ARTICLE

CHALLENGES AND CONTROLLING STRATEGIES OF MOSQUITO

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ABSTRACT

In recent era, mosquito-borne deadly diseases are accounting approximately about 17% of all the infectious diseases. Although Malaria is the principal focus of the scientists, other deadly diseases like dengue and chikungunya are endemic in many developing countries. Though, synthetic mosquito repellents are controlling the mosquito population but they possess a lot of disadvantages to pregnant women and children. Thus, the focus has been shifted towards plant based repellents and plant derived essential oils which show efficacy with no side-effects. Research is also going on focusing the development of an anti-parasite vaccine. To this end, though, there is no licensed vaccine at present but a lot of progress is seen in this field recently. Another area of research has been focused on sterile insect techniques and transgenic mosquitoes in order to suppress the whole disease spreading female vector population. The progress in the field of molecular biology has facilitated greatly to disrupt and exploit the mosquito's life cycle. This review highlights all the approaches investigated to control mosquito-borne diseases with a fair discussion on challenges faced in this regard.

KEY WORDS
Mosquito-borne diseases, parasites, vectors, infectious diseases.

INTRODUCTION

In this highly socialized and globalized world, Mosquito borne diseases are one of the major causes of deaths every year. Mosquito-borne diseases account for about 17% of all the infectious diseases, causing more than 1 million deaths annually [1, 2, 3]. According to a survey, it is estimated that about 2.5 billion people in over 100 countries are at a risk of acquiring dengue fever alone. It is also found according to survey that malaria alone causes 600000 deaths globally every year and the saddest part is that most of them are children [1, 2]. Other mosquito-borne diseases such as dengue, West Nile virus, Japanese encephalitis etc. also cause deaths of many people every year globally. Although many of these mosquito-borne diseases are preventable if there is awareness about protective measures.

In epidemiology, a vector is any agent (person, animal or microorganism) that carries and transmits an infectious pathogen into another living organism. Mosquito-borne diseases spread through bites of infected mosquito species (vector), which mainly includes Aedes, Culex and Anopheles. These Mosquito-borne diseases are highly wide spread in few parts of the world whereas they are found in minimum numbers in certain countries of the world. They are most prominent during a particular time of the year while they are less prevalent in other times. Most vector-borne diseases exhibit a distinct seasonal pattern, which clearly suggests that they are highly sensitive to weather. Rainfall, temperature, and other weather variables also affect in many ways to both the vectors and the pathogens they transmit. Thus, the probability of transmission may or may not be increased by higher temperatures [1, 2, 4]. There are various factors which make a huge impact and bring a drastic change in the diverse spread of these vector borne-diseases in the world.

So a review of all the existing approaches towards mosquito-borne diseases is indeed necessary for people especially those who are living in the endemic areas to prevent themselves from mosquito-borne diseases. As there has been a lot of focus on different methods to exploit the vector's life cycle in preventing the disease, many improvements have been achieved by the scientists, which has been discussed in this study.

MOSQUITO LIFE CYCLE AND TRANSMISSION OF PARASITE

Despite the many differences that exist within the genus, the lifecycle of all mosquitoes is generally the same. Eggs hatch in water, where they hatch and undergo the transition to larvae. There are four aquatic larval stages. It is followed by an aquatic pupal stage before the adult arises. Adults feed on nectar and other sugar sources. Within days of emergence, adult males form mating swarms into which females fly to mate. The female must then take a blood meal before she is able to lay 50-200 eggs. Most adults can live up to 2 weeks in the field [5].

In humans, the parasites enter and multiply first in the liver cells and then they invade RBC's. In the blood, broods of parasites grow inside the RBC's and destroy them, releasing daughter parasites (merozoites) that continue the cycle by invading other RBC's and form gametocytes. The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites (gametocytes) are picked up by a female mosquito during its blood meal, they start another different cycle of growth and multiplication inside mosquito. The male and female gametocytes form a zygote and then form an oocyst. After 10-18 days, the parasites are found in the form of sporozoites in the mosquito's salivary glands. When the female mosquito takes a blood meal on another human, the sporozoites are injected with the mosquito's saliva into human and start another human infection when they parasitize the liver cells [5].

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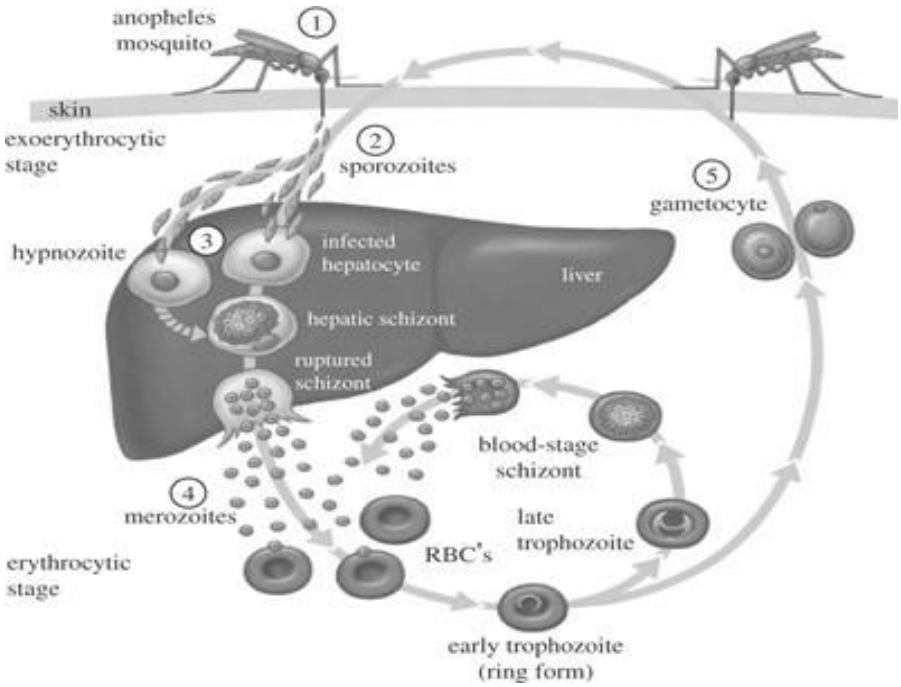

Fig. 1: Transmission and life cycle of malarial parasite, Wikimedia commons [5, 6].

METHODS USED IN CONTROLLING MOSQUITO POPULATION

Several approaches towards mosquito borne diseases have been investigated worldwide which are mainly classified into two types- genetic and non-genetic. While most of the non-genetic approaches are methods used for controlling mosquito population before the technology overtook. These approaches are highly effective in some areas and have been even employed now-a-days in many parts of the world. While genetic approaches are able to prevent and cure mosquito-borne diseases by highly cost effective interventions [3].

Non genetic approaches

Environmental control

Vector control strategies in this method involves suppressing the mosquito population through environmental modifications and manipulations like draining of stagnant water from wetlands, removal of mosquito's potential breeding habitats, and installation of meshes in homes [7, 8]. This approach has some success in controlling the mosquito population in Rome, Israel, India, Brazil, Egypt, and Zambia and is still used and recommended as an alternative approach in a few areas [9-11]

Chemical control

Early mosquito management relied on the use of Paris green (copper aceto arsenite) and petroleum by-products. It was discontinued because of their high toxicity and pollution of water sources [7, 12]. DEET (N,N-Diethyl-meta-toluamide) is a synthetic mosquito repellent that has been effective against several species of mosquitoes, however its repeated use disrupts natural biological systems. This pesticide has been reported toxic and allergic reactions in infants, children and pregnant women. It has also been found that mosquitoes gain resistance towards these synthetic repellents after a certain period of time [13-16]. Insecticide-treated bed nets alone have been regarded as an excellent tool to reduce malaria transmission in highly endemic countries, especially by reducing child mortality and morbidity [17-19].

Biological control

The western mosquito fish Gambusia affinis is a species of freshwater fish, also known commonly as mosquito fish. There is also an eastern mosquito fish G. holbrooki. The name "mosquito fish" was given because the diet of this fish sometimes consists of large numbers of mosquito larvae, relative to body size. Mosquito fishes have been introduced directly into ecosystems in many parts of the world as a bio control to lower mosquito populations which in turn negatively affected many other species in each distinct bioregion [20-23, 7].

Another type of biological approach in use against mosquitoes, is the use of entomo-pathogenic fungi. This method involves spraying mosquito's resting sites with suspension of fungal spores. Upon exposure, the

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fungi readily invades and multiplies inside the mosquito, killing it within 15 days, and thus reducing the parasite's transmission intensity [24, 25]. Laboratory and field studies have identified two different fungal species, Beauveria bassiana and Metarhizium anisopliae, that are effective against A. Gambia. B. thuringiensis israelensis and B. sphaericus are other two environmental friendly alternatives, given that the toxins they produce are non-toxic to other species and do not persist for a long period of time in the environment [7]. Hurdles for this approach mainly includes fungal spore viability, fungal specificity, and the development of resistance in the mosquitoes [26, 27].

Essential oils and natural mosquito repellents

Essential oil obtained from plants play an important role as xenobiotics due to presence of mono and sesqui-terpenes along with other volatile carbons [26]. In this method of controlling mosquitoes, certain plants which were previously described to have mosquito repelling properties are selected and they are subjected to hydro-distillation using a Clevenger type apparatus. Then the essential oils obtained are used in arm cage studies, where the arm of human hand treated with the essential oil is kept into a cage full of mosquitoes of a particular genus. The efficiency of the oil is tested by watching the number of mosquitoes falling on the human hand at appropriate time intervals [15].

In certain experiments, leaves are dried and grounded using a mechanical blender. Then, they were extracted using polar solvents like benzene or ethyl acetate, which were again tested for mosquito repellence using arm cage studies or to check the larvicidal activity [14].

Genetic approaches

In order to develop new approaches, there is a need to grow a broader knowledge of vector biology which can be done by sequencing the whole genome of the mosquito to know about the vector's biological processes that can be helpful to exploit and disrupt/eradicate these mosquito borne diseases.

Sterile insect technique (SIT)

The Sterile Insect Technique, also identified as the Sterile Insect Release Method, is a method for the management of key insect pests like mosquitoes, flies. The Sterile Insect Technique is defined as "a method of pest control using area-wide releases of sterile insects to reduce reproduction in a field population of the same species". It is therefore a type of "birth control" in which wild female insects of the pest population do not reproduce when they are inseminated by released, radiation-sterilized males. In this type of autocidal control, sequential releases of the sterilized insects in adequate sterile to wild male over flooding ratio's lead to a reduction in pest population numbers. Effective control using sterile insects is achieved with area-wide integrated pest management (AW-IPM) programmes.

At present many genetic control methods are investigated for the control of mosquitoes, which include release of wolbachia infected mosquitoes and other strategies [28]. Sterile insect technique (SIT) is also another popular technique for vector control development. There are two types of approaches – conventional and transgenic. SIT involves release of sterile males which can reduce population by creating persistent infertility [29]. Conventional method includes creation of dominant lethal in gametes due to irradiation which is generally induced by gamma rays. This method generally involves somatic damage that impairs male mating competitiveness against wild males [30]. Transgenic approach involves insertion of dna encoding for I-PpoI, a homing endonuclease extracted from physarum polycephalum. The expression of I-PpoI eliminates holandric X chromosomes thus causing male bias progeny. Transgenic I-PpoI mosquitoes carry a visible fluroscent dye (RFP) and marked fluorescent spermatozoa which facilitates male competitiveness and survival [31]. Infertility was induced in I-PpoI male's cage populations of stable-age distribution mosquito populations. The experiments conducted in large cages particularly confirm the suitability of the beta2-Ppo2 strain for consideration in sterile insect technique programs [32]. But life history analysis of the beta2-Ppo1 and beta2-Ppo2 lines revealed retarded development of the transgenic individuals and low adult eclosion in transgenic males [32].

Transgenic mosquitoes

The genetic transformation of mosquitoes to make them refractory to Plasmodium infections is regarded as a potential strategy to control malaria transmission. Three key components are needed for a successful mosquito transformation [33]. First, an efficient germ line transformation system has to exist. Secondly, suitable promoters that will drive stage, tissue, and sex-specific expression of anti- Plasmodium genes need to be selected [34]. Thirdly successful mosquito transformation in the selection of effector genes that either impair parasite development or serve as parasiticidal agents with 100% efficiency and a low fitness cost [35].

Means of driving a refractory construct through a vector is the most problematic one [36]. Transposable elements such as the p elements are gaining attention at present as they are able to spread quickly globally by replicating within a host genome and hence inherited in off-spring's genome [37]. They are successfully implemented in Drosophila but the same cannot be repeated in A. gambiae as its activity decline substantially with increasing size and over time. MEDEA (maternal-effect dominant embryonic arrest) elements are under development which are selfish genetic elements that spread rapidly by

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distorting its own off-spring's ratio. MEDEA encodes a maternally expressed toxin and zygotically expressed antidote. The toxin causes the death of the progeny [38]. Several refractory genes are to be found out to improve the efficacy of refractoriness and reduce the probability that give resistance to anti-pathogen genes will emerge in plasmodium population [38]. MEDEA elements should also be introduced into mosquito populations and their ill-ailments should also be found out by conducting large cage trials [38].

Previous research in controlling malaria was done on plasmodium parasite and anopheles mosquitoes. The drug and vaccines developed have failed to induce sufficient long lasting protective immunity. The spray of pesticides also failed because the mosquitoes develop resistance to the pesticides. So currently SIT (sterile insect technique) is gaining importance which involves release of large number of sterile individuals to cause mosquito population suppression [39]. Germ line transformation of vectors has become more common and this technology involves germ line insertion of transposons containing genes of interest in the presence of transposase enzyme [39]. The ability to rear the mosquito population of a particular species. The ability to induce sterility. Many mechanisms include chemo-sterilization, cytoplasmic incompatibility but most commonly used mechanism is the exposure to gamma radiations, which causes chromosomal breaks in the germ cells. A wide knowledge on population dynamics is also required and viability as transgenic SIT mosquitoes. Irrelevant transposon combination also causes transgene instability [39].

As we need to separate the 2 sexes prior to release, mechanical separation methods cause physical damages and reduced competitiveness constantly [40]. Alternative methods include making genetic sexing strains but it also has fitness problems, semi-sterility, and also absence of complete sterility [39]. Transgenic markers like EGFP, RFP are used to allow sex separation based on fluorescence. The sperms of male were marked with EGFP+. Mechanical-opting sorting system is used to differentiate them phenotypically. Many insect resistance genes are also used in males [41]. In case of females, many dominant lethal genes are induced, which control the transcriptional process of certain genes, instead of gene of interests are transcribed. Using transgenesis to create strains of Anopheles suitable for SIT could potentially offer several advantages over current approaches, in that the basic design of transgenic constructs designed for other insects should be rapidly transferable to mosquitoes, and induction of sterility as a product of the transgenic modification could obviate the requirement for radiation and its associated deleterious effects [39].

Transgenic strategies of controlling mosquitoes can be used in two ways. It is population suppression and population replacement. However approaches aimed at population suppression is assumed to lose transgenes after the releases are end due to environmental non-compatibility [42]. So here Skeeter buster model is being used for suppressing adult female mosquitoes. A transgene encoding a toxic protein is controlled by a promoter which turns on expression in female flight muscles. Females with dead flight muscles cannot feed, mate, fly and they gradually die [43]. Construction of this FK strain proposes a deployment method where eggs would be transported throughout a city. Local stakeholders would then be responsible for hatching eggs in suitable habitats so that FK adults would emerge but only males would be viable. These males would then mate with wild-type females, whose female progeny would not develop into viable, virus-transmitting adult females [43]. A. aegypti is particularly amenable to this approach because its eggs are desiccation resistant and can remain viable for several months, making them easy to transport, distribute, and hatch simply by immersing in water [44]. Here they show that population elimination might be an unrealistic objective in heterogeneous populations. They demonstrate that substantial suppression can nonetheless be achieved if releases are deployed in a uniform spatial pattern using strains combining multiple lethal elements, illustrating the importance of detailed spatial models for guiding genetic mosquito control strategies [44].

Transgene introduction in mosquitoes for population suppression or inhibition of ookinete development is also an attractive approach for the control of vector borne diseases. But the problem is that these transgenes are facing fitness problems gradually over time due to various factors [40]. So here a transgenic A. stephensi that express a catalytically inactive phospholipase gene (mPLA2) under the control of a midgut promoter is created. They created two transgenic lines of Anopheles stephensi, a natural vector of Plasmodium falciparum, which constitutively secrete a catalytically inactive phospholipase A2 (mPLA2) into the midgut lumen to interfere with Plasmodium ookinete invasion. Experiments show that both transgenic lines expressing mPLA2 significantly impair the development of rodent malaria parasites, but only one line impairs the development of human malaria parasites. In addition, when fed on malaria-infected blood, mosquitoes from both these transgenic lines are more fecund than non-transgenic mosquitoes. Consistent with these observations, cage experiments with mixed populations of transgenic and non-transgenic mosquitoes, show that the percentage of transgenic mosquitoes increases when maintained on Plasmodium-infected blood. Expression of an anti-Plasmodium effector gene gives transgenic mosquitoes a fitness advantage when fed malaria-infected blood.

Vaccines

Inactivated sporozoite immunization studies in 1942 showed a hope of combining both cellular and humoral immune responses against malaria of domestic fowl [45]. Almost at the same time, Freund was developing a powerful adjuvant that showed decent efficacy in malaria studies [46]. However, today, there is still no licenced vaccine against malaria or any other parasitic disease of humans and no deployed subunit vaccine for any parasitic disease [47, 48]. Most of the vaccines that are available today belong to

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one of three categories-attenuated microbes, killed microbes or protein subunits. Attenuated viruses that protect against a cross-reactive pathogen originate with Jenner's use of a related poxvirus to prevent smallpox. After about a century, killed microbes were introduced and several such vaccines, e.g. polio vaccine, have been used widely. More recently, conjugate vaccines against encapsulated bacterial pathogens have been developed and have been hugely successful in reducing the incidence of some diseases. However, protein based licensed subunit vaccines are very few and these are particulate. Recombinant proteins that are not particulate have rarely become effective human vaccines. Parasitologists trying to develop vaccines can hardly ever safely grow and manufacture whole parasites in sufficient numbers to induce immunity, although recently this has been attempted for malaria [49]. Instead, a large number of antigens have been expressed, mainly as proteins and less often from vector systems to try to generate protective immunity. Parasite vaccines generally face the challenge of generating immunity with an immunogen that reflects only a tiny fraction (less than 1%) of the composition of the organism, a challenge that has been met only rarely in vaccinology [50]. Encouragingly, in at least one system, vaccination of pigs and cattle against Taenia, vaccination with a single antigen has prevented cysticercosis causes by cestode parasites [51].

Although most residents develop natural immunity to malaria in endemic areas, this usually few years of exposure and is imperfect. Natural immunity predominantly targets a wide variety of blood-stage antigens and no antigen appears to be especially important in providing protection [52]. Vaccine candidates selected for vaccines are malaria antigens as they are the targets of natural immunity and significant genetic polymorphism, and a key blood-stage antigen, P. falciparum erythrocyte membrane protein-1 (PfEMP1), even shows temporal switching of variant expression. Plasmodium parasites express substantial stage-specificity of antigen so candidate vaccines for one stage of the life cycle are unlikely to impact on another stage.

RTS,S, a P. falciparum CS protein was showing great efficacy in sporozoite challenge studies in a novel adjuvant [53]. This RTS,S candidate has led the way for many years, showing efficacy in progressively younger subjects and in a variety of epidemiological settings culminating in an ongoing licensure trial [54]. Blood-stage vaccine candidates continue to struggle with adjuvant formulations and limited immunogenicity [49].

However, some important questions remain about the efficacy and utility of this vaccine for malaria control, not all of which will be answered by the current phase III trial. These include the level of efficacy against severe malaria, which could be higher than that against clinical malaria, the duration of protection provided by the vaccine, which was limited with the AS02 formulation both in phase IIa and phase IIb studies, and the cost-effectiveness and community acceptability of deploying a vaccine with limited efficacy [55].

Whole parasite vaccines

A major effort has been made to develop a pre-erythrocytic vaccine comprising whole sporozoites as a response to limited efficiency achieved by sub-unit vaccine candidates and RTS,S [49]. Defective schizonts can be produced by irradiated sporozoites within the liver which invade them. This can express antigens that induce a protective immune response but the main disadvantage of defective schizonts is that they cannot rupture to release the merozoites that would normally invade RBCs and continue the infection. Comparing with animal models, complete protection is readily obtainable through the activity of induced CD8þ T cells that clear infected human liver cells but this remains to be demonstrated. However, significant efficacy has yet to be reported from these studies and it remains unclear whether a needle and a syringe can substitute for a mosquito and its salivary gland fluids in generating adequate immunogenicity and efficacy in human [56]. Even if high-level efficacy can be achieved using this approach, the challenges of cost of manufacture and distribution of parasite vials in liquid nitrogen tanks in developing countries suggest that efficacy of this approach will need to be considerable higher than that of other malaria vaccines for it to be deployed widely [57].

Vectored vaccines

The other major approach to induce pre-erythrocytic immunity is to employ vectored vaccines, aiming mainly to induce cellular immunity against the liver-stage of P. falciparum. Irradiated sporozoite induced immunity in animal models is mainly due to CD8þ T cells and appears to target multiple antigens [57]. Using chimpanzee adenoviruses (ChAds) encoding the Thrombospondin-related adhesion protein (TRAP) pre-erythrocytic antigen to prime an immune response that is then boosted by another viral vector, modified vaccinia virus Ankara (MVA) encodes the same TRAP insert [45]. This particular prime-boost approach, first discovered in malaria, leads to much higher T cell responses than single vector immunization, and extensive studies have shown its utility in pre-clinical models [58]. A related prime-boost approach is being developed by the US Naval Medical Research Centre, but here the priming vector is plasmid DNA, and a human adenovirus, Ad5, is used to boost the immune response [59].

Blood stage vaccines

In contrast to major progress in several areas of pre-erythrocytic vaccine development, results with blood-stage vaccines have been more mixed and progress is generally slower [60]. A number of candidate

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vaccines have progressed to clinical testing but they have shown limited efficacy against clinical malaria. MSP1 and AMA1 in particular are the antigens for vaccine candidates. Almost all of these candidate vaccines have been a protein given with an adjuvant designed to induce protective antibodies. Some approaches have focused on inducing antibodies that impair parasite growth, as can be demonstrated in the in vitro assays [5], whereas others have aimed to induce antibodies which achieve their effect in collaboration with effector cells and which can be measured with greater difficulty, in a functional assay of ADCI of parasite growth [61]. There have been three particular challenges for the development of blood-stage vaccines. One is difficulty in expressing conformationally correct large antigens and scaling up the methods needed to do this to the extent that would allow large-scale manufacture. A second challenge has been the only modest antibody responses achieved even with a range of new adjuvants. The third difficulty has been posed by the extensive polymorphism of many leading candidate blood-stage antigens [45].

Mosquito stage vaccines

The principle of this vaccine is that immunization with gametocyte or ookinete antigens could reduce or ablate oocyst development in the mosquitoes [62]. Membrane feeding assays are getting standardized that allow sera from vaccinated animals or humans to be evaluated for their ability to reduce or prevent transmission. The in vivo use of transgenic parasites has supplemented to assess the efficacy of antibodies induced by P. falciparum and P. vivax antigens in preventing transmission of rodent parasites [63, 64]. These systems provide means of rapidly assessing the likely efficacy of sera from vaccines participating in phase I trials of mosquito-stage vaccines, a major advantage of this approach to malaria vaccine development. In view of the potential of this approach to the prevention of malaria, it is surprising that it has been so little supported. The main hurdle has been the concern that deployment of such a transmission- blocking vaccine would prove impractical. This type of mass vaccination has been undertaken on occasion for other diseases but may prove logistically challenging.

Table 1: Comparison of different approaches to control mosquito borne diseases on the basis of effectiveness [65-69]

Method	Principal target	Approach type	Affordability	Effectiveness
Environmental control	Mosquitoes	Non-genetic	Cheap	Substantially high but slow
Biological control (Fungi)	Mosquitoes	Non-genetic	Cheap	Slow and less effective
Biological control (Fish)	Mosquitoes	Non-genetic	Cheap	Less effective
Essential oils	Mosquitoes	Non-genetic	Cheap	Considerably effective for a short span
Chemical control	Mosquitoes	Non-genetic	Considerably high	Highly effective with many disadvantages
Transgenic mosquitoes and SIT	Parasites	Genetic	High	Research in progress
Vaccines	Parasites	Genetic	High	Research in progress

CONCLUSION

Although several approaches towards controlling these deadly mosquito borne diseases are being investigated in recent times, but it is indeed necessary to look for other factors such as affordability and availability for the common person. So a combination of all these approaches can control these mosquito borne diseases unless these vectors gain resistance to them. A lot of research work is to be done in developing anti-parasite vaccines and by reducing the harmful-vector populations effectively using transgenic techniques which are major prospects of the post genomic era. As the eradication of harmful vector populations using transgenic technologies might take a very long time in order to protect ourselves we need to use many traditional non-genetic approaches like draining of stagnant water, use of mosquitocidal nets and use of essential oil mixtures for our well-being. The main important step to be initialized now is to develop a perfect model for combating these vector population which comprises eradicating future vector population using transgenic technologies and protecting ourselves by developing anti parasite vaccines and other non-genetic approaches.

CONFLICT OF INTEREST
There is no conflict of interest.

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REFERENCES

[1] WHO, Vector borne diseases. [2014] World Health Organisation, Geneva.

[2] Ferguson NM. [2018] Challenges and opportunities in controlling mosquito-borne infections. Nature, 559:490-497. doi: 10.1038/s41586-018-0318-5

[3] WHO, World Malaria report. [2014] World health organisation, Geneva.

[4] Gubler DJ, Reiter P, Ebi KL, Yap W, Nasci R, Patz JA. [2001] Climate Variability and Change in the United States: Potential Impacts on Vector- and Rodent-Borne Diseases. Environ Health Perspect, 109:223-233. doi: 10.1289/ehp.109-1240669.

[5] Ellis RD, Mullen GE, Pierce M, Martin LB, Miura K, Fay MP, Long CA, Shaffer D, Saul A, Miller LH, Durbin AP. [2009] A Phase 1 study of the blood-stage malaria vaccine candidate AMA1-C1/Alhydrogel with CPG 7909, using two different formulations and dosing intervals. Vaccine, 27:4104-4109. doi: 10.1371/journal.pone.0002940.

[6] Hill AVS. [2011] Vaccines against malaria. Philosophical Transactions of the Royal Society B: Biological Sciences, 366:2806–2814. https://doi.org/10.1098/rstb.2011.0091

[7] Walker K, Lynch M. [2007] Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. Med Vet Entomol, 21(1):2-21.

[8] Shiff C. [2002] Integrated approach to malaria control. Clin Microbiol Rev, 15(2):278-293.

[9] Su X, Hayton K, Wellems TE. [2007] Genetic Linkage and Association Analyses for Trait Mapping in Plasmodium Falciparum. Nat Rev Genet, 8(7):497-506.

[10] Utzinger J, Tozan Y, Singer BH. [2001] Efficacy and cost-effectiveness of environmental management for malaria control. Trop Med Int Health, 6(9):677-687.

[11] Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BG. [2002] Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa. Lancet Infect Dis, 2(10):618-627.

[12] Rozendaal JA. [1997] Vector Control: Methods for Use by Individuals and Communities. World Health Organization; Geneva. https://doi.org/10.1016/S0169-4758(98)01304-0.

[13] Norris EJ, Coats JR. [2017] Current and Future Repellent Technologies: The Potential of Spatial Repellents and Their Place in Mosquito-Borne Disease Control. Int J Environ Res Public Health, 14:E124.

[14] Krishnappa K, Elumalai K, Dhanasekaran S, Gokulakrishnan J. [2012] Larvicidal and repellent properties of Adansonia digitata against medically important human malarial vector mosquito Anopheles stephensi (Diptera: Culicidae). J Vector Borne Dis, 49(2):86-90.

[15] Govindarajan M, Sivakumar R. [2011] Mosquito adulticidal and repellent activities of botanical extracts against malarial vector Anopheles stephensi Liston (Diptera: Culicidae). Asian Pac J Trop Med, 4(12):941-947.

[16] Oh J, Bowling JJ, Carroll JF. [2012] Natural product studies of U.S. endangered plants: Volatile components of Lindera melissifolia (Lauraceae) repel mosquitoes and ticks. Photochemistry, 80:28-36. doi: 10.1016/j.phytochem.2012.05.001..

[17] de Castro CM, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J, Keiser J, Singer BH. [2004] Integrated urban malaria control: a case study in dares salaam, Tanzania. Am J Trop Med Hyg, 71(2):103-117.

[18] Potikasikorn J, Chareonviriyaphap T, Bangs MJ, Prabaripai A. [2005] Behavioral responses to DDT and pyrethroids between Anopheles minimus species A and C, malaria vectors in Thailand. Am J Trop Med Hyg, 73(2):343-349.

[19] WHO, Insecticide-treated mosquito nets: a WHO position statement. [2006] WHO Geneva.

[20] WHO technical report series 936. [2006] World health organisation, Geneva.

[21] Fillinger U, Knols BGJ, Becker N. [2003] Efficacy and efficiency of new Bacillus thuringiensis var israelensis and Bacillus sphaericus formulations against Afro-tropical anophelines in Western Kenya. Trop Med Int Health, 8(1):37-47.

[22] Russell TL, Brown MD, Purdie DM, Ryan PA, Kay BH. [2003] Efficacy of VectoBac (Bacillus thuringiensis variety israelensis) formulations for mosquito control in Australia. J Econ Entomol, 96(6):1786-1791.

[23] Das PK, Amalraj DD. [1997] Biological control of malaria vectors. Indian J Med Res, 106:174-197.

[24] Blanford S, Chan BH, Jenkins N, Sim D, Turner RJ, Read AF, Thomas MB. [2005] Fungal pathogen reduces potential for malaria transmission. Science, 308(5728):1638-1641.

[25] Scholte EJ, Knols BG, Takken W. [2006] Infection of the malaria mosquito Anopheles gambiae with the entomopathogenic fungus Metarhizium anisopliae reduces blood feeding and fecundity. J Invertebr Pathol, 91(1):43-49.

[26] Kanzok SM, Lorena JM. [2006] Entomo-pathogenic fungi as biological insecticides to control malaria. Trends Parasitol, 22:49-51. https://doi.org/10.1016/j.pt.2005.12.008.

[27] Scholte EJ, Knols BG, Takken W. [2004] Auto dissemination of the entomopathogenic fungus Metarhizium anisopliae amongst adults of the malaria vector Anopheles gambiaess. Malar J, 3:45. doi: 10.1186/1756-3305-4-171.

[28] Hoffmann AA, Montgomery BL, Popovici J, Ormaetxe I, Johnson PH, Muzzi F, et al. [2011] Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. Nature, 476(7361):454-457.

[29] Davidson G. [1964] Anopheles gambiae a complex of species. Bulletin of the World Health Organization, 31(5):625-634.

[30] Bakri A, Mehta K, Lance DR. [2005] Sterilizing insects with ionizing radiation. Sterile insect technique: principles and practice in area-wide integrated pest management. Dordrecht: Springer, 233-237. doi: 10.1007/1-4020-4051-2_9

[31] Windbichler N, Papathanos PA, Crisanti A. [2008] Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in Anopheles gambiae. https://doi.org/10.1371/journal.pgen.1000291

[32] Klein TA, Windbichler N, Deredec A, Burt A, Benedict MQ. [2012] Infertility resulting from transgenic I-Ppol male Anopheles gambiae in large cage trials. Pathog Glob Health, 106(1):20-31.

[33] Lorena JM. [2003] Interrupting malaria transmission by genetic manipulation of anopheline mosquitoes. J Vector Borne Dis, 40(3-4):73-77.

[34] Catteruccia F, Godfray HC, Crisanti A. [2003] Impact of genetic manipulation on the fitness of Anopheles stephensi mosquitoes. Science, 299(5610):1225-1227.

[35] Ribeiro JM, Kidwell MG. [1994] Transposable elements as population drive mechanisms: Specification of critical parameter values. J Med Entomol, 31(1):10-16.

[36] Smith RC, Kizito C, Rasgon JL, Lorena JM. [2013] Transgenic Mosquitoes Expressing a Phospholipase A2 Gene Have a Fitness Advantage When Fed Plasmodium falciparum-Infected Blood. https://doi.org/10.1371/journal.pone.0076097.

[37] Charlesworth B, Sniegowski P, Stephan W. [1994] The evolutionary dynamics of repetitive DNA in eukaryotes. Nature, 371(6494):215-220.

[38] Chen CH, Huang H, Ward CM, Su JT, Schaeffer LV, Guo M, Hay BA. [2007] A synthetic maternal-effect selfish genetic element drives population replacement in Drosophila. Science, 316(5824):597-600.

[39] Nolan T, Papathanos P, Windbichler N, Magnusson K, Benton J, Catteruccia F, Crisanti A. [2011] Developing transgenic Anopheles mosquitoes for the sterile insect Technique Genetica, 139(1):33-39.

[40] Calkins CO, Parker AG. [2005] Sterile insect quality. Sterile insect technique. Principles and practice in area-wide integrated pest management. Dordrecht, Springer, 269-296.

[41] Catteruccia F, Benton JP, Crisanti A. [2005] An Anopheles transgenic sexing strain for vector control. Nat Biotechnol, 23(11):1414-1417.

[42] Benedict MQ, Robinson AS [2003] The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol, 19(8):349-355.

[43] Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, Berendonk TU, et al. [2010] Female-specific flightless phenotype for mosquito control. Proc Natl Acad Sci USA, 107(10):4550-4554.

[44] Legros M, Xu C, Okamoto K, Scott TW, Morrison AC, Lloyd AL, Gould F. [2012] Assessing the Feasibility of Controlling Aedes aegypti with Transgenic Methods: A Model-Based Evaluation. https://doi.org/10.1371/journal.pone.0052235.

[45] Sandoval RA, Berthoud T, Alder N, Siani L, Gilbert SC, Nicosia A, Colloca S, Cortese R, Hill AV. [2010] Prime-boost immunization with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and poly-functionality of protective malaria CD8þ T-cell responses. Infect Immun, 78(1):145-153.

[46] Russell PF, Mohan BN. [1942] The immunization of fowls against mosquito-borne Plasmodium gallinaceum by injections of serum and of inactivated homologous sporozoites. J Exp Med, 76(5):477-495.
[47] Freund J, Sommer HE, Walter AW. [1945] Immunization against malaria: vaccination of ducks with killed parasites incorporated with adjuvants. Science, 102(2643):200-202.
[48] Knox DP, Redmond DL. [2006] Parasite vaccines- recent progress and problems associated with their development. Parasitology. 133:S1-S8. doi: 10.1017/S0031182006001776
[49] Hoffman SL, Billingsley PF, James E, Richman A, et al. [2010] Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. Hum Vaccin, 6(1):97-106.
[50] Anders RF, Adda CG, Foley M, Norton RS. [2010] Recombinant protein vaccines against the asexual blood stages of Plasmodium falciparum. Hum Vaccin, 6(1)39-53.
[51] Lightowlers MW. [2010] Eradication of Taenia solium cysticercosis: a role for vaccination of pigs. Int J Parasitol, 40(10):1183-1192.
[52] Marsh K, Kinyanjui S. [2006] Immune effector mechanisms in malaria. Parasite Immunol, 28(1-2):51-60.
[53] Stoute JA, Slaoui M, Heppner DG, et al. [1997] A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. RTS, S Malaria Vaccine Evaluation Group. N Engl J Med, 336(2):86-91.
[54] Ballou WR, Cahill CP. [2007] Two decades of commitment to malaria vaccine development: GlaxoSmithKline Biologicals. Am J Trop Med Hyg, 77(6):289-295.
[55] Kester KE, Cummings JF, Anyinam O, et al. [2009] Vaccine Evaluation Group. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/ AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis, 200(3):337-346.
[56] Vaughan AM, Wang R, Kappe SH. [2010] Genetically engineered, attenuated whole-cell vaccine approaches for malaria. Hum Vaccin, 6(1):107-113.
[57] Doolan DL, Hoffman SL. [1997] Pre-erythrocytic stage immune effector mechanisms in Plasmodium spp infections. Philos Trans R Soc Lond B Biol Sci, 352(1359):1361-1367.
[58] Capone S, Reyes-Sandoval A, Naddeo M, Siani L, Ammendola V, Rollier CS, Nicosia A, Colloca S, Cortese R, Folgori A, Hill AV. [2010] Immune responses against a liver stage malaria antigen induced by simian adenoviral vector AdCh63 and MVA prime-boost immunisation in non-human primates. Vaccine, 29(2):256–265.
[59] Bruder JT, Angov E, Limbach KJ, Richie TL. [2010] Molecular vaccines for malaria. Hum Vaccin, 6:54-77. https://doi.org/10.3389/fimmu.2018.02769.
[60] Goodman AL, Draper SJ. [2010] Blood-stage malaria vaccines-recent progress and future challenges. Ann Trop Med Parasitol. 104(3):189-221.
[61] Druilhe P, Spertini F, Soesoe D, Corradin G, et al. [2005] A malaria vaccine that elicits in human antibodies able to kill Plasmodium falciparum. https://doi.org/10.1371/journal.pmed.0020344.
[62] Takala SL, Coulibaly D, et al. [2009] Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. Sci Transl Med. doi: 10.1126/scitranslmed.3000257.
[63] Carter R, Chen DH. [1976] Malaria transmission blocked by immunisation with gametes of the malaria parasite. Nature, 263(5572):57-60.
[64] Ramjanee S. [2007] The use of transgenic Plasmodium berghei expressing the Plasmodium vivax antigen P25 to determine the transmission-blocking activity of sera from malaria vaccine trials. Vaccine, 25(5):886-894.
[65] Fontenille D, Lochouarn R. [1999] The complexity of the malaria vectorial system in Africa. Parassitologia, 41(1-3):267-271.
[66] Fontenille D, Cohuet A, Awono-Ambene PH, Antonio-Nkondjio C, Wondji C, Kengne P, et al. [2003] Systematics and biology of Anopheles vectors of Plasmodium in Africa, recent data. Med Trop (Mars), 63(3):247-253.
[67] Perich MJ, Tidwell MA, Williams DC, Sardelis MR, Pena CJ, Mandeville D, Boobar LR. [1990] Comparison of ground and aerial ultra-low volume applications of malathion against Aedes aegypti in Santo Domingo, Dominican Republic. J Am Mosq Control Assoc, 6(1):1-6.
[68] Marshall JM, Taylor CE. [2009] Malaria Control with Transgenic Mosquitoes. https://doi.org/10.1371/journal.pmed.1000020.
[69] Ramirez JL, Garver LS, Dimopoulos G. [2009] Challenges and Approaches for Mosquito Targeted Malaria Control. Curr Mol Med, 9(2):116-130.