

# Advanced Techniques to Control Mosquitoes and their Disease Transmission: A Review

S. Renuka 

Central Research Laboratory, Meenakshi Medical College Hospital and Research Institute, Meenakshi Academy of Higher Education and Research (Deemed to be University) Kanchipuram, Tamil Nadu, India.

## Abstract

Mosquitoes transmit life-threatening diseases to humans. The conventional mosquito control techniques that have been focused on population reduction by the application of insecticides or through source reduction by removing the larval habitat have become limited, and it has been evidenced by increased disease burden globally. This review focuses on advanced techniques that reduces and modify the mosquito population and limit their disease transmission by releasing the modified mosquitoes into the environment and that are presently under development and have the potential of controlling the mosquito-borne diseases.

**Keywords:** *Anopheles, Aedes, Culex, CRISPR/Cas9, RNAi, Mosquito Control, SIT, Wolbachia*

\*Correspondence: renuraj17@gmail.com

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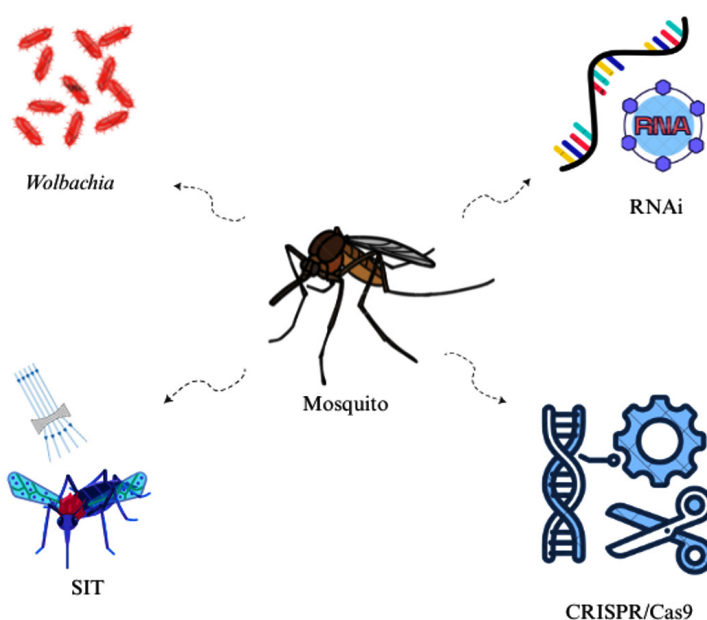
## INTRODUCTION

Mosquito-borne diseases (MBDs) are the major global health concern.<sup>1</sup> *Culex*, *Anopheles*, and *Aedes* are the major vector mosquitoes that transmit pathogens causing filariasis, malaria, dengue, and chikungunya, respectively. Among MBDs, the parasitic infection such as malaria is transmitted by the *Anopheline* mosquito, it affects around 249 million people and causes 608000 deaths every year globally. Dengue is the most widespread viral infection transmitted through *Aedes* mosquitoes and over 132 countries are under risk of contracting dengue. The estimated dengue cases are around 96 million and there are 40000 deaths every year.<sup>2</sup> The other arbovirus diseases, such as chikungunya, Zika, and yellow fever, are of major concern.<sup>2</sup> Growing resistance in vector mosquitoes against currently available insecticide and the toxicity of chemical insecticides on ecosystems has encouraged researchers to opt for the alternative vector control methods.<sup>3</sup> As an alternative method, biotechnology offers several innovative techniques for mosquito control, focusing on reducing mosquito density or their capability to transmit diseases.<sup>4</sup> The most prominent techniques used are sterile

insect technique (SIT),<sup>5</sup> release of insects having a dominant lethal (RIDL),<sup>6</sup> Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9),<sup>7</sup> *Wolbachia* bacteria-based control,<sup>8</sup> RNA interference (RNAi),<sup>9</sup> and Paratransgenesis.<sup>10,11</sup> This review provides the information on advanced techniques such as CRISPR/Cas9, RNAi, SIT, and *Wolbachia* used for vector control (Figure 1).

### Clustered regularly interspaced short palindromic repeat and their associated protein (CRISPR/Cas9)

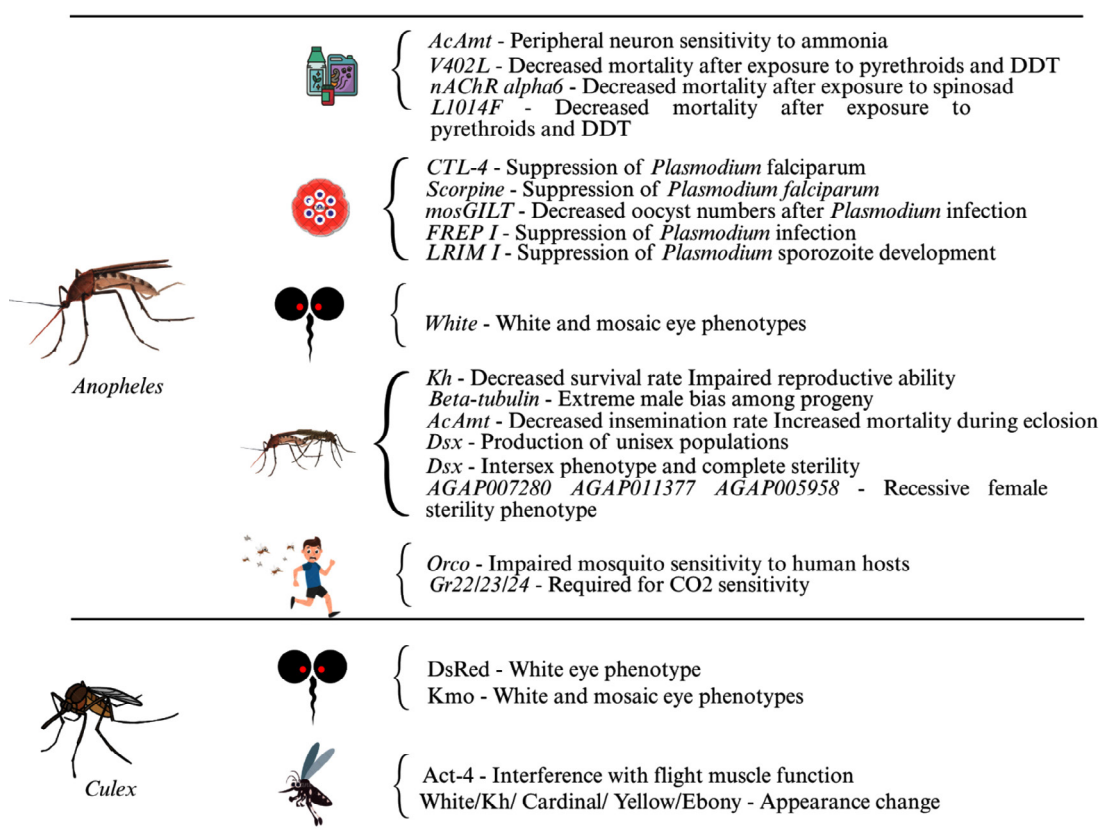
'Genome editing', a branch of genetic engineering, is a technique in which the target genome is modified by removing/inserting nucleotide sequences in the living system.<sup>12</sup> CRISPR is one such genome editing tool found in prokaryotes, it is an adaptive immunity system that defends them against viruses or bacteriophages. CRISPR was first found during analysis of the gene alkaline phosphatase in *Escherichia coli* in 1987 by Japanese scientists.<sup>13</sup> Later, the key role of CRISPR in the prokaryotic adaptive immune system against bacteriophages was experimentally proven during 2007.<sup>14</sup> The CRISPR system comprises two essential proteins components, such as guide RNA (gRNA) and Cas9 proteins. The CRISPR/Cas9 method has



**Figure 1.** Advanced methods used to reduce or modify the vector mosquito population

three steps; they are detection, cleavage, and repair. Initially, the synthesized single guide RNA (sgRNA) identifies the sequence of interest in the target genome, and it forms a complementary base pair. In the second stage, Cas9 nuclease creates double-stranded breaks, and they are repaired by cellular mechanisms. CRISPR/Cas9 is the most promising genome editing method employed in different disciplines of science. It is widely employed in agriculture to increase the nutritional content of the food grains. CRISPR/Cas9 is being used to investigate cancers, HIV (human immunodeficiency virus), and gene therapy. CRISPR/Cas9 based gene drive mechanisms have been employed for mosquito vector control through population suppression or population replacement. Over the past decade, the CRISPR/Cas9 based technique has become a promising and potential method for effective control of vector mosquitoes.<sup>15-19</sup>

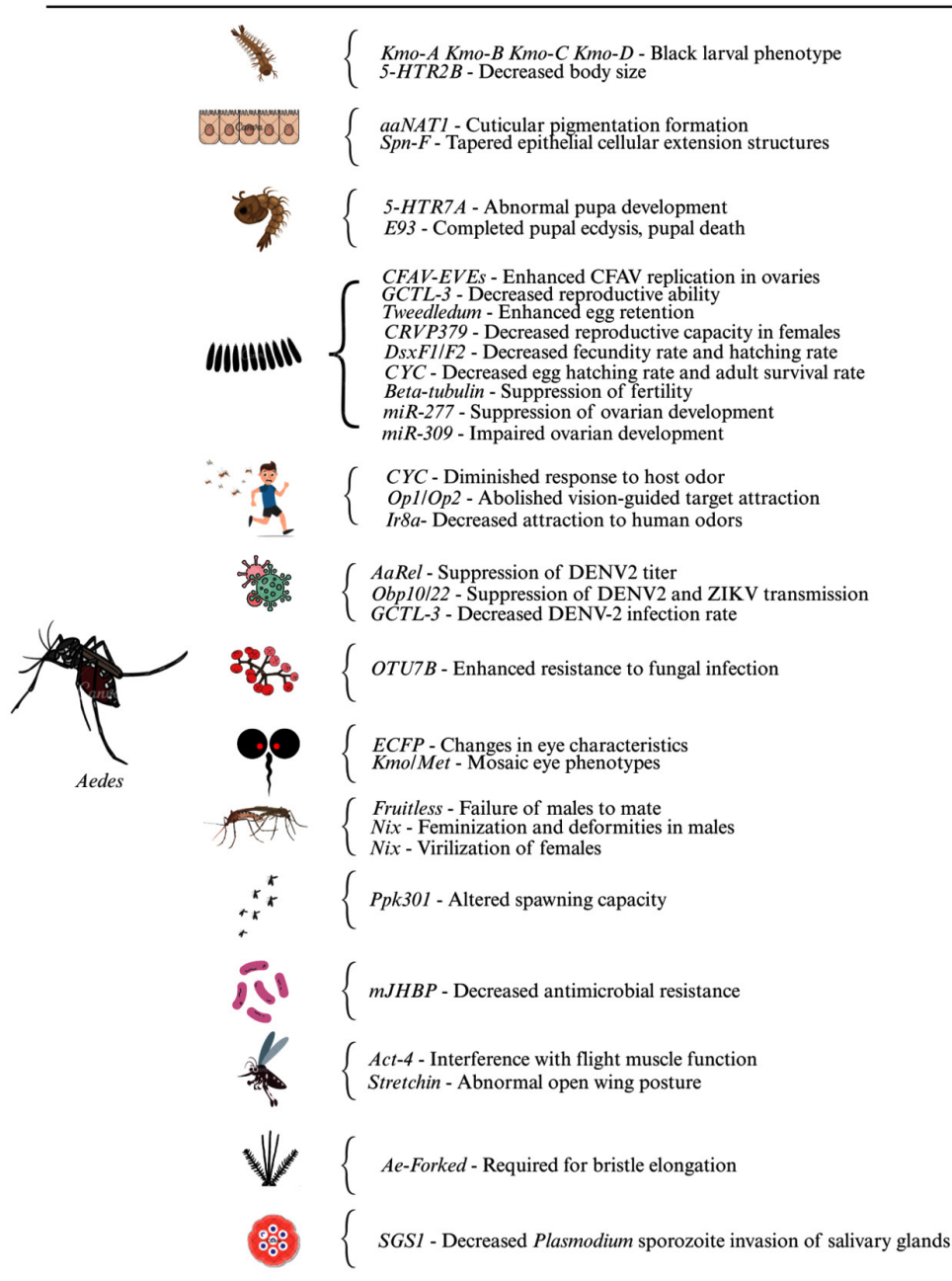
Traditional methods for controlling mosquito-borne diseases are becoming less effective due to vaccine development challenges and growing insecticide resistance. As a result, gene editing technologies like CRISPR-Cas9 are being explored as innovative alternatives. Researchers are targeting mosquito genes that influence pathogen reproduction, immune responses, and vector capacity (Figures 2 and 3). For malaria, genes like *FREP1*, *LRIM1*, and *CTL4* in *Anopheles* mosquitoes have been edited to reduce *Plasmodium* infection and transmission while also affecting mosquito development and reproduction.<sup>20-22</sup> Similar strategies have been applied to *Aedes aegypti* for arboviruses like dengue and Zika, with genes like *Obp10*, *Obp22*, *AaRel1*, and *GCTL-3* being modified to suppress virus levels or transmission.<sup>23-26</sup> Additionally, manipulating endogenous viral elements (EVEs) in mosquito genomes has shown potential in controlling viral



**Figure 2.** CRISPR/Cas9 targeted genes and their effects on *Anopheles* and *Culex* vector mosquitoes

replication through natural immune pathways.<sup>27</sup> CRISPR-Cas9 and gene drives disrupt mosquito fertility and suppress the mosquito population. In male mosquitoes, genes like  $\beta 2$ -tubulin,<sup>28,29</sup> fruitless,<sup>30</sup> and Nix<sup>31</sup> have been edited to impair fertility, disrupt mating behavior, or increase the number of male progeny. In the female mosquito,

genes such as *tweedledee* and *tweedledum*,<sup>32</sup> *CRVP379*,<sup>33</sup>  $\beta$ -Tubulin 85D,<sup>34</sup> core clock *CYC*,<sup>35</sup> *kynurense hydroxylase kh*,<sup>36</sup> *dsx*<sup>37,38</sup> were targeted to interfere with egg development and reduce fertility. Deletion of *CYP9M10* gene in *Ae. aegypti* greatly reduces resistance to pyrethroids<sup>18</sup> and mutation in the *mJHBP* gene increases mosquito



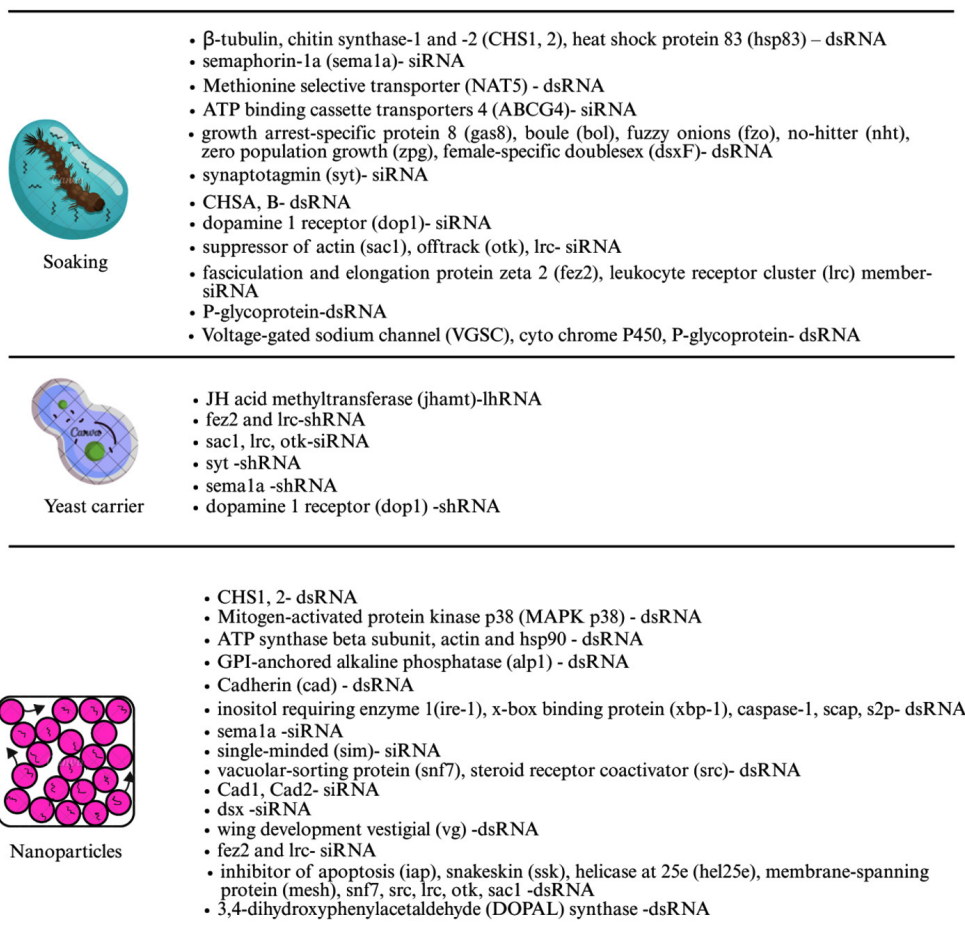
**Figure 3.** CRISPR/Cas9 targeted genes and their effects on *Aedes* vector mosquitoes

susceptibility to bacterial infection, and it leads to sepsis.<sup>39</sup> In *Culex quinquefasciatus*, the *kmo* gene, which is responsible for eye pigment, was the first target for CRISPR/Cas9-mediated gene knockouts in embryos, and it resulted in homozygous mutants with white-eye phenotypes in the following generation alone.<sup>40</sup> *Culex Hsu* cell lines were used to express Cas9 and sgRNA using *Culex*-optimized plasmid. This system well edited the immune-related targets such as *Dicer-2* and *PIWI4*, enabling the study of the antiviral gene function.<sup>41</sup> Cas9 expressing *Culex* cell lines were established to validate the immune genes such as *dicer 2*, *argonaute 2b*, *vago*, *piwi5*, *piwi6a*, and *cullin4a* roll in antiviral responses.<sup>42</sup> CRISPR-based split-gene drive system was developed to study the super-Mendelian inheritance rates in

*Cx. quinquefasciatus*.<sup>43</sup> These findings highlight the potential of gene editing in the control of mosquito-borne disease transmission.

### Ribonucleic Acid interference (RNAi)

RNAi is a well known *in vivo* technique that reduces the mRNA transcripts through post-transcriptional modification with the help of a sequence that is complementary with double-stranded RNA.<sup>44</sup> The two core proteins such as dicer and argonaut proteins, are involved in this pathway. The dicer protein is an endonuclease, which identifies the dsRNA and processes it into small RNAs. Later, the argonaut protein takes these small RNAs and searches for target complementary messenger ribonucleic acid (mRNA). In this process, the target mRNA will



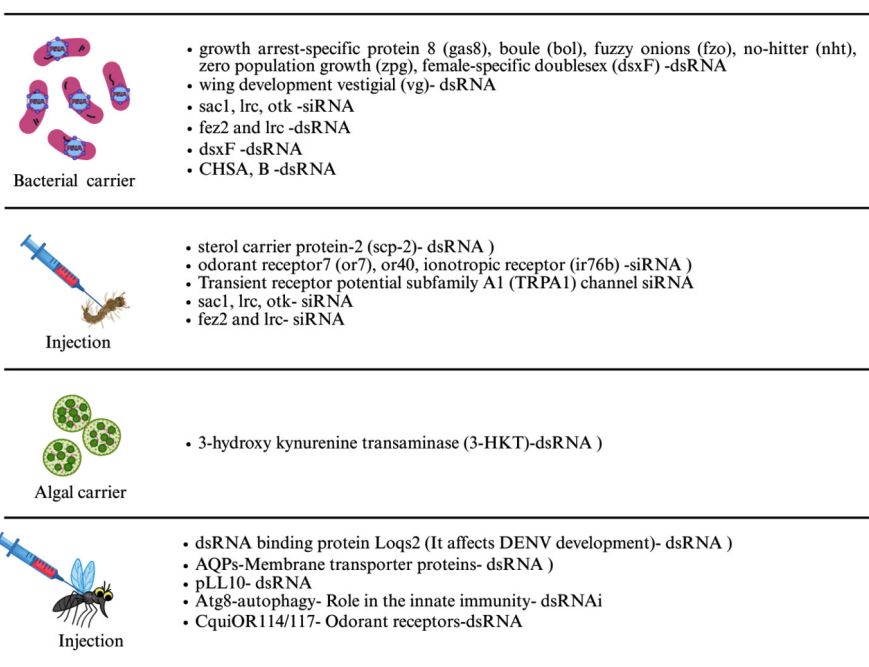
**Figure 4.** Different delivery techniques such as soaking, yeast carrier and Nanoparticles used to introduce RNAi into the host mosquito

degrade, or its translation will halt. This process is called posttranslational gene silencing.<sup>45</sup> The RNAi technique was first developed to manipulate the gene expression in the nematode, *Caenorhabditis elegans*,<sup>46</sup> later this technique was successfully used to control the agricultural insect pests by using host-induced gene silencing (HIGS), spray-induced gene silencing (SIGS), and virus-induced gene silencing (VIGS) methods.<sup>47</sup> As the RNAi technique is successfully employed in agriculture to control insect pests, researchers consider implementing this technique in mosquito control as a biopesticide. In mosquitoes, RNAi is one of the promising techniques to modify the mosquito gene expression (endogenous) and suppress the gene that encodes for pathogens *in vivo*. It disrupts the mosquito physiology by suppressing the gene required for blood feeding, reproduction, behavior pattern, longevity, and vector status, so thereby the burden of mosquito-borne diseases could be reduced.<sup>48</sup>

RNAi is a promising, species-specific method for mosquito control. By knocking down essential genes or those related to sex determination or fertility, RNAi can kill mosquitoes

or reduce their reproduction without disturbing other species.<sup>47</sup> Interfering RNAs (iRNAs), like small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and double-stranded RNAs (dsRNAs), are delivered through soaking, feeding, injection, or using nanoparticles. Oral delivery is preferred for large-scale use. RNAi can also support genetic control programs by improving sexing strategies for male-only mosquito releases.<sup>49</sup> Insects, including mosquitoes, possess the RNAi machinery, and RNAi is widely used to study the function of the gene and mosquito-pathogen interactions, mainly through adult-stage injections.<sup>50</sup> Genome sequencing of major mosquito species enabled broader RNAi applications. More recently, RNAi has been successfully applied to mosquito larvae, with gene silencing confirmed by molecular and phenotypic changes like mortality. Delivery method is crucial for RNAi success. Two main delivery categories exist: non-vector mediated (e.g., soaking, injection, nanoparticles)<sup>44</sup> and vector mediated (e.g., using bacteria, yeast, algae, and viruses)<sup>51</sup> (Figures 4 and 5).<sup>49</sup>

Injection is an effective method for delivering dsRNA into mosquitoes, especially

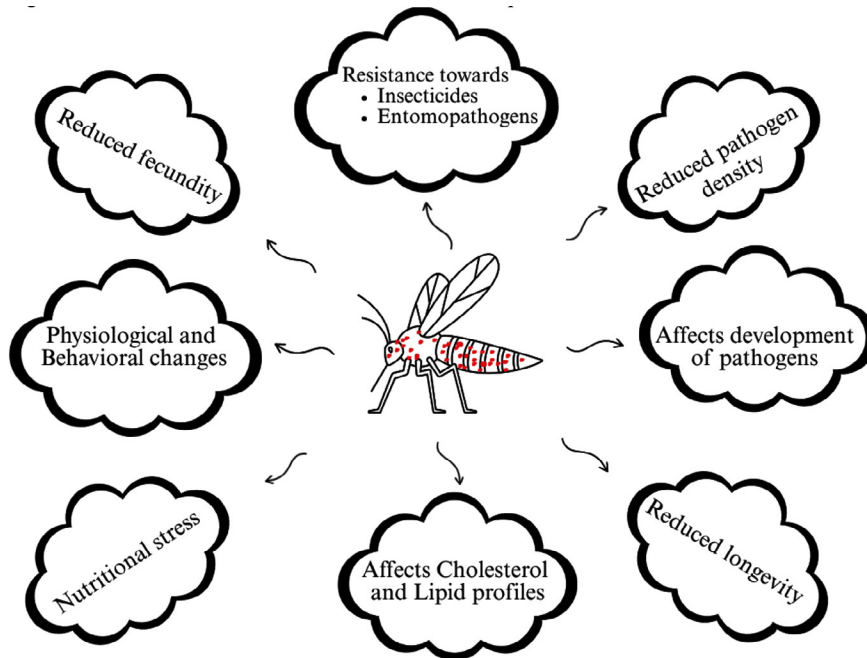


**Figure 5.** Different delivery techniques such as injection, algal and bacterial carriers used to introduce RNAi into the host mosquito

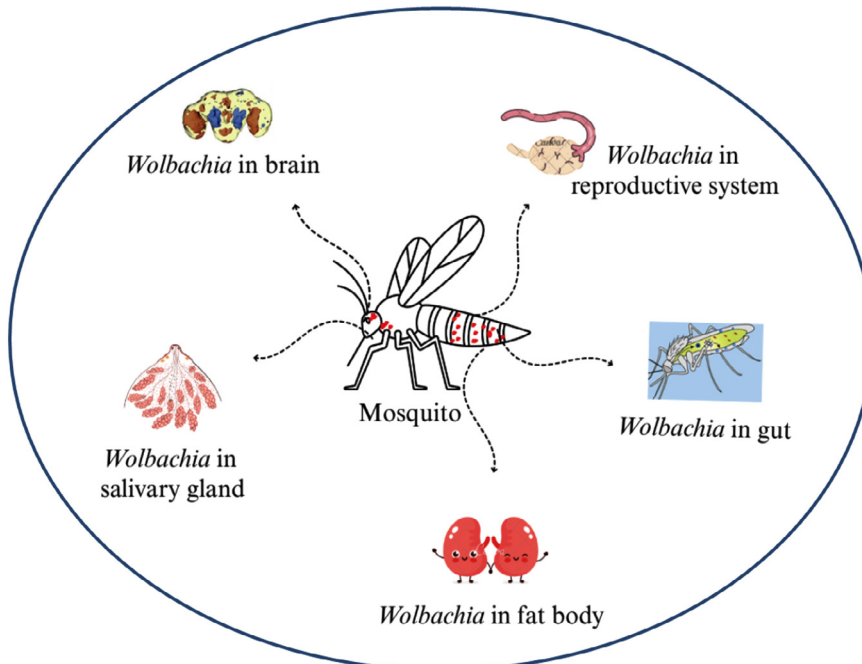


in larvae and adult stages, as it ensures direct entry into the hemocoel and bypasses barriers like the cuticle and gut.<sup>52</sup> The cold-anesthetizing mosquitoes were injected with a small quantity

(nanoliter) of dsRNA into the thorax using fine glass needles to knock down the target genes in mosquitoes. Most of the studies were conducted using adult mosquitoes to block the pathogen



**Figure 6.** Effects of *Wolbachia* infection in vector mosquito



**Figure 7.** Infection of *Wolbachia* at different body parts of the vector mosquito

replication.<sup>53</sup> Yeast has proven to be an effective RNAi delivery system and attractant for mosquito larvae and females.<sup>54-57</sup> For adult mosquito control, sugar-based baits (Attractive Targeted Sugar Baits (ATSBs)) show promise for oral delivery of RNAi,<sup>58,59</sup> while other strategies like viral vectors and symbiotic microbes are under development. RNAi could also be used to block disease transmission or alter mosquito behavior.<sup>51</sup> RNAi can cause both local and systemic effects and may influence both larvae and adults, making it a promising tool for mosquito control strategies.

### Sterile insect technique (SIT)

SIT is a safe and species-specific insect population control method that works on the systematic release of a large number of sterilized male insects into the ecosystem.<sup>60</sup> Sterile males mate with wild females, resulting in no offspring, thereby reducing the reproductive potential of the target insect. The release of sterile males in adequate quantities over a prolonged duration can result in considerable suppression or even local elimination of the targeted species.<sup>61</sup> SIT has been effectively implemented in a larger scale. During 1989, SIT was successfully employed in the United States, Mexico, Central America, and Libya to eradicate the screwworm fly *Cochliomyia hominivorax*.<sup>62</sup> Some other insect populations that were reduced using SIT technology were the *Ceratitis capitata*,<sup>63</sup> *Pectinophora gossypiella*,<sup>64</sup> and *Cydia pomonella*<sup>65</sup> from America, Africa, Europe, and Asia. The magnitude of these operations can be considerable; for instance, the El Pino facility in Guatemala generates approximately two billion sterile male *C. capitata* weekly, which is roughly equivalent to 20 tons, primarily for deployment in California and Central America.<sup>60</sup> SIT has demonstrated itself to be a cost-effective method to suppress or eradicate the insect populations.

The success of SIT encouraged researchers to implement the technique in vector control programs to reduce or to replace the mosquito populations. The preliminary study using SIT against mosquitoes was initiated during 1959 on the genera *Culex*, *Anopheles*, and *Aedes*. The trails were impeded due to lack of information on vector biology and ecology.<sup>66</sup> Sterility in mosquitoes was induced by chemicals, due to difficulty in adjusting the chemical concentration in the sterility process,

later it was replaced with irradiation.<sup>67</sup> Repeated SIT attempts improved the technique and marked it as a standard method for vector control.<sup>68</sup> SIT, when applied in urban settings against pupae and adult mosquitoes of *Aedes albopictus*, has achieved significant outcomes, inducing 70%-80% sterility in field populations.<sup>69</sup> A large number of irradiated males need to be released into the surrounding area to facilitate a 10:1 or 5:1 ratio of sterile:wild mosquitoes to compete with wild males in mating with wild females.<sup>70</sup> A behavioral and survival study on sterile *Ae. aegypti* mosquitoes revealed reduced flying distance and decreased survival time from 54 days to 27 days when the mosquitoes were sterile with 90 gray.<sup>71,72</sup>

SIT is highly species-specific, it lowers the population of the target mosquito species alone, which reduces the population or eradicates it overtime. With the success of SIT in vector control, the World Health Organization/Special Programme for Research and Training in Tropical Diseases (WHO/TDR) and the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (FAO/IAEA) released the guidelines for pilot testing of SIT. Pilot studies against *Ae. aegypti* and *Ae. albopictus* are in progress in Brazil, Cuba, Malaysia, Mexico, the USA, Thailand, Singapore, France, Germany, Greece, Italy, Mauritius, and Spain, respectively.<sup>73,74</sup>

### Wolbachia

*Wolbachia* is often isolated from mosquito gut; it is an obligate, Gram-negative gut parasitic bacteria. It was first isolated from gut of *Cx. pipiens* during 1924 by Hertig, later the bacteria was named *Wolbachia pipientis*.<sup>75</sup> Over the past two decades, several *Wolbachia* strains have been isolated from mosquitoes. *Wolbachia* forms endosymbiotic relationships with mosquitoes, ranging from parasitism to mutualism. As a parasite, *Wolbachia* interferes in mosquito physiology, immunity, and mosquito development, and it also reduces the reproductive ability and longevity of the mosquito, but its mutualism increases mosquito resistance towards viral infection.<sup>76,77</sup> *Wolbachia* transmits vertically through eggs into the next generation, and it transmits horizontally by infection. Horizontal transmission was frequently recorded in *Aedes* and *Culex* mosquitoes but not reported from



*Anopheles* and *Ae. aegypti*.<sup>78</sup> Physiological and behavioral changes have been reported from *Wolbachia*-infected *Culex*, it alters the host temperature preference, for instance, *Culex* infected with supergroup A *Wolbachia* prefers cold temperature, while supergroup B-infected mosquitoes prefer warm.<sup>79</sup> *Wolbachia* increases the insecticidal resistance in mosquitoes, field-collected mosquitoes infected with high-density *Wolbachia* showed higher resistance towards insecticides compared to infection with lower density *Wolbachia*. Similarly, *wPipSJ*-infected *Cx. quinquefasciatus* showed resistance towards infection of entomopathogenic bacteria (Figure 6).<sup>80,81</sup>

The *Wolbachia* technique has become a promising, alternative method to control mosquito-borne diseases, particularly in *Aedes*. It was found that infected female mosquitoes can mate with uninfected males and give rise to *Wolbachia*-infected offspring.<sup>82</sup> In contrast, infected male mosquitoes mating with uninfected females give rise to the production of non-viable eggs.<sup>83</sup> *Wolbachia* colonizes in the midgut, fat body, brain, and salivary gland of mosquito, but more prevalence was found in the reproductive tissues of the mosquito (Figure 7).<sup>84</sup> Mosquito host-seeking behavior and oviposition would affect when *Wolbachia* infects the mosquitoes brain.<sup>85</sup> Caragata *et al.* reported that *Wolbachia* brings nutritional stress to the mosquitoes, and it alters the cholesterol and lipid profiles, which increases the nutritional competition between *Wolbachia* and other pathogens as a result, it shortens the mosquito longevity (Figure 6).<sup>86</sup>

In *Anopheles*, it was first isolated from reproductive tissue of field-collected *Anopheles gambiae*, later from *An. funestus*, followed by *An. stephensi*.<sup>87-89</sup> It was demonstrated that *Wolbachia* colonizes throughout the body parts of the mosquitoes, and it affects the development of the *Plasmodium* parasite inside the mosquito. *Wolbachia* showed varied effects on malaria parasites depending on their species, *P. berghei* oocyst density was increased in the midgut of *An. gambiae* infected with the *Wolbachia* strain *wAlbB*, while oocyst development was suppressed when mosquitoes were infected with the *wMelPop* strain.<sup>90</sup> *wAlbB* reduced fecundity and male mating ability in *An. stephensi*, and it decreased parasite

density.<sup>91</sup> Reduced *P. falciparum* sporozoite was found in field-collected *wAnga*-infected *An. coluzzii*, and it was evidently reported that *Wolbachia* infection decreases the mosquito longevity and provides resistance to *Anopheles* mosquitoes against pathogen infection.<sup>92</sup>

## CONCLUSION

The conventional, insecticide-mediated vector control strategies are unable to control vector-borne disease due to their inefficiency and increased resistance in the vector mosquitoes. A number of advanced techniques have proven potential, and they may become an effective intervention in reducing the disease transmission. These advanced techniques either modify the mosquito population or suppress them. *Wolbachia*, SIT, RIDL, and IIT are the techniques that have been tested at the field level, and most of these techniques do not require reapplication; therefore, they reduce the application cost, and they are also environmentally safe. These techniques have significant potential in controlling the mosquito population and limiting their vector capability, making them potential alternatives to conventional methods. The current research on these techniques has become vital in identifying new techniques that would be used effectively in controlling the disease transmission. However, the implementation of these techniques requires a thorough understanding of interactions between mosquitoes, pathogens, and the environment, and the evaluation of the associated risks and benefits should also be monitored.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

**ETHICS STATEMENT**

Not applicable.

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