing mosquito larvae that regularly visit the water surface for breathing. These are ideal control measures for small water tanks and small ornamental garden ponds where the application of chemical larvicides needs to be avoided. Alternatively, bottom feeders like *Lepidocephalichthys thermalis* (common spiny loach) have shown negligible larvivorous potential since their swimming movements disturb the settled larvae. It has also been reported that column feeders like *Oreochromis mossambicus* (mossambique tilapia) show a moderate larvivorous potential (Ekanayake *et al.*, 2007). Surendran *et al.* (2008) have also recommended *O. mossambicus* as a suitable biological control agent to control *Aedes* and *Anopheles* larvae. *Gambusia* sp. (mosquito fish) are surface feeders and are popularly used in mosquito larval control in several countries because of their greater efficacy on predation (Benelli *et al.*, 2017). However, their presence has been considered to have a negative impact on the native biodiversity of aquatic systems as they become invasive and prey upon native fish species and amphibians (Mischke *et al.*, 2016). *Poecilia reticulata* (guppies) and *Aplocheilichthys dayi* (nalahandaya) have been recommended as the best candidates for biological control of *Aedes* larvae in Sri Lanka (Ranathunge *et al.*, 2021).

Although the biological control of mosquito larvae using vertebrates has been mainly associated with fish, the potentiality of amphibians in this regard has also been documented. Predatory capacity of the tadpoles of frogs and toads on mosquito larvae has been studied under different conditions (Bowatte *et al.*, 2013). Direct observations have substantiated that five tadpole species *Polypedates*, *Bufo*, *Ramanella*, *Euphlyctis* and *Hoplobatrachus* are *Aedes* egg predators under laboratory conditions. With about seven thousand frog species worldwide, living in a diversity of aquatic habitats where fish cannot reach, the role of tadpoles in the biological control of mosquitoes can be more

significant than is currently understood (Bowatte *et al.*, 2013). However, it has been suggested that tadpoles are generally herbivores and their predatory behaviour is merely due to competition since both mosquito larvae and tadpoles are detritus feeders (Weterings, 2015).

Use of microorganisms and their derivatives as biological control agents

Naturally occurring pathogenic microorganisms such as fungi, protozoans, viruses and bacteria can also be considered as potential agents for mosquito biological control. Toxins derived from some of these microbial agents are popularly used as alternatives for synthetic larvicides. *Bacillus thuringiensis var. israelensis* (*Bti*) and *B. sphaericus* (*Bs*), gram-positive spore-forming bacteria species, are used as bacterial larvicides in many countries against *Anopheles* (Derua *et al.*, 2019), *Aedes* (Boyce *et al.*, 2013) and *Culex* (Dylo *et al.*, 2014) mosquitoes. Although *Bs* has shown a better larval control in polluted water, their success in controlling *Aedes* larvae has been very poor (Rozendaal, 1997). The *Bti* products are available in the market as wettable powder and granules, consisted of dead bacteria, living spores and toxic crystals. Quick deposition of the material to the bottom has been identified as a drawback and slow-releasing briquettes and dunks, which float on the water, have been introduced to overcome this. Resistance development against bacterial larvicides caused by long-term application represents a serious threat to their success (Wirth, 2010).

Infective spores produced by entomopathogenic fungi such as *Lagenidium*, *Coelomomyces* and *Culicinomyces* can penetrate the mosquito cuticle and release toxins that kill the mosquito (Scholte *et al.*, 2004). It has been shown that the rate of resistance development against fungal toxins is much slower than that against synthetic insecticides (Knols *et al.*, 2010). It is anticipated that modern genetic engineering tools will assist in improving the fungal efficacy to control mosquito-borne disease vectors (Scholte *et al.*, 2004).

Plant-based mosquitocides and green-fabricated nanoparticles

Another approach for mosquito control is employing bioactive attributes of plant-derived products (PDPs) which are of low mammalian toxicity and short environmental persistence. Azadirachtin (extracted from leaves and seeds of neem *Azadirachta indica*) and pyrethrum (extracted mainly from the flower *Chrysanthemum*) are the well-known PDPs used in mosquito control (George *et al.*, 2014). Various plant

extracts such as papaya (*Carica papaya*) leaf and seed extracts have shown mosquitocidal activities (Sesanti *et al.*, 2014). In addition, a number of plant-based essential oils and extracts with mosquitocidal properties have been described (Shaalan *et al.*, 2005). Herbal mosquito repellents are becoming popular due to their environmental friendliness, low cost and high effectiveness. Many researches have indicated that the natural plant extracted essential oils and other products exhibit better properties than synthetic ones (Kulkarni, 2017).

Today, the nano-biotechnology has revolutionized the field by synthesizing plant-mediated fabrications of nanoparticles to control mosquitoes (Benelli *et al.*, 2016). Various green-synthesized metal nanoparticles (metal nanoparticles with bioactive agents such as plant extracts and microorganisms) have been made available. Silver-(protein-lipid) nanoparticles (Ag-PL NPs) fabricated using the seed extract of *Sterculia foetida* (Indian almond tree) have shown a very high efficacy against larvae of malaria vector *An. stephensi*, dengue vector *Ae. aegypti* and filariasis vector *C. quinquefasciatus* (Rajasekharreddy & Pathipati, 2014). *Sargassum muticum* (a seaweed) treated silver nanoparticles have reduced the egg hatchability of the same three mosquito species by 100 % (Madhiyazhagan *et al.*, 2015).

RELEASING MOSQUITOES TO REDUCE TRANSMISSION OF MOSQUITO-BORNE DISEASES

Human population growth, increased globalization, rapid urbanization and geographic range expansion of mosquito species, together with increased vector resistance to insecticides and ineffective vector control programmes have led to the unprecedented global emergence of mosquito-borne diseases seen today. To effectively limit or prevent future outbreaks, intervention of novel strategies like releasing genetically modified mosquitoes or transgenic mosquitoes is essential. Two different endpoints are targeted by releasing mosquitoes: population reduction and replacement of vector population, with strains that are refractory to pathogen development and transmission (Figure 2).

Sterile insect technique (SIT)

The sterile insect technique (SIT) involves sterilization of male insects by radiation or chemicals to generate chromosomal aberrations/ dominant lethal mutations in sperms and release them in the wild. Any mating with released sterile males results in no progeny production

leading to population suppression (Figure 2A). This strategy is associated with mass rearing of male insects and releasing them in very high proportion to wild population. Continuous release of sterile males will eventually eliminate the target insect population. The technique has been successfully implemented to eradicate the screwworm fly *Cochliomyia hominivorax* from North America to the Darien Gap in Panama (Wyss, 2000). Even though a SIT field trial carried out in Italian

urban areas to control *Ae. albopictus* resulted in 70–80% sterility in the target population (Bellini *et al.*, 2013), the technique is not widely recognized in mosquito control programmes. Accurate selection of male mosquitoes prior to release, irradiating large numbers of male mosquitoes, unavailability of a marker system to monitor the released mosquitoes, fitness disadvantage shown by irradiated males and continuous release have been identified as major drawbacks of SIT.

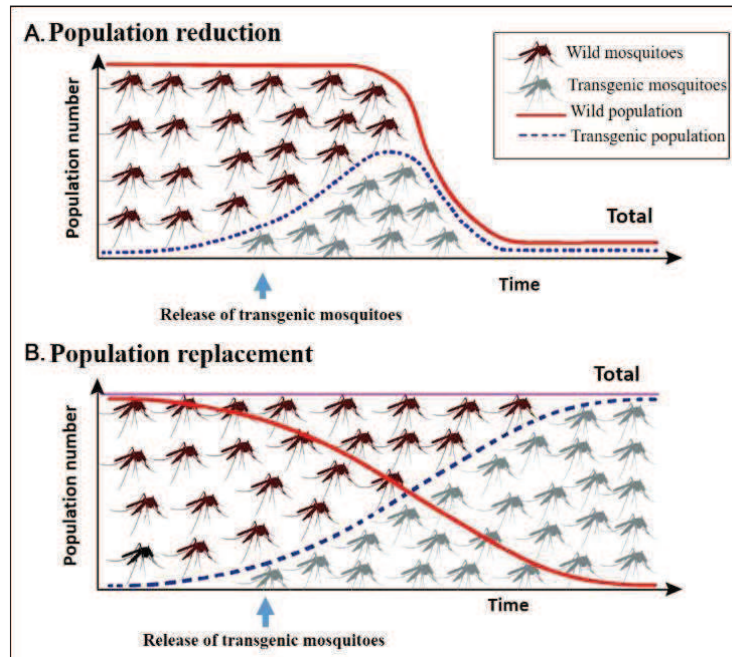


Figure 2: Graphical illustration of population reduction and population replacement by releasing transgenic mosquitoes.

A. Population reduction: conventional sterile insect technique (SIT), releasing mosquitoes carrying dominant lethal (RIDL) gene and *Wolbachia* mediated incompatible insect technique (IIT) are used to reduce a mosquito vector populations. B. Population replacement: release of transgenic mosquitoes and *Wolbachia* infected mosquitoes that are refractory to pathogen development and disease transmission can replace a mosquito population.

Releasing mosquito vectors carrying dominant lethal (RIDL) gene

Release of insects carrying a dominant lethal (RIDL) gene, a promising approach for reducing vector populations, was pioneered by the Oxitec (www.oxitec.com). In this system, transgenic insects carry a female-specific lethal dominant and repressible gene system (Thomas *et al.*, 2000). Only males are released into the environment to mate with wild females so that the

subsequent female progeny die in their immature stages or are flightless. The lethal gene is repressed using an antidote (tetracycline) so that female mosquitoes can be reared to adulthood in the laboratories in the presence of tetracycline for reproduction (Figure 3). This system also carries a fluorescence marker to monitor RIDL mosquitoes. A sustained series of field releases of OX513A *Ae. aegypti* males in a suburb of Brazil reduced the local *Ae. aegypti* population by 95% over a period of one year (Carvalho *et al.*, 2015).

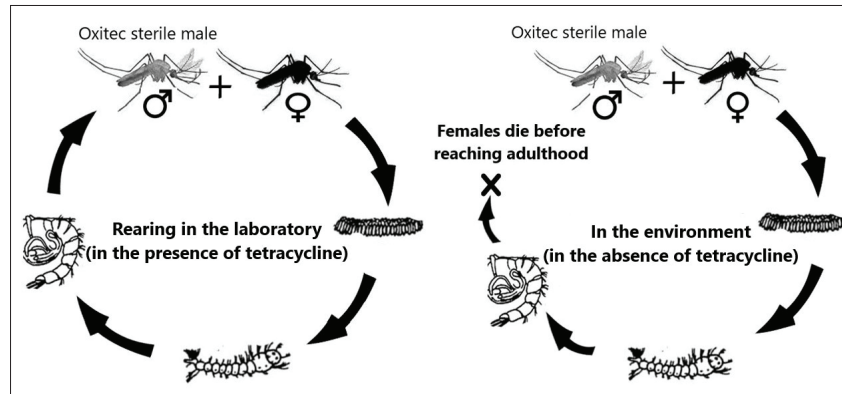


Figure 3: Oxitec release of insects carrying a dominant lethal (RIDL) gene method. Transgenic mosquitoes carry a female-specific lethal dominant gene which can be repressed using an antidote (tetracycline) so that female mosquitoes can be reared for reproduction in the laboratories in the presence of tetracycline.

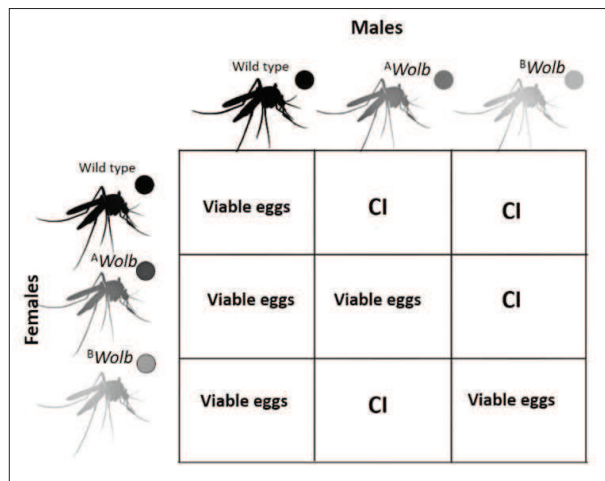


Figure 4: *Wolbachia* induced cytoplasmic incompatibility (CI)

Releasing *Wolbachia* infected mosquitoes

The *Wolbachia*-based mosquito control has emerged as another novel and promising tool in mosquito control. *Wolbachia* are maternally inherited endosymbiotic bacteria that are naturally occurring in many arthropods including mosquitoes. *Wolbachia* infection causes reproductive abnormalities including feminization, male-killing and cytoplasmic incompatibility (CI) (Gotoh *et al.*, 2006). Because of CI, viable offsprings are produced when *Wolbachia*-infected females mate with uninfected males or males infected with the same *Wolbachia* strain. If a *Wolbachia*-infected male mates

with an uninfected female, or if a *Wolbachia*-infected female mates with a male infected with a different strain of *Wolbachia*, no offspring will be produced (Figure 4). The *Wolbachia*-induced CI was proposed as a potential tool for mosquito control as this can lead to a population reduction.

Various *Wolbachia* strains have been isolated from their natural hosts and transinfected to different mosquito vectors. It has been reported that different strains of *Wolbachia* in mosquitoes inhibit the transmission of pathogens such as dengue (DENV) (Hedges *et al.*, 2008), chikungunya virus (CHIKV) (Aliota *et al.*, 2016), yellow fever virus (YFV) (Van den Hurk *et al.*, 2012), malaria parasites (Hughes *et al.*, 2011) and Zika virus (ZIKV) (Dutra *et al.*, 2016). Even though *Wolbachia* infection and associated disease control has been experimented with different vector mosquito species of the genera *Culex*, *Aedes* and *Anopheles* (Benelli *et al.*, 2016; Yen & Failloux, 2020; Wang *et al.*, 2021), the major focus has always been on *Ae. aegypti*, the primary vector of dengue. Transinfection of *Wolbachia* in *Ae. aegypti* is considered as a major success in dengue vector control since *Wolbachia* infection in *Ae. aegypti* is extremely rare in nature (Nugapola *et al.*, 2017; Yen & Failloux, 2020).

Incompatible insect technique (IIT) uses infection of *Wolbachia* to prevent viable progeny production. When *Wolbachia*-infected males are released to a non-infected target population, a nonviable progeny will be produced leading to a population reduction. The IIT requires multiple release of mass number of *Wolbachia*-infected

males. An IIT approach releasing of male *Wolbachia*-infected *Ae. aegypti* resulted in 92–98% reduction of the wild type population in Singapore (The Project *Wolbachia* – Singapore Consortium, 2021).

The IIT can be undermined by accidental release of females infected with the same *Wolbachia* strain. To overcome this, a combination of the radiation-based SIT and IIT can be advocated where female mosquitoes, if any, are exposed to radiation to ensure sterility (Lees *et al.*, 2015) (Figure 5). Release of a triple infected *Ae. albopictus* (infected with three strains of *Wolbachia*) with a pupal irradiation to negate the effect of unintentional release of females, resulted in almost complete eradication of *Ae. albopictus* population in a field setting in China (Zheng *et al.*, 2019).

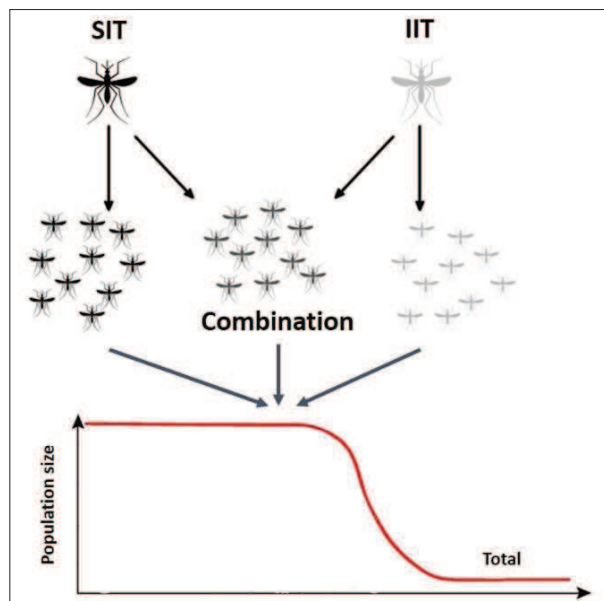


Figure 5: The three techniques, radiation-based sterile insect technique (SIT), incompatible insect technique (IIT) and the combined SIT/ IIT technique, to reduce vector populations

Wolbachia infection can also be used for vector population replacement strategy. Spreading *Wolbachia* into vector populations by releasing infected females can be regarded as a population replacement as shown in Figure 2B. Here, *Wolbachia* block the pathogen development and shorten the life of the mosquitoes reducing disease transmission. The population replacement strategy has been effectively used in Australia by infecting *Ae. aegypti* populations with the *wMel* strain to reduce dengue transmission (Hoffmann *et al.*, 2011). Infection

with *wMelPop* resulted in almost complete blocking of transmission of DENV in field populations in Australia and Vietnam (Nguyen *et al.*, 2015).

Since *Wolbachia* infection is maternally inherited, it can rapidly be spread across the population acting as a gene drive system (Segoli *et al.*, 2014). Further, the *Wolbachia*-based gene drive system has its potential application to introduce foreign genes also to the symbiont- a process called paratransgenesis, to spread them into a vector population (Hayiril & Martelli, 2019). Release of mosquitoes to suppress or replace vector populations can be combined with auto-dissemination (Caputo *et al.*, 2012) where released adults are used to disseminate substances such as IGRs. When IGR infested males are released, they will contaminate females and eventually IGR will be disseminated to breeding sites. This approach has been successfully implemented in Peru reducing *Ae. aegypti* populations (Devine *et al.*, 2009).

Releasing transgenic mosquitoes with gene drive systems

Transgenic strains of mosquitoes have been developed with refractory genes (genes encoding anti-pathogen effector molecules) that reduce the mosquito vector competency. In order to effectively spread a refractory gene in a wild population, it can be coupled with a gene drive system which is capable of increasing the likelihood of the gene to be passed onto the next generation overriding normal inheritance.

Moving genes into a population is possible with gene drive systems developed through Maternal-effect dominant embryonic arrest (*Medea*) (Chen *et al.*, 2007) and Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 systems (Doudna & Charpentier, 2014). CRISPR/Cas9 system is a highly effective tool for precision genome-editing (Kistler *et al.*, 2015) and can be used for population suppression and population replacement. This can be used to modify a vector population to express anti-pathogenic effector molecules as a population replacement strategy (Adolfi *et al.*, 2020). Current gene-drives also employ a CRISPR-based DNA cleavage mechanism to copy themselves from their location on one chromosome to the same site on another chromosome. This can alter the balance of naturally occurring genetic variants and is referred to as allelic-drive. Inclusion of additional CRISPR components to cut the unfavoured allele but not the preferred allele can favour the inheritance of one allele over another. When this process occurs in germ

cells the unfavoured allele is corrected by copying the gene sequence of the intact preferred allele. A fascinating CRISPR gene-drive system developed in mosquitoes had the ability to be transmitted in a highly efficient “super-Mendelian” fashion to >99% of progeny and carried a gene cassette conferring resistance to malarial parasites (Grantz *et al.*, 2015). This allelic-drive process has been used to replace an allele conferring resistance to DDT with the native insecticide susceptible allele in the fruit fly *Drosophila melanogaster* indicating the potential of restoration of insecticide susceptibility in mosquito populations reversing insecticide resistance (Kaduskar *et al.*, 2022).

CONCLUSIONS AND FUTURE PERSPECTIVES

Various strategies and techniques have been used to control mosquitoes aiming to reduce transmission of mosquito borne diseases. Each method has its own merits and demerits. Even though the control programmes still heavily depend on conventional insecticides, an urgent need has been arisen to limit their usage mainly due to resistance development and environmental concerns. Biological control strategies including the use of natural enemies and plant- and microorganism- based bioactive compounds are eco-friendly but often have operational difficulties in large-scale use, and are hence recommended for integrated vector control approaches. Two novel technologies, RIDL and *Wolbachia* mediated mosquito control, have given promising results in controlling wild mosquito populations reducing disease transmission. Advancement in gene drive systems will allow us to effectively spread refractory genes and insecticide susceptible alleles into mosquito populations overriding normal inheritance to aid in mosquito borne disease control. Enrichment of our knowledge on mosquito biology and the changing behaviour of mosquito species are of paramount importance in developing new molecular tools for effective mosquito control. Technological advances provide novel approaches to solve old problems. Such is the case with mosquito control!

Conflict of interest

The authors declare that there is no conflict of interest.

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