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Biotechnological advances in RNA interference for mosquito control: Delivery platforms, gene targets, and field prospects (2015–2025)

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ABSTRACT

Mosquito-borne diseases, such as malaria, dengue, and chikungunya, persist globally due to the emergence of resistance to major classes of insecticides among mosquitoes. This resistance, alongside the need to reduce pesticide overuse, necessitates the development of alternative vector control strategies. RNA interference (RNAi) is a vector control method that acts by silencing specific genes vital for the development, reproduction, survival, and pathogen transmission of disease vectors. This review evaluates existing RNAi studies for vector control, focusing on its application, delivery methods, effectiveness, challenges, and future directions. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines were used to retrieve research articles from databases. The results indicate RNAi's potential in silencing key genes in the mosquitoes' lifecycle, immunity, fecundity, and survival. For instance, RNAi silenced genes crucial for malaria parasite development in *Anopheles gambiae* and reduced *Aedes aegypti's* susceptibility to the dengue virus. Various delivery methods, including microinjection, soaking, oral, and transgenic approaches, were employed, each with pros and cons for large-scale use. RNAi is a potentially powerful alternative vector control tool. However, further advancement is required for the proper delivery of interfering RNA species, cost-effectiveness, and field application.

1. INTRODUCTION

Diseases caused by mosquitoes remain a significant public health concern. Approximately 17% of infectious diseases are attributed to mosquito-borne infections. According to the World Health Organization (WHO) [1], around 700,000 deaths worldwide are caused by mosquito-borne pathogens. Malaria is a major disease, particularly in tropical and subtropical regions. It is caused by *Plasmodium* parasites that are transmitted through the bite of female *Anopheles* mosquitoes; specifically, *Anopheles gambiae* and *Anopheles funestus* species in Africa [2,3]. Current prevention strategies have not reduced the number of cases of malaria, dengue, yellow fever, Zika virus, and chikungunya, particularly with malaria alone reported to have over 249 million cases in 2023 [4]. Several diseases resurged after being eliminated in the post-COVID-19 era, which shows how urgently we need to develop effective and sustainable control strategies [5]. Mosquitoes belonging to the genera *Anopheles*, *Aedes*, and *Culex* carry parasites and viruses such as *Plasmodium*, DENV, CHIKV, Zika, and West Nile Virus [6,7]. The development of resistance to insecticides has reduced the efficacy of traditional methods of control, such as insecticide-treated nets, larval source management, and indoor residual spraying [8,9]. For example, in sub-Saharan Africa, the three leading agents of malaria (*A. gambiae*, *Anopheles arabiensis*, and *A. funestus*) are now acquiring behavioral changes and resistance to insecticides. These patterns are indicators of the need to identify novel control agents that can replace or supplement existing ones. Several genetic approaches, such as vector population replacement and transmission-blocking, are being explored [10-12]. In addition, lethal genes or microbial agents are introduced into mosquito populations as potential vector control tools [13,14].

RNAi serves effectively in the controlled silencing of genes in disease vectors. Figure 1 illustrates its pathway. It involves the post-transcriptional degradation of target messenger RNA (mRNA) by double-stranded RNA (dsRNA) molecules. This process was first demonstrated in the nematode, *Caenorhabditis elegans* [15,16]. Two core proteins are involved: Dicer, which processes dsRNA into small interfering RNAs (siRNAs), and Argonaute (Ago), which guides siRNAs to complementary mRNA for degradation [17]. In mosquitoes, RNAi has successfully silenced genes essential for survival, reproduction, development, and vectorial capacity. This offers an avenue for controlling mosquito populations or disabling them as disease carriers [8,18]. siRNAs capable of causing effective lethality at both larval and adult stages have been identified, showing high species specificity and minimal risk to non-target organisms [19,20].

RNAi technology has gained attention in both laboratory research and agricultural pest control. Its potential as a species-specific,

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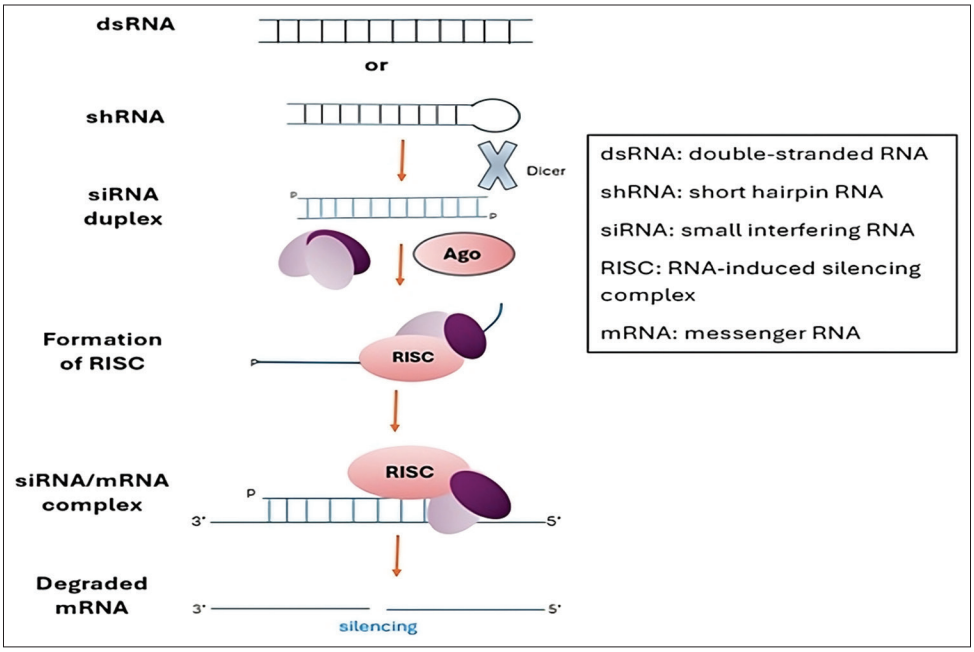

Figure 1: Illustration of the RNA interference pathway.

environmentally safe bioinsecticide is being explored in vector biology [8,21,22]. While CRISPR/Cas9 represents an advanced genome-editing tool, [23] RNAi presents a more immediate and non-transgenic method for gene function studies and population suppression in mosquitoes. Reviews by Balakrishna Pillai *et al*. [18] and Munawar *et al*. [24] outline basic RNAi applications through mosquito developmental stages, emphasizing genes controlling insecticide resistance and vector-pathogen interactions.

In this review, we critically assess the application of RNAi for controlling mosquito vectors. We emphasized how it is used to silence genes regulating vital physiological and behavioral traits related to reproduction, development, or insecticide resistance. We explore delivery methods, study designs, and the observed impacts on mosquito survival, fecundity, and vectorial capacity. Furthermore, we discuss emerging synthetic biology tools such as CRISPR interference (CRISPRi) and RNAi nanocarriers, which are now integral to vector control biotechnology. Finally, we identify research gaps and challenges, including those limiting real-world applications, and highlight candidate gene targets for future RNAi-based biopesticide control agents.

2. METHODS

This systematic review was registered on PROSPERO with registration ID, CRD420251109160. The primary databases used for this systematic review search included PubMed and Web of Science. Some articles were obtained from the reference lists of articles retrieved through the database search, followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines for writing a systematic review. The step-by-step processes followed to identify the included studies are illustrated in Figure 2.

2.1. Search Strategy

The electronic retrieval method was used for the literature search, and the search was performed using each database's advanced search tool with relevant terms such as "RNA interference," "gene silencing," "mosquito," and "vector control." Specific search strings were used for each database, including:

PubMed: ("RNA interference" OR RNAi OR "gene silencing" OR "dsRNA" OR "siRNA") AND (*Anopheles* OR *Aedes* OR mosquito*) AND (malaria OR dengue) AND ("vector control" OR "disease transmission" OR "malaria control" OR "dengue control" OR "arbovirus control")

Web of Science: TS = ("RNA interference" OR RNAi OR "gene silencing" OR dsRNA OR siRNA) AND TS = ("*Anopheles gambiae*" OR "*Aedes aegypti*" OR mosquito*) AND TS = ("vector control" OR "disease control").

2.2. Eligibility Criteria

Criteria type	Inclusion	Exclusion
Study design	Original research and open-access articles	Reviews, editorials, opinion pieces, or letters
Population	Studies on mosquitoes	Studies focused on other insects
Intervention	The use of RNA interference	Other methods
Outcome	Biological effects, gene silencing success, and vector population reduction	Studies without biological effects
Timeframe	Studies published from 2015 to 2025	Studies earlier than 2015

2.3. Study Selection and Data Extraction

The search results were uploaded into the Microsoft Excel 2024 program for proper documentation and screening. From each of the studies, two authors (POJ and TIB) extracted the following information in a tabular form: Mosquito species used, target genes, methods of interfering RNA delivery, the developmental stage of the mosquito, the study design, and the major observed outcomes. Discrepancies were resolved by either reaching a consensus or involving a third author

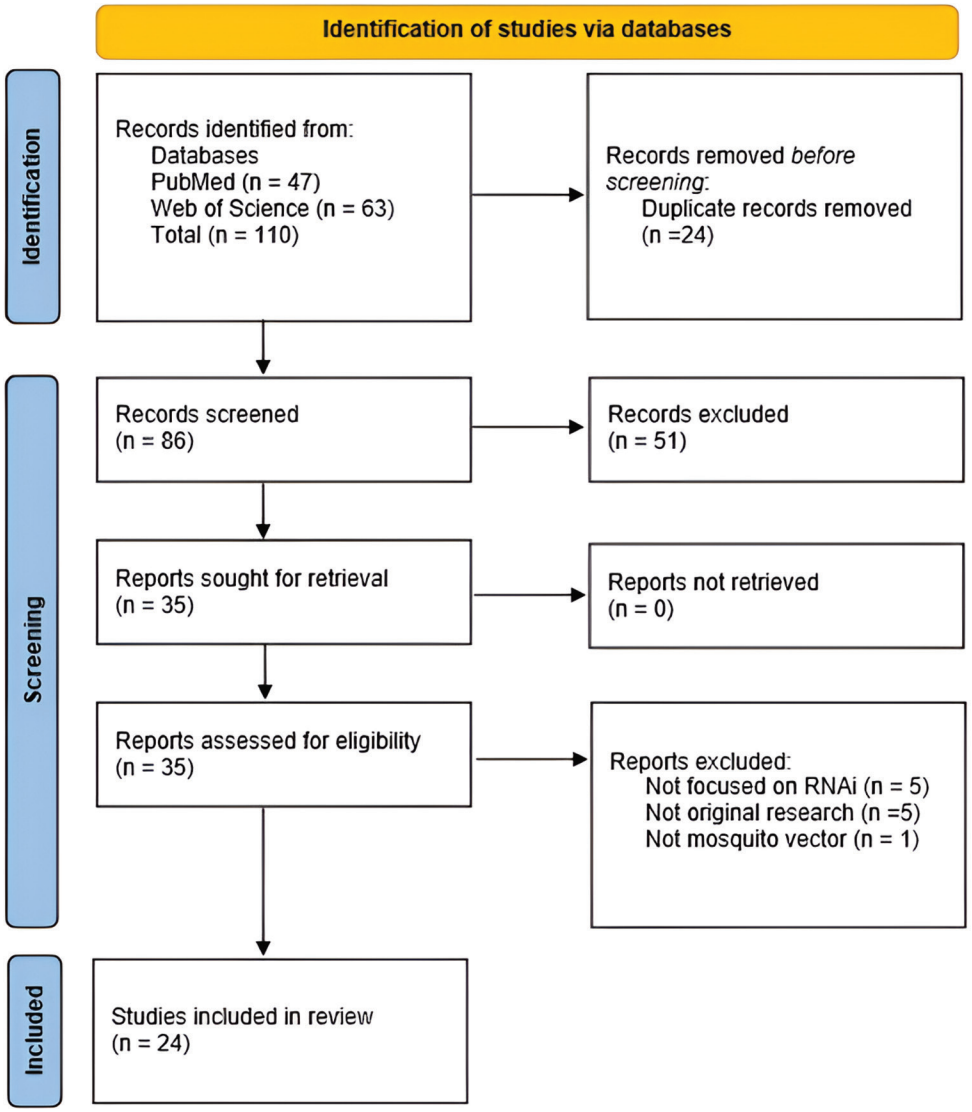

Figure 2: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the study selection process.

(AHA). After completing the data extraction, the authors performed a comprehensive analysis of each study, noting their successes and limitations as well as highlighting areas for future research.

2.4. Risk of Bias in the Review Process

Risk of bias in the review process was assessed using the Risk of Bias in Systematic Reviews (ROBIS) tool. The overall risk was judged to be moderate [Table 1], primarily due to the lack of formal appraisal of each included studies. No formal appraisal of individual studies was done because they varied widely in design, mosquito species, and observed outcomes, ranging from laboratory-based experiments to semi-field trials. However, ROBIS allowed us to transparently assess the overall review process. Eligibility criteria, selection strategy, and data extraction were clear and consistently applied.

Table 1: Summary of ROBIS assessment.

Domain	Risk	Reason
Eligibility and selection	Low	Clear criteria and dual screening
Data handling	Moderate	No individual study bias appraisal was conducted, but an overall study bias appraisal was conducted
Synthesis	Low	Confidence grading applied
Overall	Moderate	Transparent process, overall appraisal was conducted

3. RESULTS

A total of 110 articles were retrieved from the initial search. After removing duplicates and screening titles and abstracts, 86 articles remained [Figure 2]. Next, articles were screened by reading full texts, and 51 articles did not meet the inclusion criteria. Additional screening was conducted to ensure the studies focused on mosquito species, had a target gene, and used clear methods that could be easily replicated. Eleven articles were excluded, leaving a total of 24 articles. These 24 articles met all the inclusion criteria and were included in this study.

The current studies covered different mosquito species, namely *Aedes aegypti*, *A. gambiae*, *Aedes albopictus*, *Culex quinquefasciatus*, *A. arabiensis*, *A. funestus*, *Anopheles stephensi,* and *Culex pipiens pallens*. Several of the studies assessed the application of RNAi technology on multiple species simultaneously [20,25,26]. Each of the studies targeted one or more genes responsible for physiological

and neurological functioning, reproductive regulation, and overall survival of mosquitoes. For instance, Mysore *et al*. [25], Mysore *et al*. [20], and Mysore *et al*. [26] targeted *Rbfox1*, *Shaker,* and *Sema1a* genes, respectively, which are known to be associated with neural development in mosquitoes. Other genes that were targeted in the studies include genes involved in reproductive regulation (*Vg-2*, *EcR*, *dsx*) [27-29], and detoxification mechanisms such as cytochrome P450s [30-32]. Additional targets included midgut and chitin synthesis-related genes [33,34], and immune modulators [35,36].

3.1. Methods of Interfering RNA (iRNA) Delivery

The most common method for delivering iRNA in many studies was microinjection, due to its accuracy and effectiveness. However, it had some limitations. Oral delivery method was the second most employed delivery system, used in studies by Mysore *et al*. [26], Prates *et al*. [37], Fei *et al*. [38], among others. This method has been proven to be more suitable for field application but faces challenges of RNA instability, degradation, and variability in uptake by mosquito

Table 2: RNAi studies targeting the larval stage of mosquitoes.

Mosquito species	Target gene (s)	Gene function	Delivery method	Study type	Main effects observed	Reference
A. aegypti, A. gambiae, A. albopictus, and Culex quinquefasciatus	*Shaker*	Neuronal potassium channel for neural signaling	Microinjection, ATSB, *S. cerevisiae* (baker's yeast)	Laboratory and Semi-field	- Severe neural and behavioral defects and high levels of adult mortality - High larval mortality	[20]
A. aegypti, *A. albopictus*, *A. gambiae,* and *C. quinquefasciatus.*	*Sema-1a*	Neural development and axon guidance proteins	Oral: yeast expressing shRNA	Laboratory, Semi-field, and simulated field trials	90–100% larval mortality	[26]
A. aegypti, *A. albopictus*	*Sem-1a*, *fasciculation and elongation protein zeta2*, and *leukocyte receptor cluster member 8 homolog, beta-tubulin*	Sex differentiation, neural development, gut RNases	Oral, Soaking	Laboratory experimental study	Minimal knockdown, no significantly higher larval death compared with the control	[37]
A. aegypti	Chitin synthase A and B	Chitin synthesis on exoskeleton/midgut	Oral: *E. coli* lysate expressing dsRNA	Laboratory experimental study	Larval mortality, deformities	[33]
A. aegypti	*3-hydroxykynurenine transaminase*	Tryptophan metabolism and redox balance	Oral: transgenic Chlamydomonas (microalgae)	Laboratory and semi-field trial	High larval mortality	[38]
A. stephensi	*ABCG4*	ABC transporter detoxification	Soaking	Laboratory experimental study	Increased permethrin susceptibility	[39]
A. albopictus	*CHS-2*	Midgut chitin synthesis and peritrophic membrane integrity	Microinjection	Laboratory experimental study	- Peritrophic membrane disruption - No larval mortality	[34]
A. gambiae	Female doublesex (*AgdsxF)*	Sex determination (female-specific)	Oral: *E. coli* bacteria	Laboratory experimental study	Reduced female emergence by > 66%	[29]
C. quinquefasciatus	*CYP325BC1, CYP9M12*	Cytochrome P450s involved in insecticide detoxification	Microinjection	Laboratory experimental study	Increased malathion susceptibility	[30]
A. gambiae	*Maf-S*	Transcription factor for detoxification enzymes	Microinjection	Laboratory experimental study	Increased insecticide susceptibility	[31]
A. aegypti, *A. gambiae*	Various neural and developmental genes (e.g., *Sac1, lrc, otk*)	Neural and developmental genes	Oral delivery of shRNA via genetically engineered *S. cerevisiae* yeast tablets	Laboratory experimental study	Effective gene silencing and high larval mortality in both *A. aegypti* and *A. gambiae*	[42]
A. aegypti, *A. albopictus*, *A. gambiae*, *C. quinquefasciatus*	*Rbfox1*	RNA-binding protein involved in neural development	Yeast ATSB, soaking	Laboratory and simulated semi-field studies	- *Rbfox1* silencing induced high mortality in both larvae and adult mosquitoes (up to 93%) - Broad activity against multiple mosquito species	[25]

Sema-1a: *Semaphorin-1a*, RNAi: RNA interference, *A. aegypti*: *Aedes aegypti*, *A. albopictus*: *Aedes albopictus, A. gambiae*: *Anopheles gambiae*, *C. quinquefasciatus*: *Culex quinquefasciatus*, *S. cerevisiae: Saccharomyces cerevisiae*, dsRNA: double-stranded RNA, *E. coli*: *Escherichia coli*, ATSB: Attractive targeted sugar bait

larvae or adults [20,37,39]. Soaking-based delivery was used in a few studies involving mosquito pupae or larvae, with varying success. For example, Prates *et al*. [37] observed minimal larval knockdown, which did not significantly increase larval death compared with controls. These studies highlighted that oral, soaked, and injected RNAi treatments largely failed to replicate previous reports of strong gene knockdown or mortality. In addition, Arshad *et al*. [40] found that knockdown efficiency in pupae was variable and that this technique

Table 3: RNAi studies targeting the pupal stage of mosquitoes.

Mosquito species	Target gene (s)	Gene function	Delivery method	Study type	Main effects observed	Reference
Aedes aegypti	*CYP4G35*	Cuticular hydrocarbon biosynthesis and desiccation resistance	Soaking	Laboratory experimental study	No significant pupal mortality when compared with the control	[40]

RNAi: RNA interference

Table 4: RNAi studies targeting the adult stage of mosquitoes.

Mosquito species	Target gene (s)	Gene function	Delivery method	Study type	Main effects observed	Reference
A. aegypti	*Xanthine Dehydrogenase 1*	Nitrogen metabolism	Microinjection	Laboratory experimental study	Decreased fecundity and increased mortality	[44]
A. aegypti	Zika virus (NS3/4A region)	Viral protein – immune target	CRISPR/Cas9 transgenesis with inverted repeat RNA	Laboratory experimental study	Engineered mosquitoes showed~90% resistance to ZIKV	[41]
A. albopictus	Vitellogenin 2	Vitellogenin: Egg development. Associated with vitellogenesis and linked to host-seeking behavior	Oral feeding, Microinjection	Laboratory experimental study	Altered host-seeking behavior	[27]
A. aegypti	*Dcr2, R2d2*	RNAi machinery for antiviral defense	Transgenic overexpression using the midgut-specific AeCpA promoter	Laboratory experimental study	Increased immunity, decreased virus susceptibility	[36]
A. gambiae	Arginase, *Elf1, Elf2, HSP*	Immunity and stress response proteins	Microinjection	Computational and Experimental studies	- Elf2 and HSP knockdown reduced survival - Arginase knockdown reduced *Plasmodium* infection	[43]
A. funestus	*EcR*	Ecdysone receptor for oogenesis and longevity	Nano injection	Laboratory experimental study	*EcR* knockdown decreased lifespan, impaired oogenesis, and reduced fertility	[28]
A. aegypti, *A. albopictus*, *A. gambiae*, *C. quinquefasciatus*	*Rbfox1*	RNA-binding protein involved in neural development	Yeast ATSB, soaking	Laboratory and simulated semi-field studies	- *Rbfox1* silencing induced high mortality in both larvae and adult mosquitoes (up to 93%) - Broad activity against multiple mosquito species	[25]
A. arabiensis	*FN3D1*, *GPRGr9*	Immune/gut homeostasis regulators	Microinjection	Field-linked Laboratory study	Decreased longevity (reversed by antibiotics)	[35]
C. pipiens pallens	*miR-4448* and its target *CYP4H31*	MicroRNA-regulated detoxification enzyme CYP4H31, involved in metabolic detoxification	Oral, Microinjection	Laboratory experimental study	Increased deltamethrin susceptibility	[32]
A. aegypti	Dyspepsia (*SLC16*)	Solute carrier in iron metabolism	Microinjection	Laboratory experimental study	Decreased fecundity, impaired digestion	[45]
A. arabiensis	*Akirin*	- Transcription cofactor in innate immunity - Embryonic development	Microinjection	Laboratory experimental studies	Reduced longevity, fecundity, and fertility	[46]
A. gambiae	*A. gambiae* aquaglyceroporin 3	Transports water, glycerol, and urea for the survival of *Anopheles* and the development of *Plasmodium* in the mosquito	Microinjection	Laboratory experimental studies	Reduced survival at 39°C, reduced *P. falciparum* oocysts development	[47]

RNAi: RNA interference, *EcR*: Ecdysone receptor, *A. aegypti*: *Aedes aegypti*, *A. albopictus*: *Aedes albopictus, A. gambiae*: *Anopheles gambiae*, *A. funestus*: *Anopheles funestus*, *C. quinquefasciatus*: *Culex quinquefasciatus*, *A. arabiensis*: *Anopheles arabiensis*, *C. pipiens pallens*: *Culex pipiens pallens*, *P. falciparum*: *Plasmodium falciparum*

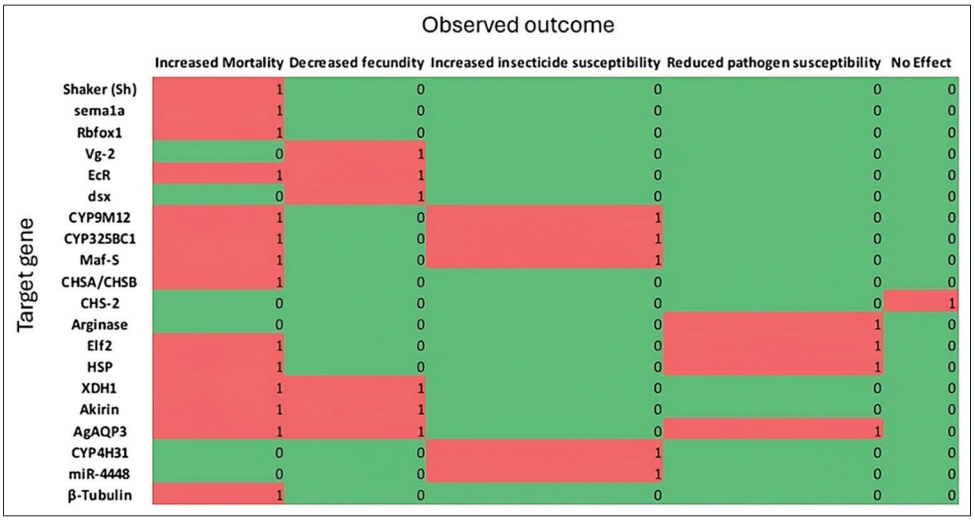

Figure 3: Frequency of reported outcomes for RNA interference–targeted genes across studies.

Table 5: Certainty of evidence grading.

Intervention	Overall confidence	Reason
RNAi via microinjection	Moderate	Consistent effects in laboratory settings
RNAi via oral delivery	Moderate	Low uptake and inconsistent outcomes
RNAi targeting neural genes	High	Strong and consistent mortality results
RNAi targeting detoxification enzyme genes	High	Good and consistent mortality results

RNAi: RNA interference

might be ineffective in older pupae compared with its success in larvae. Transgenic expression systems using microorganisms such as *Escherichia coli* [29,33], *Saccharomyces cerevisiae* [20,25,26], and microalgal [38] to express dsRNA, along with attractive targeted sugar bait (ATSB) formulations, were used in a limited number of studies [25,41], showing high efficacy but requiring further field validation and regulatory evaluation.

3.2. RNAi Gene Targeting by Mosquito Developmental Stage

3.2.1. Larval stage

The larval stage was the most frequently targeted, with interventions focused on inducing mortality, disrupting development, or sensitizing larvae to insecticides. Gene silencing of *Shaker*, *sema1a*, and *Sac1* led to high mortality due to neural dysfunction [20,26,42]. Chitin synthesis-related genes were targeted to impair exoskeletal or peritrophic membrane formation, leading to deformities or digestive defects [33,34]. Genes involved in insecticide detoxification were suppressed to restore insecticide susceptibility [30,31,39] [Table 2]. Multiple gene approaches using microbial carriers or nanoparticles also yielded up to 100% larval mortality in some studies [38]. The methods of delivery of iRNA species used on the larval stage were mostly oral (yeast or bacteria), soaking, or microinjection.

3.2.2. Pupal stage

Only one study focused on the pupal stage, mainly using the soaking method. Arshad *et al.* [40] showed that soaking newly molted pupae in dsRNA targeting the *CYP4G35* gene caused a lasting knockdown into adulthood. However, the effectiveness of the knockdown depended on the sex of the mosquitoes, with females showing a range of 60–99% and males 79–98%, and the effect being short-lived. It was also observed that pupal mortality was not significantly different from mortality in the control group [Table 3].

3.2.3. Adult stage

Several studies targeted adult mosquitoes, focusing on reducing reproduction, suppressing pathogen transmission, or shortening lifespan [Table 4]. For example, silencing *Vg-2*, *EcR*, and *dsx* genes led to reduced fecundity, oviposition, or mating success [27-29]. Knockdown of immune-related genes such as *HSP*, *Elf2*, arginase, and *FN3D1* impaired survival or reduced mosquitoes' susceptibility to *Plasmodium* [35,43]. Transgenic overexpression of *Dcr2* and *R2d2* enhanced antiviral defense against dengue and Zika viruses [36], while neural knockdown of *Rbfox1* caused mortality in both larval and adult mosquitoes [25].

3.3. Efficacy of Gene Silencing

High mortality was reported in RNAi treatments targeting neural [20,26] and detoxification genes [30,31], with several studies noting increased susceptibility to insecticides following gene knockdown [32,39]. In reproductive targets, outcomes included reduced egg production and shortened lifespan [28,44]. Transgenic overexpression of RNAi components [36] or the use of symbiotic microbes [33,38] showed enhanced pathogen resistance and larvicidal effects, respectively. However, variable RNAi efficiency was a commonly noted limitation [34,37]. Figure 3 is a frequency plot showing the observed effects after silencing specific genes in mosquitoes. The red color indicates a positive outcome for that feature, whereas the green color means the effect was absent.

Most of the studies included in this review were conducted entirely under laboratory conditions, with only four studies involving semi-field trials [20,25,26,38], and one including a computational study [43].

3.4. Certainty of Evidence Assessment

Evidence certainty was assessed using a simplified GRADE approach. Confidence in each intervention was based on risk of bias, consistency,

Table 6: Comparative characteristics of microbial RNAi delivery platforms for mosquito control.

Intervention	RNAi expression	Cost	Biological safety	Stability	Field deployability	Reference
Chlamydomonas reinhardtii	Moderate	High (potentially due to alga culture)	Limited biosafety evaluation	Moderate (light sensitivity)	Limited due to aquatic delivery	[48]
Escherichia coli	High	Low	Safety concern exists	Moderate due to sensitivity to ultraviolet and temperature	Moderate to low, as it requires a cold chain	[49]
Saccharomyces cerevisiae	Moderate	Moderate	Generally regarded as safe	High	High (wide mode of delivery)	[50]

RNAi: RNA interference

directness, precision, and publication bias. Table 5 summarizes the overall confidence levels for selected interventions.

4. DISCUSSION

RNAi is an innovative approach in the field of functional genomics research and has been used to control pests by silencing specific genes. When genes vital to the survival of mosquitoes are silenced, it can lead to increased mortality of the mosquito species. Researchers have employed RNAi to suppress mosquito genes to determine the impact of gene silencing on the mosquitoes [7]. In this review, we examined RNAi as an emerging technology with potential applications as a novel vector control agent. From the studies, it can be deduced that RNAi can be applied to disrupt various physiological processes within mosquito vectors. Laboratory experimental analyses were used by most of the studies to silence genes responsible for immune regulation, neural function, xenobiotic detoxification, and reproduction. Silencing of these genes compromised mosquito survival, reduced fecundity, lowered susceptibility to pathogen infection, impaired pathogen development, and increased insecticide susceptibility [8,18,22]. From the studies, the strongest impact of silencing targeted genes in various mosquito species was increased mortality. Silencing the *AgAQP3* gene resulted in increased mortality, decreased fecundity, and reduced pathogen susceptibility [Figure 3], suggesting this gene as a good target for future RNAi interventions.

The majority of the studies analyzed showed success in targeting specific genes across the three developmental stages of mosquitoes. Research on neural genes, particularly *Shaker*, *sema1a*, and *Rbfox1*, showed that silencing them induced mortality in both larvae and adult mosquitoes [Figure 3], demonstrating their important roles in mosquito survival [20,25,26,42]. Similarly, cytochrome P450 genes, involved in insecticide detoxification, such as *CYP9M12*, *CYP325BC1*, and the transcription factor *Maf-S*, were effectively silenced using interfering RNA. This process ultimately resulted in increased insecticide susceptibility [30,31]. In addition to the identified gene targets, reproductive and immune regulatory genes such as *Vg-2*, *EcR*, *dsx*, *Elf2*, and *HSP* were also targeted by interfering RNA, resulting in reduced fecundity, impaired oogenesis, decreased pathogen susceptibility, and increased mortality in mosquito vectors [27,28,35,43,44]. These studies have revealed that RNAi can be applied not only to impair mosquito development but also to decrease their chances of being infected by various pathogens.

4.1. Interfering RNA Delivery Methods

The most widely used method of dsRNA delivery remains the microinjection method, which exhibits excellent precision and consistency. However, this technique has several drawbacks, such as the requirement for skilled personnel and notable differences in injection efficiency across species. Variations in key factors such as needle choice, injection site, optimal volume, dsRNA concentration, and amount supplied can influence the final outcome [18]. In addition, this method is laborious and limited in field applications. Next was the oral administration approach, often involving genetically engineered microorganisms such as *S. cerevisiae* (yeast), *E. coli* (bacteria), and *Chlamydomonas* (microalgae) as delivery agents. The outcome of this method yielded mixed results. Studies by Mysore *et al*. [20,25,26], Lopez *et al*. [33], and Fei *et al*. [38] concluded that although engineered microbes could successfully express dsRNA to silence specific genes, issues such as degradation, instability, and variability in dsRNA uptake were frequently reported, and this limited the consistency of oral delivery systems. Table 6 presents comparative characteristics of microbial RNAi delivery platforms for mosquito control. Despite these challenges, several past studies reported significant successes in gene silencing. However, a recent study by Prates *et al*. [37] found minimal knockdown effects and no notable larval mortality when using oral and soaking methods to deliver dsRNA targeting multiple neural and structural genes. Similarly, Zhang *et al*. [34] found that silencing midgut chitin synthesis genes disrupted the peritrophic membrane but did not cause larval death, indicating variable effectiveness depending on gene targets and delivery methods. Soaking, mainly used in larvae and pupae, showed varying success rates. For example, one study reported that soaking recently molted pupae in dsRNA targeting the cytochrome gene had minimal impact on pupal mortality and an even lesser effect on older pupae [40]. This reveals that although soaking is a relatively cheaper and easier method, its efficiency is largely dependent on the developmental stage of the mosquito vector.

Innovative delivery systems such as ATSBs-dsRNA and transgenic RNAi expression systems have been explored in some studies. Williams *et al*. [41] and Mysore *et al*. [25] found that these methods demonstrated high effectiveness in inducing gene knockdown and mosquito mortality. However, to fully adopt these approaches, thorough field validation, biosafety assessments, including their impact on non-target organisms, and regulatory measures are necessary before real-world implementation.

4.2. Emerging Advances in Gene Silencing Technologies for Mosquito Vector Control

Synthetic biology tools are transforming mosquito control by enabling precise, multifaceted strategies that address the limitations of traditional methods. RNAi, CRISPR-based systems (including CRISPRi and gene drives), nanocarriers, and RNA stabilization techniques are increasingly combined to develop sustainable molecular solutions against vector-borne diseases. CRISPR/Cas technologies have advanced research in insect biology and vector control, facilitating heritable, population-wide modifications. In this framework, CRISPRi provides non-cleavage, reversible gene repression through dCas9-sgRNA complexes that silence genes without making permanent changes. Although research on CRISPRi in mosquitoes is limited, successful applications of dCas9 in *A. aegypti* indicate potential for reversible gene regulation [45].

4.2.1. Nanoparticle delivery systems

RNAi has great potential in modern vector control strategies, but its effectiveness is limited by the rapid degradation of dsRNA in the gut. The instability of naked RNA molecules when exposed to heat, RNases, or ultraviolet radiation has led to growing interest in nanoparticle-based delivery systems [46]. Nanoparticles are increasingly being used in medicine for drug delivery and siRNA therapy [24]. They are non-microbial methods for delivering RNAi triggers, particularly dsRNA, that have found application in mosquito vector control.

4.2.1.1. Chitosan nanoparticles

Chitosan, a biodegradable material, has been extensively used for delivering drugs; however, it has also found recent applications in dsRNA delivery in insects [24,47]. It protects dsRNA from degradation by nucleases and maintains stability in the high pH of the insect gut, and also possesses antimicrobial properties that prevent the degradation of dsRNA by microorganisms [48]. The use of chitosan nanoparticles (CNP) complexed with dsRNA to induce RNAi in mosquito larvae was pioneered by Zhang *et al*. [49]. This complex is formed through the self-assembly of positively charged chitosan amino acids and negatively charged dsRNA phosphate groups. When incorporated into mosquito food, CNP/dsRNA successfully downregulated chitin synthase genes in *A. gambiae* [49]. Research by Dhandapani *et al*. [50] demonstrated that cross-linking chitosan with sodium tripolyphosphate increased the efficacy of CNP binding, thereby enhancing the stability and delivery of dsRNA. This ultimately led to improved gene knockdown and reduced larval mortality. The function of several genes, including wing-development vestigial genes, cadherin, and many more, has been extensively studied in mosquito research using RNA interference (RNAi) via nanoparticles [24].

4.2.1.2. Other nanoparticles and liposomes

Other nanoparticles, such as silica nanoparticles (SNPs) and carbon quantum dots (CQDs), have been investigated as dsRNA delivery vehicles in addition to chitosan. According to comparative research, CQDs caused more gene silencing and larval mortality than CNP and SNPs, presumably due to their quick diffusion throughout the insect body and stability in the extremely high pH of the mosquito gut [51].

Double-stranded RNA has also been effectively delivered to mosquito larvae through liposomes, a lipid form of nanoparticles; preliminary research has shown that these particles can downregulate genes such as MAPK p38 in *A. aegypti* larvae [52]. Liposomes may decrease dsRNA breakdown and improve distribution via gut cells, but they may also show some larval toxicity based on exposure duration and concentration [24].

4.2.2. Symbiont-based delivery system

A promising alternative is the microbial expressivity of RNAi delivery compared to synthetic and injection-based methods. Notably, biologically engineered *S. cerevisiae* expressing shRNAs against Notch pathway genes has been reported to increase the mortality of larvae of both *A. gambiae* and *A. aegypti* [25]. Similarly, designed *E. coli* can serve as a chassis for synthesizing dsRNA and potentially provide a scalable and affordable strategy for silencing target genes in mosquitoes. The dsRNA produced can induce RNAi effects in the target organisms [53]. Specifically, a study by Whitten and colleagues in 2016 showed that genetically modified gut symbionts could stably colonize insects, establish long-term bacterial expression of dsRNA, and generate a strong gene knockdown resulting in phenotypic control [54]. *Rhodococcus rhodnii* in *Rhodnius prolixus* and *Pantoea agglomerans* in *Frankliniella occidentalis* were engineered to target vital genes, such as vitellogenins and tubulin. Their results showed a notable decrease in fecundity and survival, and the modified bacteria remained within the insect gut and could transmit horizontally. These characteristics also demonstrate how symbiont-based RNAi has the potential to address major limitations of traditional microbial platforms, such as the temporary expression of dsRNA and challenges with outdoor delivery. Although symbiont-based RNAi has not yet been widely used in mosquitoes, its success in non-model vectors with culturable symbionts suggests it can be easily adapted to these insect hosts, targeting *Aedes* or *Anopheles* mosquitoes, and warrants further research in the future.

4.3. Comparative Analysis: RNAi vs. CRISPR-Based Silencing

RNAi operates post-transcriptionally via siRNA-RISC-guided mRNA degradation, producing temporary knockdown effects [55,56]. CRISPR/Cas9-based editing permanently modifies DNA, enabling long-term population suppression or replacement through gene drives. CRISPRi, in contrast, offers precise, reversible repression without DNA cleavage. RNAi and CRISPRi suit reversible research and interventions, whereas CRISPR/Cas9 editing underpins durable, heritable strategies [57]. Each approach faces off-target risks and potential resistance evolution, necessitating ongoing molecular optimization and field monitoring.

4.4. Bioengineering Advances for RNA Stabilization

RNA's inherent fragility limits its utility in vector control. Bioengineering efforts focus on chemical modifications (e.g., m6A, pseudouridylation, and 2′-fluorination), structural engineering (e.g., RNA nanostructures and duplexes), and protective reagents or matrices to block RNase activity and prevent degradation [58]. Nanoparticles also play a dual role, facilitating delivery while stabilizing RNA molecules. These innovations not only enhance RNAi durability in mosquitoes but also have broader implications for RNA therapeutics, including mRNA vaccines and gene therapies [59].

4.5. Gaps and Implications for Vector Control

RNAi has shown several promising outcomes from laboratory and semi-field trials, but despite that, some limitations must be addressed before it can be fully employed as a vector control tool.

1. RNA instability and off-target effects: RNA molecules used for this process can become unstable and prone to degradation, either by nucleases present in the mosquito's gut or in its environment. This is especially true for oral and soaking methods of delivery [24,37]. This problem can be solved by employing the novel and emerging tools and technologies discussed earlier
2. Lack of standardization: There was variability in mosquito strains, developmental stages, gene targets, dsRNA doses, and delivery methods across all the analyzed studies. This complicates drawing broad conclusions, comparing efficacy, or designing protocols for RNAi research. These variations could also be the cause of the differences in gene knockdown efficiency and mortality outcomes
3. Limited field validation: Only very few studies have carried out semi-field trials [20,26,38]. None of the studies reported large-scale field deployment, but most have only been validated in the laboratory and under laboratory conditions. This presents a major translational gap between laboratory research and large-scale field deployment
4. Regulatory and biosafety concerns: This is particularly important when dealing with transgenic RNAi systems and microbial dsRNA delivery methods. Regulatory agencies, such as the U.S. Environmental Protection Agency, emphasize case-by-case risk

assessments that consider dsRNA stability, sequence specificity, exposure pathways, and impacts on non-target organisms. International agreements, such as the Cartagena Protocol on Biosafety, also guide the use of genetically modified organisms, which may apply to microbial-based RNAi delivery. While concerns regarding environmental safety, non-target effects, and public acceptance will be raised, available evidence indicates that dsRNA molecules are rapidly degraded in the soil and digestive systems, and their high sequence specificity minimizes unintended effects. Many studies have reported that RNAi is a safe control tool with minimal effects on non-target organisms and the environment [8,19,20].

Despite promising results, the reproducibility of RNAi outcomes varies across mosquito studies. Several studies have reported variable knockdown efficiency and inconsistent mortality effects, even when targeting similar genes [37]. Methodological flaws, such as inconsistent dsRNA doses and a lack of standardized delivery protocols, further hinder comparability between studies. Moreover, most of the existing studies on RNAi are laboratory-based, creating a translational gap that needs to be addressed to advance RNAi as a reliable and scalable vector control strategy.

4.6. Future Directions

The main areas to focus on in the effort to implement RNAi as a vector control agent include finding safe and cost-effective ways to stabilize the iRNA species before they reach their target mRNA, identifying and standardizing the optimal dose of dsRNA that is highly effective in gene silencing, and that is also safe for the environment and non-target organisms. Biotechnological advancements, such as the bio-design of interfering RNA species that can be reproducible and have predictable outcomes, as well as the development of an RNAi toolkit that would consist of customizable promoters, silencing sequences, and delivery agents, would allow for easy prototyping and lead to greater efficiency and scalability of RNAi-based control. In addition, machine learning software can be used to predict gene targets by mining genomics and transcriptomics data. This would largely improve specificity and knockdown success rate [60].

5. CONCLUSION

RNAi is an emerging vector control strategy and a safe alternative to chemical insecticides. It works by targeting essential genes responsible for various physiological functions at different developmental stages of mosquitoes, aiming to either reduce the vector population or lower disease transmission. Integrating RNAi with advanced delivery systems and CRISPR-based tools, including CRISPRi and gene drives, offers a powerful, environmentally conscious strategy for vector control. Foundational science is strong, but real-world deployment requires further optimization of CRISPRi, with scalable and safe nanocarriers in mosquitoes. Future innovations may combine these tools, such as engineering mosquitoes to enhance in situ RNAi production or designing biodegradable gene drives. Although RNAi-based technologies hold great promise for mosquito control, translational readiness and scalability are still issues of concern. While insecticide resistance is increasing, the urgent need for alternative vector control strategies is also arising. Targeting detoxification genes with RNAi could be an effective way to reduce resistance. Turning these advancements into real-world solutions will require investments in delivery technology, regulatory frameworks, and cost management, thereby making it affordable and accessible.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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