J. Sylhet Agril. Univ. 11(2): 19-28, 2024 **DOI: https://doi.org/10.3329/jsau.v11i2.82715**

ISSN: 2308-1597

Journal of the Sylhet Agricultural University

Journal home page: http://www.jsau.sau.ac.bd

Review Paper

MICROBIAL AND PHYTOCHEMICAL APPROACHES TO MOSQUITO CONTROL: A REVIEW FOCUSING ON *LYSINIBACILLUS SPHAERICUS* AND *SPHAGNETICOLA TRILOBATA*

NA Rani[1,2], TB Robin[1,2], FMA Hossain[3,4], M Hasanuzzaman[5], KMA Zinnah[*,1,2]

[1]Faculty of Biotechnology and Genetic Engineering, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[2]Department of Animal and Fish Biotechnology, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[3]Faculty of Veterinary, Animal and Biomedical Science, Sylhet Agricultural University, Bangladesh

[4]Department of Dairy Science, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[5]Cytology and Biocontrol Research (CBR), Radiation Entomology and Acarology Division (READ), Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Commission

Article info

Article history

Received: 24.09.2024

Accepted: 23.11.2024

Published: 30.12.2024

Keywords

Mosquito, Microbial, Phytochemical, Toxin, Environment

***Corresponding author**

Kazi Md. Ali Zinnah
E-mail:
zinnah.afb@sau.ac.bd

Abstract

Mosquito-borne diseases such as malaria, dengue, Zika virus, chikungunya, and yellow fever continue to pose significant threat for global public health. Traditional chemical pesticides, is somewhat effective to control mosquito populations, but has severe environmental concerns, such as damaging ecology, harmful for beneficial non-target animals, and an alarming increase in insecticide-resistant mosquito strains. Our review sheds light on microbiological and phytochemical approaches to mosquito control as effective and environmentally sustainable option. *Lysinibacillus sphaericus*. A gram-positive soil bacterium has great effectiveness as a biocontrol candidate because it generates specific larvicidal toxins such as the binary toxin complex (BinA and BinB) and mosquitocidal toxins (Mtx). These poisons selectively target mosquito larvae midgut cells and have no major impact on non-target species, making *L. sphaericus* a vital part in integrated vector management schemes. Phytochemical approaches disrupt crucial mosquito life cycle pathways using plant-derived bioactive chemicals. *Sphagneticola trilobata*, a widely distributed tropical plant, has demonstrated substantial potential in mosquito control as it contains a great range of bioactive compounds, including flavonoids, terpenoids, saponins, and phenolic acids. These substances have larvicidal, insecticidal, and repellant activities through an involvement with mosquito digestion, hormonal balance, and neural pathways. *S. trilobata* extracts are also a sustainable, biodegradable, and environmentally friendly alternative to synthetic insecticides. This analysis highlights *L. sphaericus* and *S. trilobata's* promise as eco-friendly and effective solutions to the expanding mosquito control difficulties by investigating their mechanisms of action, practical uses, and environmental advantages.

Introduction

Mosquitoes are one of the crucial human disease vectors, that transmits germs causing malaria, dengue fever, Zika virus, chikungunya, and yellow fever. These complications have a substantial global health effect, primarily in tropical and subtropical regions where they result in greater morbidity and mortality rates. Malaria is the reason behind hundreds of thousands of death every year, small children and pregnant women are mostly effected. The impact of diseases caused by mosquito demands the urgent requirement for efficient vector control strategies (Chandra and Bhattacharjee, 2024, Patel et al., 2024). Chemical pesticides have usually been the center point for mosquito control, that cause an instant decreases in mosquito populations. However, the overuse of these synthetic pesticides has raised significant ecological and public health concern, involving environmental damage, harm to beneficial non-target creatures, and an alarming increase in insecticide-resistant mosquito strains (Rani et al., 2021).

Cite This Articale

Rani NA, Robin TB, Hossain FMA, Hasanuzzaman M and Zinnah KMA. 2024. Microbial and Phytochemical Approaches to Mosquito Control: A Review Focusing on Lysinibacillus Sphaericus and Sphagneticola Trilobata. J. Sylhet Agril. Univ. 11(2): 19-28, 2024. https://doi.org./...

Mosquito control is an escalating problem today due to the rise of mosquito-borne diseases. This has led the focus of research towards identifying novel, sustainable, and eco-friendly means of controlling mosquitos. Microbiological and plant phytochemicals have been suggested as such remedies. These approaches select the susceptible stage of mosquitos using microorganisms and plant origin chemicals having minimal environmental or non-target animal disturbances. Microbes such as *Lysinibacillus sphaericus* and plant derived *Sphagneticola trilobata* poison have proven efficacy in controlling mosquito populations. Their effectiveness, biological origins, and environment friendly nature may substitute traditional pesticides (Shajahan et al., 2022, McMillan et al., 2022, Sowmyashree et al., 2015).

Lysinibacillus sphaericus, an insecticidal bacterium found in nature, has been the center of many studies due to its larvicidal activity. It produces toxins that attack the midgut cells of mosquito larvae, mainly those of the Culex, Anopheles and Aedes genera. These toxins are highly specific and do not cause any severe effects to non-target creatures such as plants and fish. Moreover, the ability of *L. sphaericus* to survive in water bodies for long period of times increases its effectiveness as a control agent. Its mode of action is also quite distinct from chemical pesticides, which helps circumvent cross resistance – a threat that renders it a great asset in vector control integrated programs (Jamal and Ahmad, 2022, Filha et al., 2014).

Phytochemicals are another source from the plant kingdom for an abundance of bioactive materials. The creeping dicotyledonous *Sphagneticola trilobata* widely spread around tropical and subtropical regions has also been studied for its effective mosquito control capacity. This plant also produces larvicidal, insecticide and repellent bioactive materials such as flavonoids, terpenoids and phenolic acids. These compounds interfere with specific physiological functions in mosquitos such as neuronal signaling, digestive enzyme action and cuticle formation. It should also be noted that plant-based interventions like *S. trilobata* are sustainable, eco-friendly and with low toxicity levels to humans and non-targeted animals which make them important in integrated mosquito control programs (Shajahan et al., 2022, Azmee, 2014, Yooboon et al., 2019).

This study investigated the bacterial and Phytochemical approaches to mosquito control, especially focusing on *Lysinibacillus sphaericus* and *Sphagneticola trilobata*; such focus would delve into the mechanisms, efficacy, and practical applications of these agents to show that they can be used as environmentally benign and effective alternatives to chemical insecticides.

Methodology

An extensive literature review was conducted using data available on pubmed server (https://pubmed.ncbi.nlm.nih.gov/). The keywords used to search research regarding mosquito controls are: "*Lysinibacillus, Lysinibacillus sphaericus, Mosquito control, Biological control, Vector control, Plant derivatives, Biological agent, Biocontrol Resistant, Mosquito management, Sphagneticola trilobata".* The number of datasets found upon search were illustrated at **Figure 1.** These literature studies were reviewed and relevant data focusing on the microbial and phyto-control agent of mosquitoes were taken. The review mostly focused on the mechanism and potential of *Lysinibacillus* and *Sphagneticola trilobata* to control mosquito.

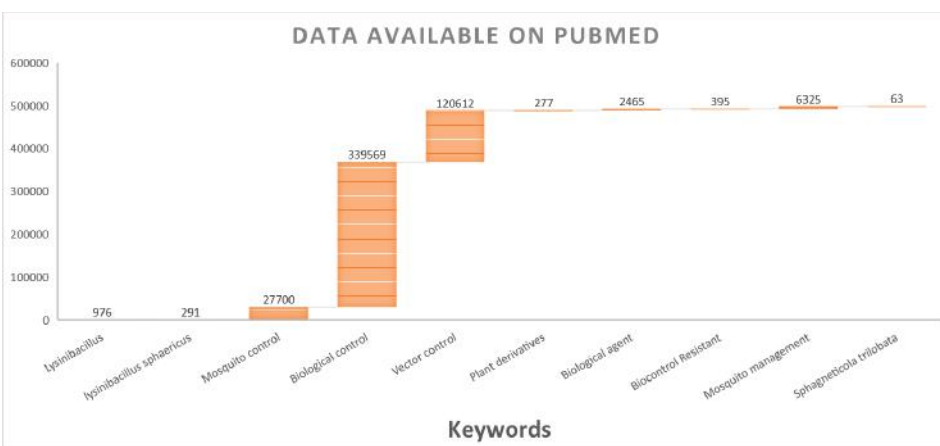

Figure 1. Keywords and available dataset found regarding the study

Results

Microbial Approaches of Mosquito Control

Microbial control agents' bacteria, fungi, viruses, and protozoa can affect mosquito life cycle. The main idea behind this is to use microorganisms or their byproducts to target mosquitos in an environmentally friendly and sustainable way. The two species particularly well-documented about being strong larvicides are *Lysinibacillus sphaericus* and *Bacillus thuringiensis* (Bt). These bacteria produce crystal proteins (toxins), which selectively act on the midgut cells in mosquito larvae; lysis of these cells leads to death. Selectivity lowers the damage done to the non-target organisms at the same time as environmental damage is minimized. Their occurrence in water makes them long-lasting, therefore effective and best suited for integrated vector management program. Also, microbial methods solve the problem of pesticide resistance by providing safer alternatives to chemical insecticides in mosquito control (Katak et al., 2023, Dalal et al., 2024).

Table 1. The microbial approaches of mosquito control

Microbial Agent	Example	Target Phase	Mode of Action	Advantages	References
Bacteria	*Lysinibacillus sphaericus*	Larvae	Produces the midgut cells-disturbing binary toxins BinA and BinB.	Very specific; environment-friendly; minimal non-target impact	(Berry, 2012)
	Bacillus thuringiensis	Larvae	Generate cytolytic and δ-endotoxins that target midgut cells.	Works at low doses; compatible Good for integrated pest management	(Land et al., 2023)
Fungi	*Metarhizium anisopliae*	Larvae, Adults	Causes systemic infection in mosquitoes by penetrating their cuticles.	Diverse host range; eco friendly	(Tiago et al., 2014)
	Beauveria bassiana	Larvae, Adults	Causes enzymatic and mechanical disruption to infect the cuticle and kill insects.	Good effect against insecticide-resistant populations	(Tawidian et al., 2023)
Protozoa	*Vavraia culicis*	Larvae	Interferes with growth and development by infecting gut cells.	Long term infection; can complement other approaches	(Lorenz and Koella, 2011)
Microbial Symbionts	*Aspergillus niger*	Larvae	Generate development-impairing mycotoxins.	Potential for dual-use as biopesticide and biofertilizer	(Singh and Prakash, 2012)
Genetically Modified Bacteria	*Escherichia coli* (engineered strains)	Larvae	Designed to generate poisons unique to mosquitoes	Targeted action; adaptable for different mosquito species	(Katak et al., 2023)

Lysinibacillus sphaericus for Mosquito Control

The member of the Firmicutes phylum, belonging to Bacillaceae family, *Lysinibacillus sphaericus* is a gram-positive spore-forming soil bacteria evolved as a great weapon against arthropod-borne diseases like that of mosquitoes (Jamal and Ahmad, 2022). It has an active genome of over 4.6 million base pairs, encoding a vast range of insecticidal compounds. The binary toxin complex formed during sporulation between BinA and BinB proteins is the most potent against mosquito larvae (Kale et al., 2013). Interestingly, research indicates that *the L. sphaericus* strains possess additional mosquitocidal toxin (Mtx) genes, thus boosting larvicidal activity. These Mtx genes, along with the binary toxin complex, create a complex genetic regulatory network inside the *L. sphaericus* genome. The interplay among these genes, therefore, influences their efficacy for larvicidal activity (Wirth et al., 2007). Knowing the complexity of this network and the specific function of each toxin is essential to optimising *L. sphaericus* as a biological control agent. In addition, whole genome sequencing studies on several strains *of L. sphaericus* have identified plasmids that could harbor more genes linked to the insecticidal characteristics. Unraveling the significance of these plasmids may lead to the identification of new insecticidal mechanisms and the customisation of *L. sphaericus* for a wider spectrum of mosquito species control (Park et al., 2010).

Toxin Genes in L. sphaericus

The genome of L. sphaericus is very small, about 4.2-4.8 Megabase pairs (Mb) in length. This small size would allow it to replicate faster and perhaps even grow more quickly in mosquito breeding sites (Jamal and Ahmad, 2022). The key toxin genes and their effects involves:

Binary Toxin Complex (BinA and BinB)

Through the process of sporulation, L. sphaericus creates a potent binary toxin complex made up of the two proteins BinA and BinB (de Maagd et al., 2003). These proteins targets towards mosquito larvae by forming a pore allowing BinB to enter the cell by interaction of BinA to the receptors in the larval midgut epithelium (Sharma & Kumar, 2022). BinB inhibits essential cellular processes, causing larval death (Kale et al., 2013).

Mosquitocidal Toxin (Mtx) Genes

The confines of the genome of L. sphaericus have discovered additional toxin genes called mosquitocidal toxin genes. The Mtx genes primarily encode toxins, of which many sizes are above 100 kDa (Mohamed et al., 2023). The addition of Mtx genes improves the larvicidal activity of *L. sphaericus* strains by expanding the range of insecticidal action against mosquito larvae (Su & Trdan, 2016).

Mode of Action of L. sphaericus

It is also known that L. sphaericus, a spore-forming and gram-positive microorganism, produces great specific binary crystal toxin during the process of sporulation, an environmentally safe means of mosquito population control. This Bin toxin is encoded by the cry4Aa and cry4Ab gene, wherein the toxin consists of two polypeptides, the BinA (41.9 kDa) and the BinB (1.4 kDa) (Soberón et al. 2009). Serine proteinases are the ones activating Bin toxin in the midguts of larvae of mosquitoes. This toxin attacks the receptors on the brush border membranes of such species as Aedes and Culex. The apsA gene generates aminopeptidase N1 (APN1) proteins which are thought to include these receptors. Once bound, a cytopathic cascade is triggered that disrupts the midgut cell membranes and causes death of the larvae (Charles et al., 1996, Filha et al., 2014). This selective and precise mechanism of action demonstrates *L. sphaericus'* promise in mosquito control.

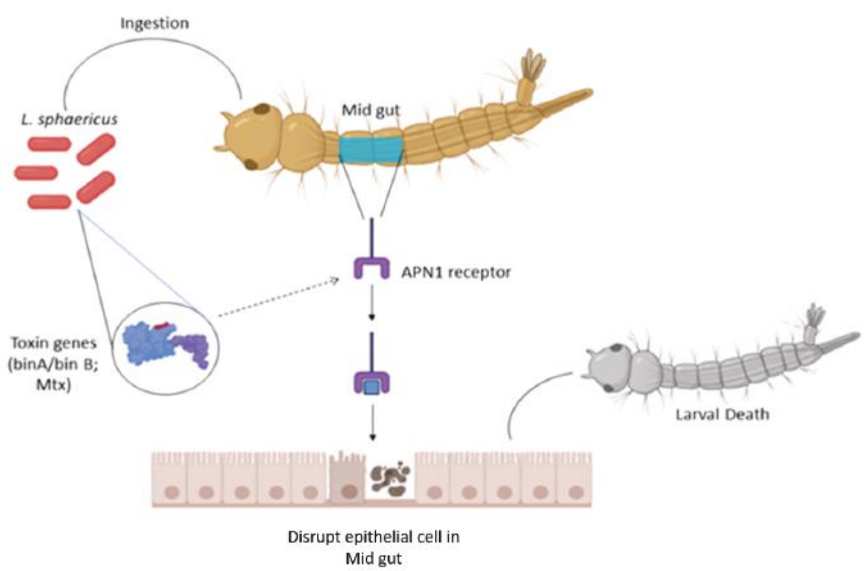

Figure 2. Mode of action of *L. sphaericus*

Phytochemical Approaches of Mosquito Control

Bioactive products of plants and employ them in sufficiently effective and sustainable ways to target mosquitoes is a great alternative. Many plant species produce secondary metabolites such as flavonoids, terpenoids, alkaloids, and phenolic acids, which have been discovered to have strong effects of larvicidal, insecticidal, and repellent actions (Shajahan et al., 2022). These bioactive substances manipulate the vital physiological processes in mosquitoes like brain signalling, digestion, and exoskeleton development, along with other processes, with the least interference to non-target animals. The plant extracts showed excellent effectiveness like *Sphagneticola trilobata*, which is killing mosquitoes' larvae and repelling the adults too. Phytochemical solutions being renewable, biodegradable, and less liable to develop resistance than synthetic insecticides are likely very attractive alternatives to use for environmentally friendly mosquito control intervention (Shajahan et al., 2022, Ghosh et al., 2012).

Table 2. The phytochemical approaches of mosquito control

Plant Source	Active Compound(s)	Target Phase	Mode of Action	Advantages	References
Neem	Azadirachtin, Nimbin, Salannin	Larvae, Adults	Prevents moulting and reproduction by upsetting the hormonal balance	Low toxicity to humans; repellent and larvicidal	(Kaura et al., 2019)
Citronella	Citronellal, Geraniol, Limonene	Adults	Act as a repellant and involves with mosquito sensory receptors	Natural and safe for humans; repellent impact	(Hsu et al., 2013)
Eucalyptus	Eucalyptol, Citronellol	Adults	Works as a repellant by interfering with mosquitoes' senses	Pleasant smell; Work against various species	(Navayan et al., 2017)
Lemongrass	Citral, Geraniol, Limonene	Adults	Uses sensory disturbance to create a repelling impact	Can be cultivated; biodegradable	(Zulfikar et al., 2019)
Lavender	Linalool, Camphor	Adults	Utilize sensory pathway disruption to act as a repellant	Non-toxic to humans and animals; nice smell	(Komansilan et al., 2021)
Sphagneticola trilobata	Flavonoids, Terpenoids	Larvae, Adults	Interferes with mosquito development, metabolism, and reproduction	Easily found; biodegradable	(Sowmyashree et al., 2015)

***Sphagneticola trilobata* for Mosquito Control**

Sphagneticola trilobata is popularly known as the Creeping Oxeye, Wedelia, or Singapore Daisy, is an ever-spreading perennial plant originating from Mexico, followed by Central America and the Caribbean. The plant is also known in other tropical and subtropical regions, as Africa and Australia. *Sphagneticola trilobata* is a low-growing herbaceous plant with a prostrate trailing stem that spread quickly to form dense mats. Its leaves are generally triseriate; thus the name of the species "trilobata," and dark green, having a glossy texture. The flower gives very beautiful daisies with a bright yellow-orange center. Flowering is abundant through the whole year, adding beautiful values to gardens and landscapes. Creeping Oxeye grows in many different environments--disturbed areas, roadsides, gardens, and agricultural land. In moist, well-drained soil, it prefers full sun and light shade. *Sphagneticola trilobata* leaves and stems are sources for traditional medicine and have been said to contain therapeutic powers (Macanawai, 2013, Hyun et al., 2021, Azmee, 2014).

Plant extracts of *Sphagneticola trilobata* are often directly used on conditions such as wounds, cuts, and infections owing to their antibacterial and anti-inflammatory actions. Gastrointestinal disturbances such as diarrhea and dysentery are treated by preparing teas or infusions with its leaves (Ali et al., 2024, Mardina et al., 2021). Phytochemical analysis revealed that the plant had an array of bioactive compounds including flavonoids, tannins, saponins, terpenoids, and alkaloids. These compounds confer medicinal potential on the plant as the flavonoids shows antioxidant and anti-inflammatory activities, tannins would provide astringent and wound healing properties, and saponins, terpenoids, and alkaloids may have some antibacterial activities

(Afzal and Rajesh, 2021, Mardina et al., 2021). It has been discovered that *Sphagneticola trilobata* extracts show broad-spectrum action against numerous Gram-positive and Gram-negative bacterium such as *Staphylococcus aureus, Escherichia coli*, as well as *Pseudomonas aeruginosa*. Antibacterial effects of these extracts target bacterial cell wall disruption, inhibition of protein synthesis, and interference with nucleic acid metabolism (Mardina et al., 2021, TOPPO et al., 2013). According to such description, *Sphagneticola trilobata* can be utilized in the healing of wounds and combatting infections. Studies have proved that several plant extracts are effective against mosquito larvae making the use of this particular plant a natural way of combatting mosquito infestation. *Sphagneticola trilobata* has bioactive compounds like flavonoids, terpenoids, and saponins, which are believed to play a role in its mosquitocidal activity (Sowmyashree et al., 2015, Poornima et al., 2024). Those plant extractions studied inflict mortality up to the significant levels in mosquito larvae, thus interrupting their development with reduced adult mosquito populations. Mechanisms include hormonal imbalance of the larvae, disturbance of cellular metabolism, and physical injury on the larval digestive systems by the active compounds (Ghosh et al., 2012).

Lemongrass (*Cymbopogon citratus*) and citronella (*Cymbopogon nardus*) are well known for their powerful mosquito-repelling abilities because they contain bioactive substances including limonene, geraniol, and citronellal that have high adulticidal and larvicidal activities. Essential oils derived from these plants have been found in studies to efficiently repel mosquitos and disrupt their reproduction cycles (Benelli et al., 2018). Sphagneticola trilobata, on the other hand, has also become a plant of promise with mosquitocidal properties. Research reveals that S. trilobata contains bioactive chemicals with insecticidal characteristics, displaying considerable impacts on mosquito larvae (Sowmyashree et al., 2015). While C. nardus and C. citratus have proven commercial applications in mosquito control, S. trilobata is a promising option that deserves more exploration for sustainable and environmentally friendly vector management.

Sphagneticola trilobata Active Compounds and Mechanism of Action

Sphagneticola trilobata is well-known for its bioactive, larvicidal, insecticidal, and repellent properties, making it a great candidate for greener options in mosquito control. The existing bioactive compounds exhibit activity with their isolated function on specific aspects of mosquito physiology and development, including flavonoids, terpenoids, phenolic compounds, steroids, essential oils, and saponins. Most notably, flavonoids such as quercetin and/or derivatives thereof can act as inducers of oxidative stress, leading to mortality via interference with the activity of enzymes. Terpenoids (monoterpenes and sesquiterpenes) act through neurotoxic mechanisms that impair brain signaling, resulting in paralysis and death (Boate and Abalis, 2020, Zuharah et al., 2021, Mony et al., 2022). The phenolic compounds gallic acid and tannins are known to affect the mosquitoes' survival by disrupting the process of cuticle larval production, further inducing dehydration and ultimately death (VERMA et al., 2024). Steroids act to inhibit the chitin production, vital for the formation of the exoskeleton,resulting into the blockage of the moulting of larvae (Lafont and Dinan, 2024). Essential oil works by disturbing the respiratory system of mosquito larvae while working as effective repellents to adult mosquitoes through masking smells of hosts and interfering with olfactory receptors through the aromatic chemicals eugenol and linalool (Pavela, 2015). Another active group of chemicals is saponins, that attacks cell membranes and cause damage in larval tissues (Chaieb, 2010).

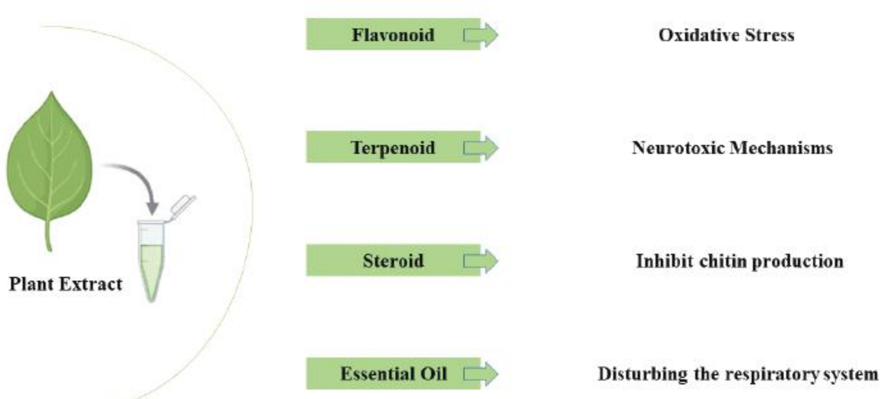

Figure 3. Mechanism of action of the active compounds of *Sphagneticola trilobata*

Larvicides made from extracts of *Sphagneticola trilobata* can be used in stagnant water bodies, which form mosquito breeding areas, and essential oils can be formulated into sprays, lotions, or incense-type products against adult mosquitos. The natural insect control measures become lucrative alternatives to chemical insecticides because they are biodegradable, pose lesser risks to non-target creatures, and are environmentally friendly. Nevertheless, the standardization of formulations remains problematic because the concentration of active components varies with the environment in which the plants are grown. Field studies are also required to verify effectiveness and safety when applied in real-world situations. More research is needed to isolate and purify the active ingredients, to study their peculiar molecular mechanisms and to evaluate any toxicological evidence to humans and other non-target groups for this to come true on *Sphagneticola trilobata*. It can also include field trials under varied environmental conditions to allow engineers to develop scalable and effective solutions. *Sphagneticola trilobata*, with its powerful bioactive chemicals and environmentally friendly characteristics, offers a promising option for long-term mosquito control measures.

Environmental factors affecting natural mosquito control strategy

The effectiveness of mosquito control methods based on plants and bacteria is significantly influenced by environmental factors. The growth and generation of bioactive compounds by mosquito-repellent plants such as citronella (Cymbopogon nardus) and Sphagneticola trilobata are directly influenced by the properties of the soil, such as pH, moisture content, and nutrient availability (Day, 2016, Karunaratne and Surendran, 2022). Soil microbial variety is crucial to the durability and efficacy of control measures, whereas nutrient-rich soil increases plant biomass and the production of essential oils, boosting their repellent qualities (Hamed et al., 2022, Basak et al., 2021). The effectiveness of mosquito control is also influenced by climate variables like temperature, humidity, and rainfall (Anoopkumar and Aneesh, 2022). While too much rain can wash away bacterial spores, decreasing their larvicidal activity, warmer temperatures increase the production of plant metabolites but may decrease bacterial persistence (Benelli et al., 2018, Anoopkumar and Aneesh, 2022). High humidity also promotes the production of essential oils and plant development, but it also expands the number of mosquito breeding grounds (Figueiredo et al., 2008). Another important consideration is exposure to sunlight, which can breakdown bacterial spores and encourage the manufacture of repellent chemicals in plants (Roberts and Paul, 2006). For long-term success, this treatment must be strategically applied in watery or shady habitats. The sustainability and effectiveness of natural mosquito management techniques can be greatly increased by using an integrated strategy that maximises these environmental elements.

Recent advancement and future aspects to combat resistance mechanism

Recent advances in combating insecticide resistance in mosquito control have resulted in the development of innovative and long-term strategies that prioritize efficacy while mitigating resistance evolution. Entomopathogenic bacteria such as Bacillus thuringiensis israelensis (Bti) and Lysinibacillus sphaericus remain important, with synergistic combinations of their toxins (e.g., Bin and Cyt1Aa) restoring efficacy in resistant populations (Silva-Filha et al., 2021, Bravo et al., 2017), and structural modifications such as PEGylation of BinA toxin improving stability and larvicidal activity (Sharma et al., 2017). Entomopathogenic nematodes, such as Romanomermis culicivorax, provide biological control by parasitizing mosquito larvae (Abagli and Alavo, 2019), while phytochemicals derived from plants like neem and eucalyptus provide environmentally friendly alternatives, which are frequently enhanced through nanoformulations (Khater, 2012). Genetic strategies, such as CRISPR-based gene drives and Wolbachia-based approaches, target mosquito populations at the molecular level (Wang et al., 2024), whereas the Sterile Insect Technique (SIT) uses sterile male releases to suppress wild populations. Integrated vector management combines these methods with rotation and mosaic strategies to delay resistance (Bouyer, 2024), which are supported by advanced monitoring techniques such as molecular markers and standardized bioassays (Oliva et al., 2021). Behavioral and physical controls, such as appealing toxic sugar baits and acoustic traps, use mosquito biology to deliver targeted interventions (Torto and Tchouassi, 2024). Mosquito management programs can adapt to resistance challenges by combining these diverse approaches biological agents, phytochemicals, genetic tools, and innovative physical controls to ensure effective and long-term disease prevention tailored to local ecological context.

Conclusion

Diseases that are transmitted by mosquito have now had a considerable influence on global health and have made way for new environmental strategies and sustainable methods of controlling these diseases and their vectors. *L. sphaericus* provides specific larvicidal toxins such as the binary toxin complex: BinA and BinB, besides mosquitocidal metabolites (Mtx), acts as a great sustainable alternative for mosquito population reduction. Same with bioactive chemicals like flavonoids, terpenoids, saponins, phenolic acids in *S. trilobata*, provides eco-sustainability option for disrupting mosquito growth.

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