Journal of Mosquito Research, 2024, Vol.14, No.6, 294-304
http://emtoscipublisher.com/index.php/jmr

Research Insight Open Access

Development and Testing of New Biopesticides for Mosquito Control

Xiaojie Liu, Zhongqi Wu
Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
Corresponding email: zhongqi.wu@jicat.org

Journal of Mosquito Research, 2024, Vol.14, No.6 doi: 10.5376/jmr.2024.14.0028
Received: 20 Sep., 2024
Accepted: 31 Oct., 2024
Published: 21 Nov., 2024

Preferred citation for this article:
Liu X.J., and Wu Z.Q., 2024, Development and testing of new biopesticides for mosquito control, Journal of Mosquito Research, 14(6): 294-304 (doi: 10.5376/jmr.2024.14.0028)

Abstract Mosquito-borne diseases pose significant global health challenges, necessitating effective and sustainable mosquito control strategies. Current control measures rely heavily on chemical pesticides, which face issues of resistance, environmental harm, and public health concerns. This study focuses on the development and testing of innovative biopesticides as eco-friendly alternatives. We provide an overview of biopesticides, their classifications, and mechanisms in mosquito control, highlighting their advantages over traditional chemical pesticides. A newly developed biopesticide, was field-tested for efficacy in reducing mosquito populations, with assessments of environmental impact and community acceptance. The results demonstrated substantial reductions in mosquito density with minimal ecological disruption. Advances in testing methodologies, including laboratory assays, semi-field trials, and molecular tools, were employed to ensure rigorous evaluation. Integration of biopesticides into broader mosquito management programs is discussed, emphasizing the need for scalable production, effective implementation, and adoption in resource-limited settings. This study underscores the potential of biopesticides to transform mosquito control, offering insights for future research, policy development, and stakeholder engagement to address emerging challenges in vector management.

Keywords Mosquito control; Biopesticides; Eco-friendly alternatives; Resistance management; Vector management programs

1 Introduction

Mosquito-borne diseases, such as malaria, dengue, chikungunya, yellow fever, Zika, and West Nile, pose significant threats to global public health. These diseases are transmitted by various mosquito species, including *Anopheles*, *Aedes*, and *Culex*, and affect millions of people worldwide, leading to substantial morbidity and mortality (Benelli et al., 2016; Benelli et al., 2018; Onen et al., 2023). The global burden of these diseases is exacerbated by the rapid spread of highly invasive mosquito species and the emergence of resistance to conventional insecticides (Ogunah et al., 2020; Ogunah et al., 2020).

Traditional mosquito control strategies primarily rely on chemical insecticides, such as organochlorides, carbamates, organophosphates, and pyrethroids. However, these methods face several challenges, including the development of insecticide resistance, environmental pollution, and adverse effects on non-target organisms (Shen et al., 2020; Ogunah et al., 2020; Ogunah et al., 2020). Additionally, the COVID-19 pandemic has disrupted vector control programs, further complicating efforts to manage mosquito populations and prevent disease transmission (Lu et al., 2023).

Given the limitations of chemical insecticides, there is an urgent need for sustainable and eco-friendly alternatives. Biopesticides, including those derived from natural products or microorganisms, offer a promising solution. These biopesticides are effective against mosquito vectors while minimizing environmental impact and reducing the risk of resistance development (Ohia and Ana, 2015; Benelli et al., 2016). Green synthesized plant-based metallic nanoparticles and entomopathogenic fungi are examples of innovative biocontrol agents that have shown potential in mosquito management (Benelli et al., 2018; Shen et al., 2020; Onen et al., 2023).

This study attempts to explore the development and application of sustainable and environmentally friendly biopesticides for mosquito control, discuss the challenges associated with traditional mosquito control methods, and provide an overview of the potential of biopesticides, including green-synthesized nanoparticles and entomopathogenic fungi, as alternative control strategies.

2 Biopesticides: Definitions and Mechanisms

2.1 Overview of biopesticides: types and classifications

Biopesticides are pest control agents derived from natural materials such as animals, plants, bacteria, and certain minerals. They are classified into three main types: microbial pesticides, plant-incorporated protectants (PIPs), and biochemical pesticides. Microbial pesticides, which include bacteria, fungi, viruses, and protozoa, are the most commonly used in mosquito control. For instance, bacterial-derived biopesticides like those from *Bacillus thuringiensis* and *Chromobacterium sp. Panama* have shown significant efficacy in controlling mosquito populations (Mnif and Ghribi, 2015; Caragata et al., 2020; Engdahl et al., 2023). Plant-based biopesticides, such as those derived from *Azadirachta indica* (neem) and *Pongamia glabra*, are also effective and environmentally friendly alternatives (Maheswaran and Ignacimuthu, 2015).

2.2 Mechanisms of action in mosquito control

Biopesticides control mosquito populations through various mechanisms. Microbial biopesticides often work by producing toxins that are lethal to mosquito larvae. For example, the bacterium *Chromobacterium sp. Panama* produces compounds that rapidly kill mosquito larvae by disrupting their mid-gut epithelial cells (Figure 1) (Caragata et al., 2020; Engdahl et al., 2023). Another mechanism involves the use of plant-derived compounds that interfere with the mosquito's development and reproduction. PONNEEM, a biopesticide made from neem and pongamia oils, exhibits strong larvicidal and ovicidal activities, significantly reducing mosquito populations (Maheswaran and Ignacimuthu, 2015). Additionally, nanopesticides, which are biopesticides formulated at the nanoscale, can enhance the delivery and efficacy of active ingredients, leading to higher mortality rates in mosquito larvae (Kalimuthu et al., 2017; Mishra et al., 2018).

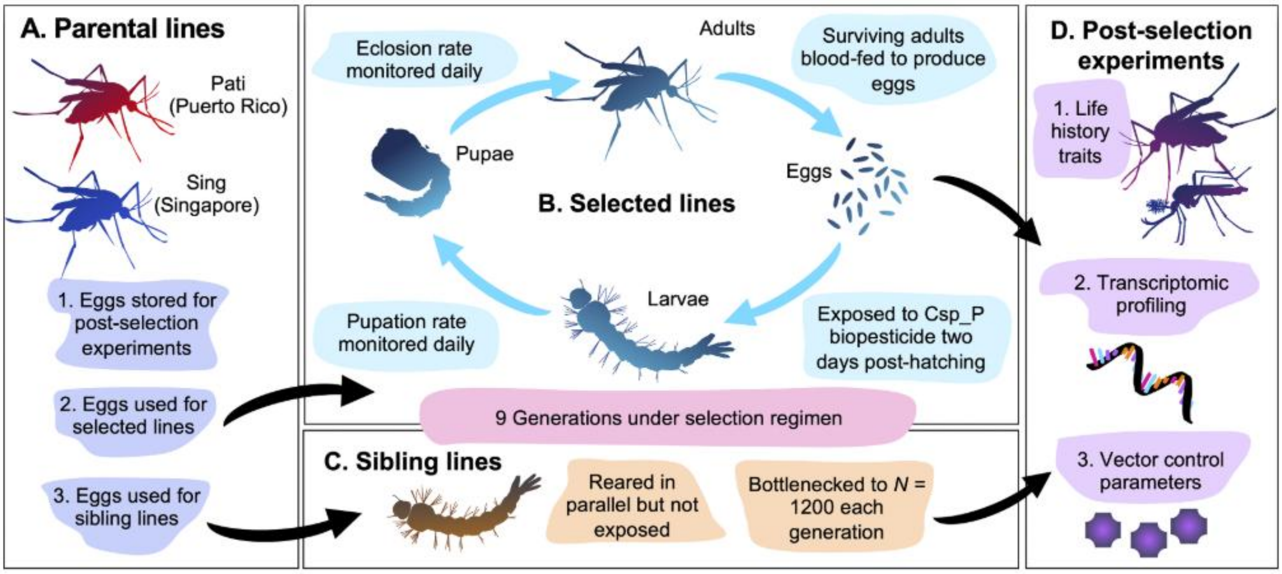

Figure 1 Schematic overview of selection process and postselection experiments (Adopted from Engdahl et al., 2023)

Image caption: (A) Parental eggs from the Pati (Patillas, Puerto Rico) and Sing (Singapore) *Ae. aegypti* populations were used to generate (B) selected (Csp_P biopesticide-exposed) and (C) sibling (bottlenecked but not exposed) lines. The selection regimen was enacted for nine generations, and during this time, developmental parameters were monitored daily. (D) After selection, life history traits were assayed (fecundity, fertility, wing length, and longevity), the transcriptome was profiled using RNA sequencing (RNA-Seq). The mosquitoes were exposed to common chemical insecticides, as well as DENV-2, in order to evaluate the consequences of multigenerational exposure to the Csp_P biopesticide (Adopted from Engdahl et al., 2023)

2.3 Advantages of biopesticides over chemical pesticides

Biopesticides offer several advantages over traditional chemical pesticides. Firstly, they are generally more specific to target pests, reducing the risk of harming non-target organisms and beneficial insects (Mishra et al., 2018). Secondly, biopesticides are biodegradable and less likely to contaminate the environment, making them a more sustainable option for pest control (Maheswaran and Ignacimuthu, 2015; Mathivanan et al., 2019).

Furthermore, the risk of resistance development is lower with biopesticides. Studies have shown that mosquitoes exposed to biopesticides like *Chromobacterium sp. Panama* do not develop resistance, unlike those exposed to chemical insecticides (Engdahl et al., 2023). Lastly, biopesticides can be integrated with other control methods, such as the sterile insect technique (SIT), to enhance overall efficacy and reduce the need for high application rates (Pleydell and Bouyer, 2019).

Biopesticides, derived from natural sources, offer a sustainable and effective alternative to chemical pesticides for mosquito control. They work through various mechanisms, including toxin production and developmental interference, and provide several advantages such as environmental safety, specificity, and reduced resistance development. Integrating biopesticides with other control methods can further enhance their efficacy in managing mosquito populations and mitigating vector-borne diseases.

3 Development of New Biopesticides

3.1 Sources of biopesticides: natural, microbial, and synthetic derivatives

Biopesticides can be derived from various sources, including natural, microbial, and synthetic derivatives. Natural biopesticides often originate from plant extracts, such as the oils of *Azadirachta indica* and *Pongamia glabra*, which have been used to create the biopesticide PONNEEM. This biopesticide has shown high efficacy against mosquito species like *Anopheles stephensi* and *Culex quinquefasciatus*, demonstrating 100% larvicidal and ovicidal activities (Maheswaran and Ignacimuthu, 2015). Microbial biopesticides, such as those derived from the bacterium *Chromobacterium sp. Panama*, have also proven effective. These microbial biopesticides can kill mosquito larvae rapidly and are particularly valuable as they do not lead to resistance in mosquito populations (Caragata et al., 2020; Engdahl et al., 2023). Additionally, synthetic derivatives, including nanopesticides, offer a promising approach. These can be synthesized using plant extracts and essential oils, providing eco-friendly and target-specific solutions for mosquito control (Mishra et al., 2018).

3.2 Screening methods for bioactive compounds

Screening for bioactive compounds involves several methods to identify effective biopesticides. One approach is the use of laboratory bioassays to test the efficacy of various compounds against mosquito larvae and adults. For instance, a testing cascade was employed to screen a panel of chemistries with novel modes of action against *Anopheles gambiae*, identifying compounds with high intrinsic activity and appropriate concentration efficacy (Figure 2) (Lees et al., 2019). Another method involves RNA sequencing to observe the genetic response of mosquitoes to biopesticide exposure, ensuring that resistance does not develop (Engdahl et al., 2023). Additionally, mathematical modeling can be used to assess the potential gains of combining biopesticides with other control methods, such as the sterile insect technique (SIT), to enhance overall efficacy (Pleydell and Bouyer, 2019).

3.3 Formulation techniques for enhanced efficacy and stability

Formulation techniques are crucial for enhancing the efficacy and stability of biopesticides. One effective formulation is the air-dried, nonlive preparation of *Chromobacterium sp. Panama*, which retains its activity even at high temperatures and can be incorporated into attractive baits for mosquito larvae (Caragata et al., 2020). Another technique involves the use of nanoemulsions, which are prepared using essential oils like neem oil and citronella oil. These nanoemulsions offer better target specificity and eco-friendly properties (Mishra et al., 2018). Additionally, the combination of biopesticides with other control agents, such as predatory copepods, can significantly enhance the control of mosquito populations. For example, the synergy between Hedychium coronarium-synthesized silver nanoparticles and predatory copepods has shown high efficacy in controlling *Aedes aegypti* larvae (Kalimuthu et al., 2017).

3.4 Regulatory considerations and safety evaluations

Regulatory considerations and safety evaluations are essential for the development and deployment of new biopesticides. In India, the biopesticide industry is regulated by the Central Insecticides Board and Registration Committee (CIBRC), which oversees the registration of microbial species and formulations (Kumar et al., 2019). Safety evaluations must ensure that biopesticides do not harm non-target organisms or the environment.

Journal of Mosquito Research, 2024, Vol.14, No.6, 294-304
http://emtoscipublisher.com/index.php/jmr

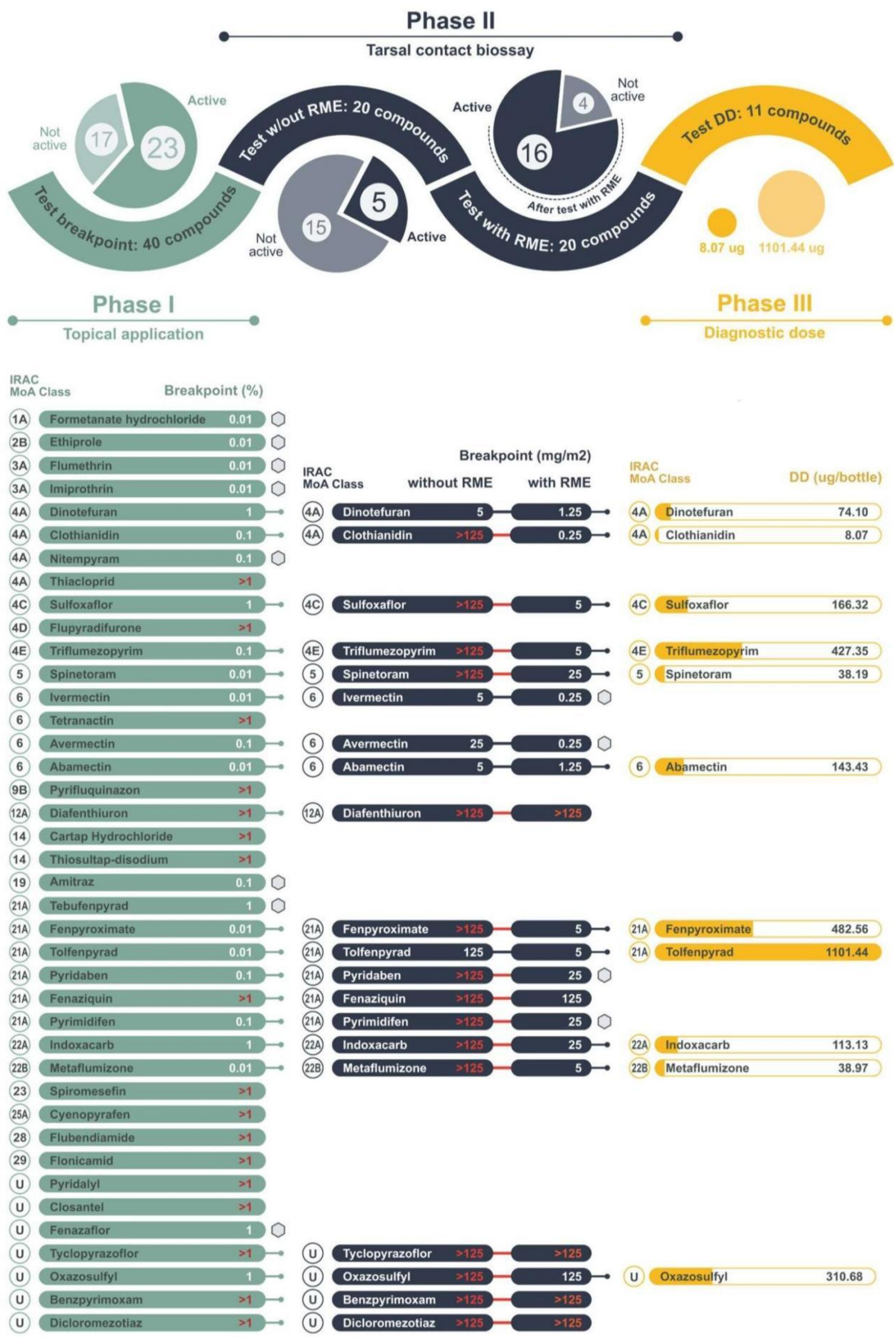

Figure 2 Testing cascade to screen existing insecticides for use against mosquito disease vectors (Adopted from Lees et al., 2019)

Image caption: Intrinsic insecticidal activity is measured by topical application of compound directly onto adult female Anopheles gambiae mosquitoes, activity through tarsal contact is measured in a bioassay with and without RME as an adjuvant, and relative potency is judged by determining the discriminating dose in CDC bottle bioassays. Activity in Phase I was defined as ≥80% mortality 24 hours after topical application, and the breakpoint denotes the concentration at which activity was first observed; '>1' means that activity was not observed even when the highest concentration, 1% active ingredient, was applied. Activity in Phase II was defined as ≥80% mortality 24 hours after tarsal exposure, and the breakpoint denotes the concentration ay which activity was first observed; '>125' means that activity was not observed even at the highest concentration tested, 125 mg/m^2. Compounds which were only active in tarsal contact bioassays (Phase II) in the presence and not the absence of RME are highlighted in red. Of 40 compounds from 20 IRAC MoA classes, and 7 compounds not listed or not classified by IRAC, 11 compounds are shortlisted for further investigation. The hexagon symbol denotes compounds which showed insecticidal activity during screening but which were not progressed for other reasons (Adopted from Lees et al., 2019)

For instance, the biopesticide PONNEEM has been shown to be biodegradable and does not affect non-target organisms like *Gambusia affinis* and *Diplonychus indicus* (Maheswaran and Ignacimuthu, 2015). Additionally, the nonlive preparation of *Chromobacterium sp. Panama* has been demonstrated to be safe and effective under natural conditions, further supporting its potential for widespread use (Caragata et al., 2020). The development of new biopesticides for mosquito control involves sourcing from natural, microbial, and synthetic derivatives, employing rigorous screening methods, utilizing advanced formulation techniques, and adhering to strict regulatory and safety evaluations. These efforts collectively contribute to the creation of effective, stable, and environmentally safe biopesticides that can play a crucial role in mitigating mosquito-borne diseases.

4 Case Study: Field Testing of *Chromobacterium sp. Panama* (Csp_P) in Mosquito Control

4.1 Description of the biopesticide: origin and active ingredients

Chromobacterium sp. Panama (Csp_P) is a biopesticide derived from an abundant soil bacterium. The preparation is an air-dried powder containing no live bacteria, which can be incorporated into an attractive bait and fed directly to mosquito larvae. This biopesticide has broad-spectrum activity against the larval forms of mosquitoes responsible for transmitting diseases such as malaria, dengue, chikungunya, yellow fever, West Nile, and Zika viruses (Caragata et al., 2020). The active ingredients in Csp_P are microbial compounds that exhibit mosquitocidal properties, effectively killing mosquito larvae at low dosages and maintaining stability under high temperatures.

4.2 Experimental design for field testing

Field testing of Csp_P was conducted in semi-field trials in Puerto Rico. The test sites included natural breeding waters where *Aedes aegypti* and *Culex* species larvae were present. Environmental conditions varied, but the biopesticide demonstrated high efficacy even under natural conditions, indicating its robustness and suitability for diverse environmental settings (Caragata et al., 2020). Population monitoring involved regular sampling of mosquito larvae from the test sites. Larval counts were conducted before and after the application of the biopesticide to assess its impact. Additionally, the longevity and developmental stages of the mosquito populations were monitored to evaluate any long-term effects of the biopesticide exposure (Engdahl et al., 2023).

4.3 Results and effectiveness in reducing mosquito populations

The field tests showed that Csp_P was highly effective in reducing mosquito populations. High mortality rates were observed in *Aedes aegypti* and *Culex* species larvae. The biopesticide maintained its efficacy over multiple generations, with no evidence of resistance development in the mosquito populations (Caragata et al., 2020; Engdahl et al., 2023). The preparation's ability to kill larvae at low dosages and its stability under high temperatures further underscored its potential as a reliable mosquito control tool.

4.4 Environmental impact assessment and community feedback

The environmental impact assessment indicated that Csp_P did not adversely affect non-target organisms, such as *Gambusia affinis* and *Diplonychus indicus*, which are commonly found in the same habitats as mosquito larvae (Maheswaran and Ignacimuthu, 2015). Community feedback was generally positive, with local residents noting a significant reduction in mosquito nuisance and a perceived decrease in mosquito-borne disease incidence. The biopesticide's biodegradable nature and lack of harmful residues were particularly appreciated by the community (Ramirez et al., 2018; Lees et al., 2019).

4.5 Challenges and lessons learned

One of the primary challenges encountered during the field testing was ensuring consistent application of the biopesticide in diverse environmental conditions. Additionally, maintaining community engagement and cooperation was crucial for the success of the field trials. Lessons learned include the importance of thorough environmental assessments to ensure non-target species safety and the need for continuous monitoring to detect any potential resistance development. The field trials also highlighted the necessity of integrating biopesticides with other vector control strategies for comprehensive mosquito management (Maheswaran and Ignacimuthu, 2015; Caragata et al., 2020; Engdahl et al., 2023).

The field testing of *Chromobacterium sp. Panama* (Csp_P) demonstrated its high efficacy in reducing mosquito populations, particularly *Aedes aegypti* and *Culex* species. The biopesticide's stability, low effective dosage, and lack of resistance development make it a promising tool for mosquito control. Environmental assessments confirmed its safety for non-target organisms, and community feedback was positive. Challenges included ensuring consistent application and maintaining community engagement, but the overall results underscore the potential of Csp_P as part of an integrated vector management strategy (Hernandez-Tenorio et al., 2022).

5 Advances in Testing Methodologies

5.1 Laboratory-based assays for toxicity and efficacy

Laboratory-based assays are essential for determining the toxicity and efficacy of new biopesticides. These assays typically involve exposing mosquito larvae or adults to various concentrations of the biopesticide and measuring mortality rates. For instance, bioassays have been used to evaluate the dose-response relationship of mosquitoes to insecticides, helping to identify the susceptibility or resistance of mosquito populations to specific compounds (Liu et al., 2023). High-throughput screening methods, such as the invertebrate automated phenotyping platform (INVAPP), have also been developed to measure larval motility and screen for new larvicides more efficiently than traditional WHO standard larval assays (Figure 3) (Buckingham et al., 2021). These laboratory-based methods provide a controlled environment to assess the initial effectiveness of biopesticides before moving to more complex testing stages.

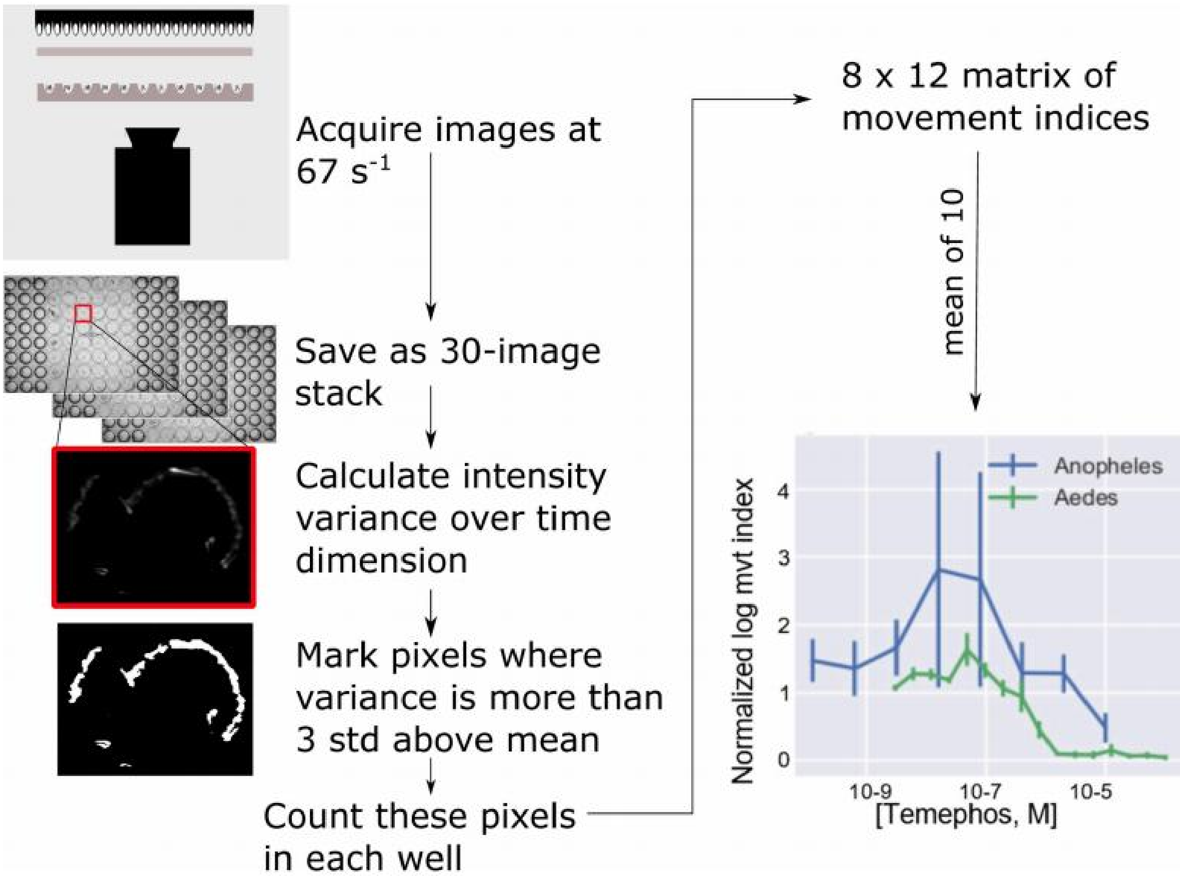

Figure 3 The procedure for automating the analysis of mosquito larval swimming (Adopted from Buckingham et al., 2021)

Image caption: In each trial, 30 images are acquired at 10 ms intervals and stored for later offline analysis. An index of the amount of movement is obtained by measuring the variance for each pixel over time. Pixels for which the variance more than 3 standard deviations from the mean variance are scored as 1, the remainder as 0. The movement index for each well is taken as the sum of these scores for that well. The output of the algorithm for quantifying movement plotted against the concentration of temephos (bottom right), a larvicide commonly used in the control of mosquitoes, is shown at the end of the pipeline. Alongside this similar data for larvae of *An. gambiae* are shown. A concentration-dependent inhibition of movement is seen in studies on larvae of *An. gambiae* and *Ae. Aegypti* (Adopted from Buckingham et al., 2021)

5.2 Semi-field and field trial designs

Semi-field and field trials are crucial for evaluating the real-world efficacy of biopesticides under natural conditions. These trials bridge the gap between laboratory findings and practical application. For example, a novel biopesticide derived from *Chromobacterium sp. Panama* was tested in semi-field trials in Puerto Rico, demonstrating high efficacy against field-derived *Aedes aegypti* populations and other mosquito species in natural breeding water (Caragata et al., 2020). Such trials help to validate laboratory results and assess the biopesticide's performance in diverse environmental conditions. Additionally, field bioassays have been used to measure the spatial distribution and effectiveness of insecticides, providing insights into the practical resistance ratios and the impact of environmental factors on biopesticide performance (Lee et al., 2023).

5.3 Incorporating molecular tools for resistance monitoring

Incorporating molecular tools into resistance monitoring is a significant advancement in the testing methodologies for biopesticides. RNA sequencing and other molecular techniques can identify genetic markers associated with resistance, providing a deeper understanding of how mosquitoes respond to biopesticide exposure. For instance, studies have shown that exposure to a Chromobacterium biopesticide did not lead to the development of resistance in *Aedes aegypti* mosquitoes, as there was no increased activity of xenobiotic metabolism and detoxification genes typically associated with insecticide resistance (Engdahl et al., 2023). These molecular tools enable researchers to monitor resistance at a genetic level, ensuring the long-term efficacy of biopesticides and informing strategies for resistance management (Brito-Sierra et al., 2019).

6 Integration into Mosquito Management Programs

6.1 Combining biopesticides with other control strategies

Integrating biopesticides into existing mosquito management programs can enhance the overall effectiveness of control strategies. For instance, combining biopesticides with traditional larviciding methods has shown promising results. The use of Hedychium coronarium-synthesized silver nanoparticles (AgNPs) in conjunction with predatory copepods like *Megacyclops formosanus* has demonstrated high control of *Aedes aegypti* larval populations, leveraging the synergistic effects of both biological agents (Kalimuthu et al., 2017). Similarly, the application of *Chromobacterium sp. Panama*-derived biopesticides has been effective against mosquito larvae, including those resistant to conventional insecticides, suggesting that these biopesticides can be integrated with other larvicides and adulticides for extended suppression of mosquito populations (Caragata et al., 2020; Engdahl et al., 2023).

6.2 Challenges in scaling up production and application

Scaling up the production and application of biopesticides presents several challenges. One significant issue is the consistency in the quality and efficacy of biopesticides when produced on a larger scale. For example, the production of PONNEEM, a biopesticide derived from *Azadirachta indica* and *Pongamia glabra*, must ensure that its larvicidal and ovicidal activities remain effective over time and under various environmental conditions (Maheswaran and Ignacimuthu, 2015). Additionally, the logistics of distributing and applying biopesticides in large areas can be complex. Truck-mounted applications of larvicides and adulticides, such as those using *Bacillus thuringiensis* var. israelensis and pyriproxyfen, have shown promise but require careful planning and execution to achieve consistent results (Unlu et al., 2018).

6.3 Strategies for adoption in resource-limited settings

Adopting biopesticides in resource-limited settings requires strategies that address cost, accessibility, and local infrastructure. One approach is to utilize biopesticides that are easy to produce and apply, such as plant-based extracts, which can be formulated without the need for sophisticated equipment (Pavela et al., 2019). Additionally, leveraging local resources and knowledge can facilitate the adoption of biopesticides. For instance, the use of nonlive preparations of *Chromobacterium sp. Panama*, which can be incorporated into attractive baits and fed directly to mosquito larvae, offers a low-cost and effective solution suitable for resource-limited settings (Caragata et al., 2020). Furthermore, community-based programs that involve local populations in the application and monitoring of biopesticides can enhance the sustainability and effectiveness of mosquito control efforts (Faierstein et al., 2019).

Integrating biopesticides into mosquito management programs can significantly enhance control strategies by combining them with other methods like larviciding and habitat management. However, challenges in scaling up production and application must be addressed to ensure consistent efficacy. In resource-limited settings, adopting cost-effective and locally accessible biopesticides, along with community involvement, can improve the sustainability and success of mosquito control initiatives.

7 Future Directions

7.1 Innovations in biopesticide discovery and formulation

The development of new biopesticides is crucial for effective mosquito control, especially given the increasing resistance to traditional chemical insecticides. Recent studies have highlighted the potential of microbial biopesticides, such as those derived from *Chromobacterium sp. Panama*, which have shown high efficacy against mosquito larvae without leading to resistance development (Engdahl et al., 2023). These biopesticides can be formulated into stable, non-live preparations that maintain their effectiveness under various environmental conditions, making them suitable for field applications (Caragata et al., 2020). Additionally, exploring fungal-based biopesticides, such as those from the genus Isaria, offers another promising avenue due to their eco-friendly nature and high pathogenicity against mosquitoes (Ramirez et al., 2018). Future research should focus on identifying and optimizing new microbial and fungal strains, improving formulation techniques to enhance stability and efficacy, and conducting large-scale field trials to validate their effectiveness under natural conditions.

7.2 Addressing resistance development in mosquito populations

Resistance to chemical insecticides is a significant challenge in mosquito control programs. Studies have shown that biopesticides, such as those derived from *Chromobacterium sp. Panama*, do not select for resistance in mosquito populations, making them a valuable tool in resistance management strategies (Engdahl et al., 2023). Additionally, combining biopesticides with chemical insecticides can exploit the vulnerabilities of resistant mosquito strains, as seen with the increased mortality of chlorpyrifos-resistant mosquitoes when exposed to *Bacillus thuringiensis* israelensis (Bti) (Delnat et al., 2019). Future efforts should include monitoring resistance patterns in mosquito populations, developing biopesticides with novel modes of action, and integrating biopesticides into existing vector control programs to delay the onset of resistance. Furthermore, innovative approaches like Late Life Acting (LLA) insecticides, which target older mosquitoes, can reduce the selective pressure for resistance and prolong the effectiveness of control measures (Heintzelman et al., 2021).

7.3 Enhancing public awareness and policy support

Effective mosquito control requires not only scientific advancements but also public awareness and policy support. Increasing public understanding of the benefits and safety of biopesticides can lead to greater acceptance and use in communities affected by mosquito-borne diseases. Educational campaigns should highlight the advantages of biopesticides, such as their eco-friendliness and reduced risk of resistance development (Ramirez et al., 2018; Namias et al., 2021). Additionally, policymakers need to be informed about the importance of supporting research and development of new biopesticides and implementing guidelines for their use. Practical insecticide-resistance monitoring guidelines are essential to ensure the efficacy of vector control programs and to adapt strategies based on local resistance patterns. Collaborative efforts between researchers, public health officials, and policymakers can facilitate the integration of biopesticides into national and international mosquito control strategies, ultimately reducing the burden of mosquito-borne diseases.

8 Concluding Remarks

The development and testing of new biopesticides for mosquito control have yielded several promising results. The novel preparation derived from *Chromobacterium sp. Panama* (Csp_P) has demonstrated high efficacy in killing mosquito larvae, including those resistant to conventional insecticides. This preparation, which is an air-dried powder containing no live bacteria, has shown broad-spectrum activity against various mosquito species responsible for transmitting diseases such as malaria, dengue, chikungunya, yellow fever, West Nile, and Zika viruses. It is effective at low dosages, retains activity under high temperatures, and has a long shelf life, making it a viable tool for mosquito control.

Additionally, studies have shown that exposure to the Csp_P biopesticide does not lead to resistance in *Aedes aegypti* mosquitoes, even after multiple generations. This is a significant finding as it suggests the long-term viability of this biopesticide in mosquito control programs. Another biopesticide, PONNEEM, derived from the oils of *Azadirachta indica* and *Pongamia glabra*, has also shown 100% larvicidal and ovicidal activities against *Anopheles stephensi* and *Culex quinquefasciatus*, with no adverse effects on non-target organisms.Moreover, the integration of biopesticides with other control methods, such as the sterile insect technique (SIT), has been shown to significantly enhance the efficiency of mosquito population control. Mathematical models suggest that boosting SIT with biopesticides can reduce the required release rates of sterile males by over 95%, providing a powerful tool for managing mosquito-borne diseases.

The development and application of sustainable biopesticides offer a promising solution for effective and environmentally friendly mosquito control. Researchers should focus on exploring biopesticides from diverse natural sources, such as sponges and their symbionts, which have shown potential for mosquitocidal compounds. Long-term efficacy studies and investigations into synergistic approaches, such as combining biopesticides with the Sterile Insect Technique (SIT) or predatory organisms, are crucial to enhancing control strategies. Policymakers can support this effort by providing regulatory approval, funding research and development, and raising public awareness about the benefits of biopesticides. Stakeholders are encouraged to adopt biopesticides within integrated pest management programs to reduce dependence on chemical insecticides, collaborate with researchers for field testing, and invest in training for effective implementation. In conclusion, leveraging biopesticides alongside existing methods presents a sustainable pathway to combat mosquito-borne diseases, requiring continued research, supportive policies, and active stakeholder engagement.

Acknowledgments

We are grateful to Dr. Wu for critically reading the manuscript and providing valuable feedback that improved the clarity of the text. We express our heartfelt gratitude to the two anonymous reviewers for their valuable comments on the manuscript.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Benelli G., Jeffries C., and Walker T., 2016, Biological control of mosquito vectors: past present and future, Insects, 7(4): 52.
https://doi.org/10.3390/insects7040052

Benelli G., Maggi F., Pavela R., Murugan K., Govindarajan M., Vaseeharan B., Petrelli R., Cappellacci L., Kumar S., Hofer A., Youssefi M., Alarfaj A., Hwang J., and Higuchi A., 2018, Mosquito control with green nanopesticides: towards the one health approach? a review of non-target effects, Environmental Science and Pollution Research, 25: 10184-10206.
https://doi.org/10.1007/s11356-017-9752-4

Bora B., Mandodan S., Lukose J., Gangmei K., Sivaprakasam M., Padmanaban H., K A., Vijayakumar A., Ashokkumar M., Krishnamoorthy V., and Subbiah P., 2022, Mosquito-borne diseases their impacts and mosquito vector control methods-A review, International Journal of Science and Technology Research Archive, 67(13): 1209-1220.
https://doi.org/10.53771/ijstra.2022.3.2.0112

Brito-Sierra C., Kaur J., and Hill C., 2019, Protocols for testing the toxicity of novel insecticidal chemistries to mosquitoes, Journal of Visualized Experiments: JoVE, 144: 21.
https://doi.org/10.3791/57768

Buckingham S., Partridge F., Poulton B., Miller B., McKendry R., Lycett G., and Sattelle D., 2021, Automated phenotyping of mosquito larvae enables high-throughput screening for novel larvicides and offers potential for smartphone-based detection of larval insecticide resistance, PLoS Neglected Tropical Diseases, 15(6): e0008639.
https://doi.org/10.1371/journal.pntd.0008639

Caragata E., Otero L., Carlson J., Dizaji N., and Dimopoulos G., 2020, A nonlive preparation of *Chromobacterium sp*., *Panama* (Csp_P) is a highly effective larval mosquito biopesticide, Applied and Environmental Microbiology, 86(11): e00240-20.
https://doi.org/10.1128/AEM.00240-20

Delnat V., Tran T., Janssens L., and Stoks R., 2019, Resistance to a chemical pesticide increases vulnerability to a biopesticide: Effects on direct mortality and mortality by predation, Aquatic toxicology, 216: 105310.
https://doi.org/10.1016/j.aquatox.2019.105310

Engdahl C., Caragata E., Tavadia M., and Dimopoulos G., 2023, Chromobacterium biopesticide exposure does not select for resistance in *Aedes* mosquitoes, mBio, 14(2): e00480-23.
https://doi.org/10.1128/mbio.00480-23

Faierstein G., Lu W., Sena A., Barbosa R., and Leal W., 2019, Conspecific and allospecific larval extracts entice mosquitoes to lay eggs and may be used in attract-and-kill control strategy, Scientific Reports, 9(1): 13747.
https://doi.org/10.1038/s41598-019-50274-1

Heintzelman J., and Madey G., 2021, Case study: end user development of an agent-based model of malaria transmission to support the design of late-life-acting insecticides for the control of malaria transmission and delay of the evolution of insecticide resistance, medRxiv, 5(28): 21257999.
https://doi.org/10.1101/2021.05.28.21257999

Hernandez-Tenorio F., Miranda A., Rodríguez C., Giraldo-Estrada C., and Sáez A., 2022, Potential strategies in the biopesticide formulations: a bibliometric analysis, Agronomy, 12(11): 2665.
https://doi.org/10.3390/agronomy12112665

Kalimuthu K., Panneerselvam C., Chou C., Tseng L., Murugan K., Tsai K., Alarfaj A., Higuchi A., Canale A., Hwang J., and Benelli G., 2017, Control of dengue and Zika virus vector *Aedes aegypti* using the predatory copepod *Megacyclops formosanus*: Synergy with Hedychium coronarium-synthesized silver nanoparticles and related histological changes in targeted mosquitoes, Process Safety and Environmental Protection, 109: 82-96.
https://doi.org/10.1016/J.PSEP.2017.03.027

Kumar K., Sridhar J., Murali-Baskaran R., Senthil-Nathan S., Kaushal P., Dara S., and Arthurs S., 2019, Microbial biopesticides for insect pest management in India: Current status and future prospects, Journal of Invertebrate Pathology, 165: 74-81.
https://doi.org/10.1016/j.jip.2018.10.008

Lee H., Shields M., Landeta A., Saldana M., Fredregill C., and Pietrantonio P., 2023, Evaluation of field resistance in field-collected mosquito *Culex quinquefasciatus* Say through quantification of ULV permethrin/PBO formulation in field bioassays, Pest Management Science, 79(10): 3934-3949.
https://doi.org/10.1002/ps.7587

Lees R., Praulins G., Davies R., Brown F., Parsons G., White A., Ranson H., Small G., and Malone D., 2019, A testing cascade to identify repurposed insecticides for next-generation vector control tools: screening a panel of chemistries with novel modes of action against a malaria vector, Gates Open Research, 3: 1464.
https://doi.org/10.12688/gatesopenres.12957.2

Liu N., Wang Y., and Li T., 2023, Assays to measure insecticide toxicity and insecticide resistance in mosquitoes, Cold Spring Harbor Protocols, 2023(7): 107705.
https://doi.org/10.1101/pdb.top107705

Lu H., Sui Y., Lobo N., Fouque F., Gao C., Lu S., Lv S., Deng S., and Wang D., 2023, Challenge and opportunity for vector control strategies on key mosquito-borne diseases during the COVID-19 pandemic, Frontiers in Public Health, 11: 1207293.
https://doi.org/10.3389/fpubh.2023.1207293

Maheswaran R., and Ignacimuthu S., 2015, A novel biopesticide PONNEEM to control human vector mosquitoes *Anopheles stephensi* L., and *Culex quinquefasciatus* say, Environmental Science and Pollution Research, 22: 13153-13166.
https://doi.org/10.1007/s11356-015-4586-4

Mathivanan A., Ravikumar S., and Selvakumar G., 2019, Bioprospecting of sponge and its symbionts: New tool for mosquitocidal and insecticidal metabolites, Biocatalysis and Agricultural Biotechnology, 19: 101158.
https://doi.org/10.1016/J.BCAB.2019.101158

Mishra P., Tyagi B., Chandrasekaran N., and Mukherjee A., 2018, Biological nanopesticides: a greener approach towards the mosquito vector control, Environmental Science and Pollution Research, 25: 10151-10163.
https://doi.org/10.1007/s11356-017-9640-y

Mnif I., and Ghribi D., 2015, Potential of bacterial derived biopesticides in pest management, Crop Protection, 77: 52-64.
https://doi.org/10.1016/J.CROPRO.2015.07.017

Namias A., Jobe N., Paaijmans K., and Huijben S., 2021, The need for practical insecticide-resistance guidelines to effectively inform mosquito-borne disease control programs, eLife, 10: e656550.
https://doi.org/10.7554/eLife.65655

Ogunah J., Lalah J., and Schramm K., 2020, Malaria vector control strategies., What is appropriate towards sustainable global eradication?, Sustainable Chemistry and Pharmacy, 18: 100339.
https://doi.org/10.1016/j.scp.2020.100339

Ohia C.M.D., and Ana G., 2015, Bio-insecticides: the one-health response to mosquito-borne diseases of public health importance, J. Biol. Agric. Healthc., 5: 22-26.

Onen H., Luzala M., Kigozi S., Sikumbili R., Muanga C., Zola E., Wendji S., Buya A., Balčiūnaitienė A., Viškelis J., Kaddumukasa M., and Memvanga P., 2023, Mosquito-borne diseases and their control strategies: an overview focused on green synthesized plant-based metallic nanoparticles, Insects, 14(3): 221. https://doi.org/10.3390/insects14030221

Pavela R., Maggi F., Iannarelli R., and Benelli G., 2019, Plant extracts for developing mosquito larvicides: From laboratory to the field with insights on the modes of action, Acta Tropica, 193: 236-271. https://doi.org/10.1016/j.actatropica.2019.01.019

Pleydell D., and Bouyer J., 2019, Biopesticides improve efficiency of the sterile insect technique for controlling mosquito-driven dengue epidemics, Communications Biology, 2(1): 201. https://doi.org/10.1038/s42003-019-0451-1

Ramirez J., Muturi E., Dunlap C., and Rooney A., 2018, Strain-specific pathogenicity and subversion of phenoloxidase activity in the mosquito *Aedes aegypti* by members of the fungal entomopathogenic genus Isaria, Scientific Reports, 8(1): 9896. https://doi.org/10.1038/s41598-018-28210-6

Shen D., Nyawira K., and Xia A., 2020, New discoveries and applications of mosquito fungal pathogens, Current Opinion in Insect Science, 40: 111-116. https://doi.org/10.1016/j.cois.2020.05.003

Unlu I., Faraji A., Williams G., Marcombe S., Fonseca D., and Gaugler R., 2018, Truck-mounted area-wide applications of larvicides and adulticides for extended suppression of adult *Aedes* albopictus, Pest Management Science, 75(4): 1115-1122. https://doi.org/10.1002/ps.5227