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Evaluating the Effectiveness of Biological Control Agents against Mosquitoes

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Abstract The resurgence of mosquito-borne diseases such as malaria, dengue, and chikungunya has necessitated the exploration of alternative control strategies due to the limitations and resistance associated with chemical insecticides. This study evaluates the effectiveness of various biological control agents against mosquitoes, focusing on eco-friendly and sustainable methods. Biological control agents, including bacteria, fungi, larvivorous fish, and predatory insects like dragonflies and damselflies, have shown promising results in reducing mosquito populations. Additionally, innovative approaches such as the use of Wolbachia bacteria and bio-nanoparticles are being investigated for their potential to disrupt mosquito life cycles and reduce disease transmission. This study highlights the need for further research to optimize these biological methods and integrate them into comprehensive vector control programs. By leveraging natural predators and microbial agents, biological control offers a viable and environmentally friendly alternative to chemical insecticides, potentially mitigating the public health threat posed by mosquitoes.

Keywords Biological control; Mosquito vectors; Eco-friendly; Wolbachia; Larvivorous fish

1 Introduction

Mosquito-borne diseases represent a significant global health challenge, affecting millions of people annually. Diseases such as malaria, dengue, Zika, chikungunya, yellow fever, and West Nile virus are transmitted by mosquitoes and have severe health and economic impacts, particularly in tropical and subtropical regions (Guarner and Hale, 2019; Côrtes et al., 2023; Onen et al., 2023). The prevalence of these diseases is exacerbated by factors such as climate change, urbanization, and the global movement of people, which facilitate the spread of both the mosquitoes and the pathogens they carry (Brugueras et al., 2020). The resurgence of these diseases in new regions and populations underscores the urgent need for effective control strategies (Achee et al., 2019).

Traditional mosquito control methods include chemical insecticides, biological control, mechanical barriers, and environmental management. Chemical insecticides, while effective, pose significant drawbacks such as high production costs, environmental toxicity, and the development of resistance in mosquito populations (Jones et al., 2020; Onen et al., 2023). Biological control methods, such as the use of natural predators, pathogens, and symbionts like Wolbachia, offer a more sustainable and environmentally friendly alternative (Anders et al., 2018; Salazar et al., 2019; Minwuyelet et al., 2023). Mechanical barriers and environmental management, including the elimination of breeding sites, are also crucial components of integrated vector management strategies (Dahmana and Mediannikov, 2020; Côrtes et al., 2023).

The increasing resistance to chemical insecticides and the negative environmental impacts associated with their use have driven the search for alternative mosquito control methods. Biological control agents, including bacteria like Wolbachia, fungi, and genetically modified mosquitoes, have shown promise in reducing mosquito populations and interrupting disease transmission (Anders et al., 2018; Achee et al., 2019; Minwuyelet et al., 2023). These methods are often more specific to target species and pose fewer risks to non-target organisms and the environment. Additionally, biological control agents can be integrated into existing vector management programs to enhance their effectiveness and sustainability (Salazar et al., 2019; Dahmana and Mediannikov, 2020).

This study systematically evaluates the effectiveness of various biological control agents against mosquitoes by assessing their impact on mosquito population dynamics, evaluating their role in reducing the transmission of mosquito-borne diseases, identifying the advantages and limitations of different control strategies, and providing recommendations for integrating these agents into comprehensive mosquito management programs, ultimately aiming to inform public health strategies and contribute to the development of more effective and sustainable mosquito control methods.

2 Types of Biological Control Agents

2.1 Microbial agents

Microbial agents such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) are widely used for mosquito control. These bacteria produce toxins that are lethal to mosquito larvae when ingested. Bti, for instance, has been shown to be effective in various environments, including mixed saltmarsh-mangrove systems, although its efficacy can be reduced by factors such as high mangrove canopy density (Johnson et al., 2020). Long-lasting formulations of Bti and Bs have been developed to extend their activity duration, which has proven effective in reducing malaria vector densities without significantly impacting non-target organisms (Derua et al., 2018). However, there are concerns about the potential indirect effects on food webs, particularly the reduction of chironomid populations, which are a key food source for many aquatic and terrestrial predators (Allgeier et al., 2019a; 2019b).

2.2 Predatory organisms

Predatory organisms, including fish and invertebrates, play a significant role in controlling mosquito populations by preying on mosquito larvae. Fish species such as *Gambusia affinis* (mosquitofish) are commonly introduced into water bodies to consume mosquito larvae. Invertebrates like dragonfly larvae and certain beetles also contribute to reducing mosquito populations. The effectiveness of these predators can vary based on environmental conditions and the availability of alternative prey. For instance, the presence of dragonfly larvae has been shown to decrease the survival rates of newt larvae in Bti-treated environments due to increased intraguild predation (Allgeier et al., 2019a).

2.3 Parasitoids

Parasitoids, including nematodes and fungi, are another group of biological control agents used against mosquitoes. These organisms infect and kill mosquito larvae or adults. For example, entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have been studied for their potential to control mosquito populations. Combining parasitoids with other biocontrol agents, such as entomopathogenic microorganisms, can enhance their effectiveness. Studies have shown that certain combinations of parasitoids and microorganisms are compatible and can improve pest control outcomes (Koller et al., 2023).

2.4 Genetic control methods

Genetic control methods involve altering the genetic makeup of mosquito populations to reduce their ability to reproduce or transmit diseases. One such method is the release of *Wolbachia*-infected mosquitoes, which can reduce the transmission of diseases like dengue and Zika by interfering with the reproductive capabilities of mosquitoes. Another approach is the Sterile Insect Technique (SIT), where sterile male mosquitoes are released to mate with wild females, resulting in no offspring. These methods have shown promise in reducing mosquito populations and disease transmission. For instance, the use of *Wolbachia*-infected mosquitoes has been effective in various field trials, demonstrating significant reductions in mosquito populations (Figure 1) (Silva-Filha et al., 2021).

Silva-Filha et al. (2021) investigates the mode of action of *Bacillus thuringiensis* israelensis (Bti) toxins, specifically Cry and Cyt proteins, in controlling mosquito larvae. The research emphasizes the crucial role of toxin-receptor interactions and toxin oligomerization in the larval gut, which leads to cell lysis and ultimately larval death. The paper discusses the structural characteristics of Cry toxins, including their receptor-binding domains, and highlights the importance of cadherin (CAD) and Cyt1Aa in facilitating the oligomerization and membrane insertion of toxins. The study suggests that Bti toxins, due to their highly specific and environmentally safe action, offer a promising alternative to chemical insecticides in mosquito vector control, addressing challenges such as insecticide resistance.

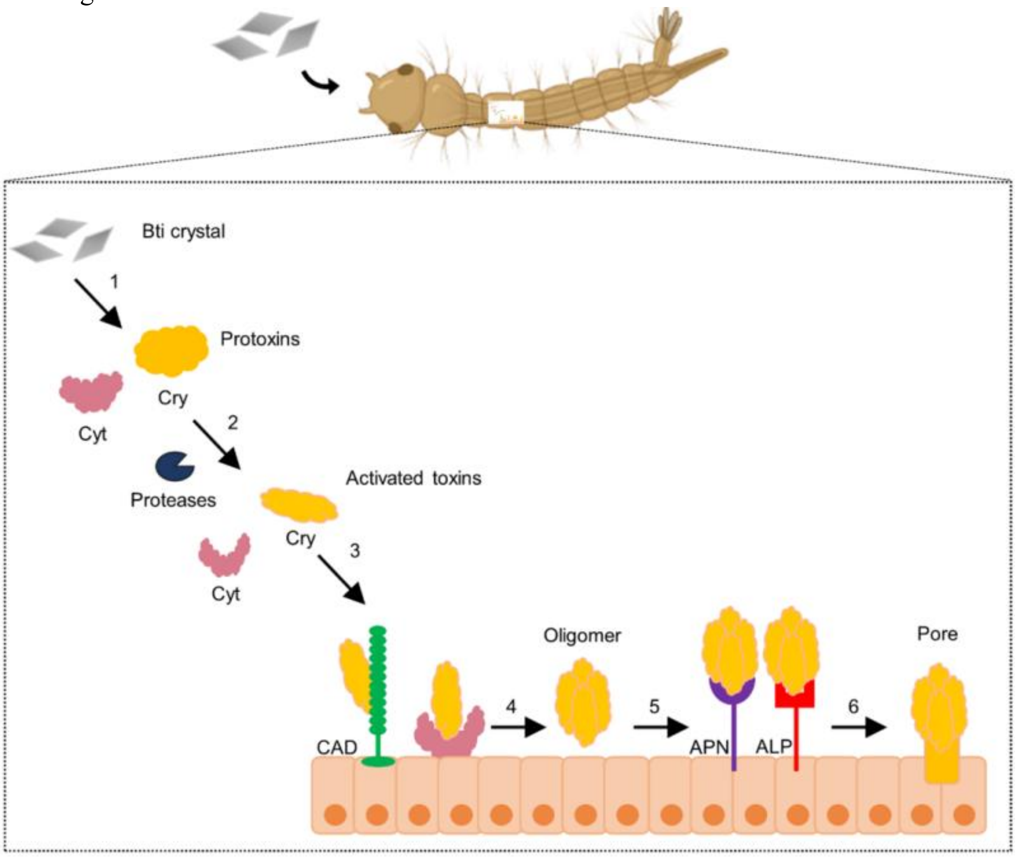

Figure 1 Schematic representation of the mechanism of action of Cry and Cyt toxins from *Bacillus thuringiensis* svar. *Israelensis* in mosquito larvae (Adopted from Silva-Filha et al., 2021)

Image caption: Crystals ingested by larvae are solubilized in the alkaline pH of the gut lumen (1). The protoxins are activated into toxins by proteases (2); and the Cry toxins can interact with a cadherin or with Cyt1Aa, which also act as a receptor (3); promoting Cry oligomerization (4). This oligomer binds with high affinity to midgut-bound receptors such as aminopeptidases-APN and alkaline phosphatase-ALP (5) and is inserted into the membrane, forming pores (6) that breakdown the cells and kill the larvae (Adopted from Silva-Filha et al., 2021)

3 Mechanisms of Action

3.1 Microbial agents: targeting larval stages

Microbial agents, such as *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus*, have been extensively used for mosquito control due to their specificity and safety for non-target organisms. These microbial larvicides produce toxins that disrupt the gut cells of mosquito larvae, leading to their death. Recent advancements in long-lasting formulations of these microbial agents have shown promising results in maintaining their effectiveness over extended periods, thereby reducing the frequency of applications needed. Studies have demonstrated that these formulations do not significantly impact the abundance, richness, or diversity of non-target organisms in treated habitats, making them an ecologically safe option for mosquito control (Derua et al., 2018).

3.2 Predators: reducing adult mosquito populations

Aquatic predators, such as dragonfly and damselfly naiads, have shown significant potential in controlling mosquito populations by preying on their larvae. Meta-analyses have revealed that these predators can reduce mosquito larval populations by up to 45% per day, indicating their effectiveness as biological control agents. However, the success of these predators in the field can vary due to habitat preferences and environmental conditions. Additionally, the use of predatory midge larvae, such as *Chaoborus flavicans*, has been explored, with studies showing high predatory impact on mosquito larvae, especially when combined with attractants like black pond dye to create population sinks (Cuthbert et al., 2019; Schiller et al., 2019; Dambach et al., 2020; Priyadarshana and Slade, 2023).

3.3 Parasitoids: infection and mortality induction

Parasitoids, such as certain species of wasps, can infect mosquito larvae, leading to their death before reaching adulthood. Although not as widely studied as microbial agents or predators, parasitoids offer a unique mechanism of action by directly targeting the larval stages and inducing mortality through infection. The integration of parasitoids into mosquito control programs requires further research to optimize their effectiveness and understand their interactions with other control methods.

3.4 Genetic methods: disruption of mosquito reproduction

Genetic methods, including the release of genetically modified mosquitoes and the use of bacteriophages to alter mosquito microbiota, aim to disrupt mosquito reproduction and reduce population sizes. For instance, bacteriophages targeting specific bacterial genera in the mosquito microbiota have been shown to affect larval development and survival, providing a novel approach to mosquito control. By manipulating the microbiota, it is possible to influence mosquito life-history traits and reduce their vector competence. This method highlights the potential of genetic and microbiota-based interventions in integrated mosquito management strategies (Barbosa et al., 2018; Alfano et al., 2019a; Tikhe and Dimopoulos, G., 2022).

4 Evaluating Effectiveness

4.1 Criteria for effectiveness in biological control

The effectiveness of biological control agents against mosquitoes is determined by several criteria, including the reduction in mosquito population, the sustainability of the control method, and the impact on disease transmission. For instance, dragonfly and damselfly naiads have shown significant predation success, reducing mosquito larvae populations by an average of 45% per day, which indicates their potential as effective biological control agents (Priyadarshana and Slade, 2023). Additionally, the genetic diversity and adaptive capacity of control agents, such as *Hydrochara affinis*, are crucial for their long-term success in various environments (Kang et al., 2020). The ability of biological agents to maintain their effectiveness over time, without leading to resistance, is another critical factor. For example, the use of entomopathogenic bacteria like Xenorhabdus and Photorhabdus is promising due to their slow rate of resistance development (Silva et al., 2020).

4.2 Field vs. laboratory trials

Field and laboratory trials are essential for evaluating the effectiveness of biological control agents, but they often yield different results. Laboratory trials provide controlled conditions to measure specific outcomes, such as the predation rate of dragonfly naiads on mosquito larvae (Priyadarshana and Slade, 2023). However, these results may not always translate to field conditions, where environmental variables and ecological interactions come into play. For instance, the effectiveness of aquatic predators in laboratory settings may not be replicated in the field due to differences in habitat preferences and ecological dynamics (Dambach, 2020). Field trials, such as those conducted with *Bacillus thuringiensis israelensis* (Bti) in Burkina Faso, demonstrate the practical application and sustainability of biological control methods over multiple transmission seasons, showing significant reductions in mosquito populations (Dambach et al., 2020).

4.3 Factors influencing success (environmental, ecological, genetic)

Several factors influence the success of biological control agents, including environmental conditions, ecological interactions, and genetic attributes. Environmental factors such as seasonality and temperature can impact the effectiveness of biological methods. For example, heatwaves can lead to the loss of Wolbachia infection in mosquitoes, reducing the efficacy of this control method (Ogunlade et al., 2023). Ecological factors, such as the presence of alternative prey and predators, also play a role. The introduction of aquatic predators must be carefully managed to avoid negative impacts on local ecosystems (Dambach et al., 2020). Genetic factors, including the genetic diversity and population structure of control agents, are crucial for their adaptability and long-term success. High genetic diversity in both natural and lab-reared populations of *Hydrochara affinis* suggests a strong adaptive capacity, making them suitable for field application (Kang et al., 2020). Additionally, the use of genetically engineered mosquitoes with gene drive technology requires careful site selection to maximize success and minimize risks (Lanzaro et al., 2021).

5 Advantages and Limitations

5.1 Benefits of biological control over chemical methods

Biological control methods offer several advantages over traditional chemical insecticides in managing mosquito populations. One of the primary benefits is the reduced risk of resistance development. Chemical insecticides, such as organophosphates and pyrethroids, have led to significant resistance in mosquito populations due to their extensive and prolonged use (Chaudhry, 2019; Senthil-Nathan, 2020; Silva et al., 2020). In contrast, biological control agents, such as entomopathogenic bacteria (e.g., Xenorhabdus and Photorhabdus), exhibit a slower rate of resistance development, making them a more sustainable option (Silva et al., 2020).

Additionally, biological control methods are environmentally benign and target-specific, reducing the negative impact on non-target organisms, including beneficial insects and other wildlife (Chaudhry, 2019; Onen et al., 2020; Senthil-Nathan, 2020). For instance, plant-derived compounds and green synthesized metallic nanoparticles are biodegradable and safe for non-target species, offering an eco-friendly alternative to chemical insecticides (Chaudhry, 2019; Onen et al., 2020). Moreover, biological control agents can be cost-effective in the long run, as they often require fewer applications and can be self-sustaining (Onen et al., 2020; Ogunlade et al., 2023).

5.2 Potential risks and limitations

Despite their advantages, biological control methods also have several limitations and potential risks. One significant limitation is the variability in effectiveness due to environmental factors. For example, the efficacy of biological agents like Wolbachia can be affected by seasonality and temperature fluctuations, which may lead to the loss of infection in mosquito populations during heatwaves (Dahmana and Mediannikov, 2020; Ogunlade et al., 2023).

Another challenge is the complexity of producing and maintaining biological control agents. For instance, the production of predatory mosquitoes like Toxorhynchites rutilus septentrionalis requires careful management to prevent cannibalism and ensure high adult yield, which can be labor-intensive and costly. Additionally, the integration of biological control agents into existing mosquito control programs may require significant changes in infrastructure and training, posing logistical challenges (Schiller et al., 2019).

Furthermore, there is a risk of unintended ecological consequences. The introduction of non-native biological control agents could potentially disrupt local ecosystems and harm non-target species (Chaudhry, 2019). Therefore, thorough risk assessments and monitoring are essential to mitigate these potential impacts.

5.3 Integration with other control strategies (e.g., integrated pest management)

Integrating biological control methods with other mosquito control strategies, such as chemical, mechanical, and environmental methods, can enhance overall effectiveness and sustainability. This integrated pest management (IPM) approach leverages the strengths of each method while mitigating their individual limitations (Chaudhry, 2019; Arias-Castro et al., 2020; Wooding et al., 2020).

For example, combining biological control agents with chemical insecticides can reduce the reliance on chemicals and delay the development of resistance in mosquito populations (Arias-Castro et al., 2020). The use of semiochemicals in odour-based traps can complement biological control by enhancing the selectivity and efficacy of traps, thereby improving mosquito surveillance and control (Wooding et al., 2020). Additionally, environmental management practices, such as eliminating breeding sites and improving water management, can further support the effectiveness of biological control agents (Chaudhry, 2019; Ogunlade et al., 2023).

6 Case Study

6.1 Overview of the selected case study region

The selected case study region is southwest Ethiopia, an area characterized by its temporary wetlands and ponds, which serve as natural habitats for both Anopheles mosquito larvae and their potential invertebrate predators. This region is particularly significant due to the high prevalence of malaria, a disease transmitted by Anopheles mosquitoes, which poses a substantial public health threat. The local environment provides a unique opportunity to explore biological control methods as an alternative to traditional insecticides, which are increasingly facing resistance issues (Eba et al., 2021).

6.2 Implementation of biological control agents in the region

In southwest Ethiopia, a study was conducted to evaluate the effectiveness of various invertebrate predators in controlling Anopheles mosquito larvae. The predators, including backswimmers (Notonectidae) and dragonflies (Libellulidae), were collected from the natural habitats within the region. Laboratory experiments were designed to determine the optimal conditions for predation, such as the appropriate larval instar, water volume, and predator density. The backswimmer emerged as the most effective predator, with a daily mean predation rate of 71.5 larvae (Table 1) (Eba et al., 2021). This implementation highlights the potential of using native aquatic predators as a biological control strategy in integrated malaria vector control programs.

Table 1 The optimal conditions for larval predation in terms of larval instar, water volume and number of predators and the number of larvae consumed (95% confidence interval) at that optimal level for the 7 different predators (Adopted from Eba et al., 2021)

Predator Family	Mosquito Instar	Water Volume	# of Predators	# of Larvae Consumed
Aeshinidae	4	2	15	109 (98;121)
Belostomatidae	2	2	15	90 (80;101)
Corixidae	2	2	15	20 (16;26)
Dytiscidae	4	3	15	168 (154;184)
Gomphidae	3	3	15	33 (27;40)
Libellulidae	4	2	15	29 (24;36)
Notonectidae	1	1	15	140 (127;154)

Note: # = number

6.3 Measured outcomes and impact on mosquito populations

The study's outcomes demonstrated that the selected invertebrate predators could significantly reduce the population of Anopheles mosquito larvae under controlled conditions. The backswimmer, in particular, showed a high predation rate, suggesting its potential effectiveness in natural settings. However, the study also noted that the effectiveness of these predators could vary based on environmental factors such as water volume and the developmental stage of the larvae. While the laboratory results are promising, further field trials are necessary to confirm these findings and assess the long-term impact on mosquito populations and malaria transmission rates in the region (Eba et al., 2021).

6.4 Lessons learned and recommendations for future applications

Several key lessons were learned from the implementation of biological control agents in southwest Ethiopia. Firstly, the selection of effective predators is crucial, as different species exhibit varying predation rates and preferences for larval instars. Secondly, environmental factors such as water volume and habitat type significantly influence the success of biological control methods. Therefore, it is essential to tailor the approach to the specific conditions of the target area. Future applications should focus on conducting extensive field trials to validate laboratory findings and ensure that the introduction of predators does not disrupt local ecosystems. Additionally, integrating biological control methods with other vector control strategies could enhance overall effectiveness and sustainability (Dambach, 2020; Eba et al., 2021).

7 Future Perspectives

7.1 Innovations in biological control technologies

The future of mosquito control lies in the continuous innovation and development of biological control technologies. Recent advancements have shown promising results in the use of genetic manipulation and symbiotic bacteria such as Wolbachia and Asaia, which are being intensively studied as alternatives to chemical insecticides (Dahmana and Mediannikov, 2020). Additionally, the use of entomopathogenic bacteria like Xenorhabdus and Photorhabdus has been highlighted for their insecticidal properties, which could be harnessed for mosquito control (Silva et al., 2020). The development of green synthesized plant-based metallic nanoparticles also presents a novel, eco-friendly approach to mosquito control, offering broad-spectrum target-specific activities against various mosquito species (Onen et al., 2020). These innovations not only provide effective mosquito control but also mitigate the issue of insecticide resistance, which has been a significant challenge in traditional mosquito control methods (Sajjad and Arif, 2019; Dahmana and Mediannikov, 2020; Singh et al., 2023).

7.2 Regulatory considerations and public acceptance

The deployment of new biological control technologies must navigate a complex landscape of regulatory considerations and public acceptance. Genetic-based solutions, such as gene drives and the release of Wolbachia-infected mosquitoes, require thorough regulatory scrutiny to ensure environmental safety and efficacy (Wang et al., 2021). Public acceptance is equally crucial, as the release of genetically modified organisms (GMOs) into the environment can be met with resistance due to safety concerns. Effective communication and education strategies are essential to address public apprehensions and highlight the benefits of these technologies. Moreover, the regulatory framework must evolve to accommodate the rapid advancements in biotechnology, ensuring that new methods are rigorously tested and approved for safe use (Parihar et al., 2020).

7.3 Opportunities for integration with emerging mosquito control methods

Integrating biological control agents with emerging mosquito control methods offers a holistic approach to vector management. Combining traditional biological control agents, such as larvivorous fish and predacious species, with modern genetic and microbial strategies can enhance the overall effectiveness of mosquito control programs (Arias-Castro et al., 2020; Eba et al., 2021). For instance, the use of Toxorhynchites rutilus septentrionalis, a natural mosquito predator, in conjunction with chemical and biological insecticides, can provide a multi-faceted approach to reducing mosquito populations (Schiller et al., 2019). Additionally, the integration of green synthesized nanoparticles with existing control measures can offer a sustainable and environmentally friendly solution to mosquito-borne diseases (Onen et al., 2020). By leveraging the strengths of various control methods, it is possible to develop comprehensive strategies that are more resilient to resistance and adaptable to different ecological contexts (Sajjad and Arif, 2019; Wang et al., 2021; Singh et al., 2023).

8 Concluding Remarks

The systematic review of biological control agents against mosquitoes reveals several promising strategies. Entomopathogenic bacteria such as Xenorhabdus and Photorhabdus have shown significant insecticidal properties, making them potential candidates for mosquito control. Dragonflies and damselflies have also demonstrated high predation success on mosquito larvae, reducing larval populations by approximately 45% per day. Additionally, green nanotechnology, particularly the use of biosynthesized nanoparticles, has emerged as an effective and eco-friendly approach to combat mosquito vectors. Plant-derived compounds, including essential oils and phytochemicals, have been identified as alternative larvicidal agents with minimal environmental impact. Furthermore, invertebrate predators like backswimmers have shown high efficacy in preying on Anopheles larvae, suggesting their potential in integrated vector management programs.

The findings underscore the importance of incorporating biological control agents into mosquito management programs. The use of entomopathogenic bacteria and biosynthesized nanoparticles offers a sustainable alternative to chemical insecticides, which are increasingly facing resistance issues. Promoting the natural predation of mosquitoes by dragonflies, damselflies, and other invertebrate predators can significantly reduce mosquito populations without adverse environmental effects. Additionally, the application of plant-derived compounds provides a cost-effective and biodegradable solution for mosquito control, aligning with the goals of eco-friendly pest management. These biological methods can complement existing chemical controls, potentially reducing the overall reliance on synthetic insecticides and mitigating resistance development.

Future research should focus on the large-scale field application and long-term efficacy of these biological control agents. Studies on the environmental impact and non-target effects of entomopathogenic bacteria and biosynthesized nanoparticles are essential to ensure their safety and sustainability. Further investigation into the behavioral and ecological interactions between mosquito predators and their prey can optimize the use of natural predators in vector control programs. Additionally, exploring the synergistic effects of combining multiple biological control agents could enhance overall control efficacy and provide a more robust solution to mosquito-borne diseases. Finally, the development of practical guidelines for the implementation of these biological methods in diverse ecological settings will be crucial for their successful integration into existing mosquito control strategies.

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Conflict of Interest Disclosure
The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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