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REVIEW OF HERBAL PLANTS FOR LARVICIDAL ACTIVITY

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ABSTRACT

Mosquito-borne diseases remain a major public health concern worldwide, particularly in tropical and subtropical regions where mosquito vectors such as *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* contribute to the transmission of diseases including malaria, dengue, chikungunya, filariasis, and other viral infections. The continuous use of synthetic insecticides has resulted in environmental contamination, toxicity to non-target organisms, and development of resistance in mosquito populations. Therefore, plant-based larvicides have gained increasing attention as eco-friendly and sustainable alternatives for mosquito vector control. This review summarizes the materials and methods commonly used in studies evaluating the larvicidal activity of medicinal plant extracts such as *Azadirachta indica* Neem, *Curcuma aromatica* Wild turmeric, and *Camellia sinensis* Tea. The reviewed procedures include collection and processing of plant materials, preparation of methanolic extracts, preliminary phytochemical screening, collection and rearing of mosquito larvae, larvicidal bioassay, and larval susceptibility testing according to standard protocols. Plant extracts are generally prepared by shade drying, powdering, methanolic extraction, filtration, evaporation, and refrigerated storage. Fourth instar larvae are commonly exposed to different extract concentrations, and mortality is recorded after 24 and 48 hours. The percentage mortality is corrected using Abbott's formula when required, while LC_{50} and LC_{90} values are calculated by probit analysis. The reviewed studies indicate that standardized bioassay methods are essential for comparing larvicidal efficacy among plant extracts. Overall, herbal larvicides show strong potential as natural, biodegradable, and environmentally safer agents for integrated mosquito vector management.

INTRODUCTION

Mosquitoes are among the most important medically significant vectors responsible for the transmission of several life-threatening diseases, including malaria, dengue fever, yellow fever, chikungunya, Zika fever, and filariasis. These diseases continue to pose major public health challenges across the world, particularly in tropical and subtropical regions where climatic conditions favour mosquito breeding and survival. Mosquitoes transmit a wide range of parasites and pathogens that cause severe morbidity and mortality in human beings as well as animals. As a result, mosquito-borne diseases remain one of the greatest global health concerns.

Mosquito-borne diseases have affected human populations since ancient times and continue to create serious health and economic burdens. These diseases are endemic in more than 100 countries and are responsible for nearly two million deaths every year. It has also been estimated that about 2100 million people worldwide are at risk of mosquito-borne infections. In India, several regions are vulnerable to mosquito-borne diseases, with 17 states and six union territories reported as endemic areas. Approximately 553 million people in India are exposed to the risk of infection, making mosquito control an important public health priority [1-2].

Control of mosquito populations is essential for reducing the spread of vector-borne diseases. Different developmental stages of mosquitoes, including eggs, larvae, pupae, and adults, can be targeted for control. Among these, the larval stage is considered an effective target because larvae are confined to aquatic breeding sites and can be controlled before they develop into adult mosquitoes. Generally, mosquito control strategies include killing adult mosquitoes, using repellents to prevent mosquito bites, and reducing mosquito population density by targeting breeding and larval habitats [3].

The repeated use of synthetic chemical insecticides has led to several problems, including environmental pollution, toxic effects on non-target organisms, and development of resistance in mosquito populations. Therefore, there is a growing need for safer, biodegradable, cost-effective, and target-specific alternatives. Plant-based products have gained considerable attention as eco-friendly mosquito control agents because they are naturally derived, biodegradable, and generally safer for humans and non-target organisms. Plant extracts contain bioactive compounds with proven insecticidal and larvicidal potential, making them suitable candidates for use in integrated vector management programs [4].

Diseases Transmitted by Mosquitoes

Mosquitoes are responsible for spreading a number of serious diseases, especially during the monsoon season when stagnant water provides favourable breeding conditions. These diseases are caused either by viruses or parasites and vary in symptoms, severity, and the mosquito species involved. Some of the major mosquito-borne diseases include dengue, malaria, chikungunya, and Zika fever [3-4].

Dengue is a viral disease caused by the dengue virus and is mainly transmitted by ***Aedesaegypti***. This mosquito usually bites during the daytime and breeds in clean stagnant water. Dengue commonly presents with sudden high fever, severe headache, pain behind the eyes, muscle and joint pain, weakness, and skin rash. In severe cases, dengue may progress to dengue haemorrhagic fever or dengue shock syndrome, which can cause bleeding, low platelet count, fluid leakage, and life-threatening complications.

Malaria is a parasitic disease caused by ***Plasmodium*** species and transmitted by infected female ***Anopheles*** mosquitoes. It is characterized by fever, chills, sweating, headache, body aches, and fatigue. If not treated promptly, malaria can lead to serious complications such as anaemia, kidney damage, and cerebral malaria. The severity of malaria depends on the species of parasite involved and the immune status of the infected person.

Chikungunya is another mosquito-borne viral disease transmitted mainly by ***Aedes*** mosquitoes. It is associated with sudden fever and severe joint pain, which may persist for weeks or months. Other symptoms may include headache, muscle pain, nausea, fatigue, and skin rash. Although chikungunya is rarely fatal, it can significantly affect quality of life due to prolonged joint discomfort and weakness.

Zika fever is also transmitted primarily by ***Aedes*** mosquitoes. It usually causes mild symptoms such as low-grade fever, rash, red eyes, muscle pain, and joint pain. However, Zika infection is of special concern during pregnancy because it may be transmitted from mother to fetus and can cause congenital abnormalities such as microcephaly. Therefore, control of mosquito breeding and prevention of mosquito bites are essential strategies for reducing the transmission of Zika and other mosquito-borne diseases.

Overall, mosquito control remains a major requirement for preventing vector-borne diseases. The use of plant-based larvicidal agents provides a promising approach for sustainable mosquito management. Herbal larvicides may reduce dependence on synthetic insecticides and support safer, eco-friendly, and effective vector control strategies.

Life Cycle of Mosquitoe with Stages [4]

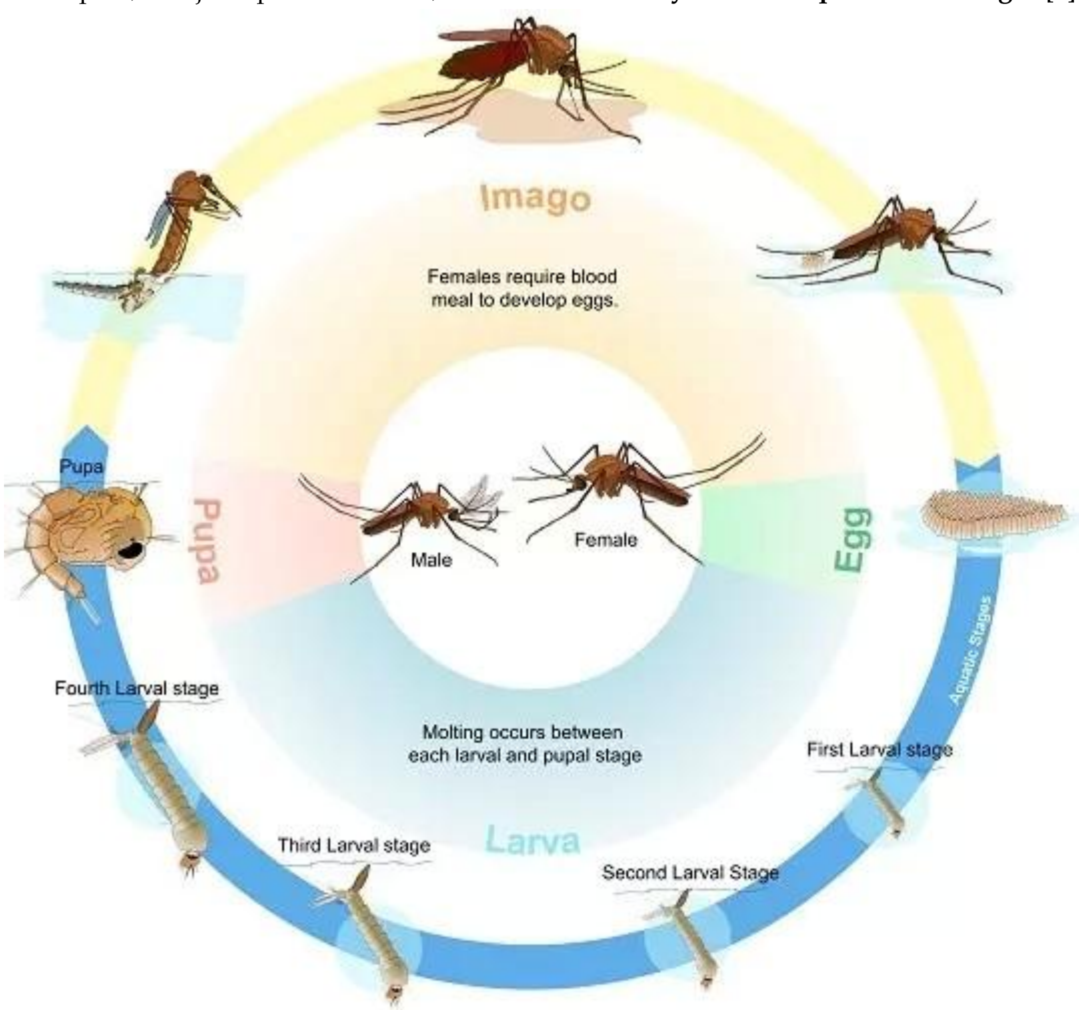

Figure 1: Mosquito life cycle

The life cycle of a mosquito consists of four main stages: egg, larva, pupa, and adult. The first stage is the egg stage (Figure 1). Some mosquitoes lay their eggs directly on the surface of water, while others lay them on the sides of containers, moist soil, or damp areas that are likely to become flooded. Water is essential for mosquito eggs to hatch. After hatching, the mosquito enters the larval stage. Mosquito larvae live completely in water and are commonly known as "wigglers" because of their wriggling movement. During this stage, the larvae pass through four growth stages called instars. They feed on small organisms and organic matter present in water, such as plankton, fungi, bacteria, and algae [5].

After the larval stage, the mosquito develops into the pupal stage. Mosquito pupae also live in water, but they do not feed during this period. Unlike the pupae of many other insects, mosquito pupae are active and can move or swim in water. This stage is a transitional stage before the mosquito becomes an adult. Finally, the adult mosquito emerges from the pupal case and leaves the water. Both male and female adult mosquitoes feed on nectar for energy. However, only female mosquitoes bite humans or animals because they require blood protein for the development of their eggs. During blood feeding, female mosquitoes may acquire pathogens or arboviruses from infected hosts. After the infection reaches their salivary glands, which may take about two weeks, they can transmit the pathogens to humans or animals through their bite. Therefore, the adult female mosquito is the main stage responsible for spreading mosquito-borne diseases.

The larval stage of a mosquito is an aquatic and legless stage that lives in water. Mosquito larvae are commonly called "wigglers" because they move in a wriggling manner. They usually remain near the surface of water, where they breathe air through a tube-like structure known as a siphon. The siphon works like a snorkel and allows the larva to obtain oxygen from the air while staying in water. During this stage, larvae feed on microorganisms and organic particles present in the water, including bacteria, algae, fungi, and plankton. As the larva grows, it undergoes moulting four times, passing through four larval stages known as instars. After completing these stages, the larva changes into the pupal stage, which is the next step before becoming an adult mosquito.

Wild ginger *(Zingiber zerumbet)* [6]

Wild ginger, botanically known as *Zingiber zerumbet* (L.) Smith, belongs to the family Zingiberaceae (Figure 2). It is also commonly known as bitter ginger or shampoo ginger. This plant has been studied for its larvicidal activity against different mosquito species due to the presence of essential oils and several bioactive phytochemical constituents. The larvicidal potential of *Zingiber zerumbet* is mainly attributed to zerumbone, which is a major sesquiterpene compound present in its rhizome essential oil. Other important compounds reported in wild ginger include α-pinene, camphene, borneol, 1,8-cineole, limonene, and β-caryophyllene. These compounds may contribute to the toxic effect against mosquito larvae by interfering with their growth, development, and survival. *Zingiber zerumbet* has shown larvicidal activity against important mosquito vectors such as *Aedesaegypti*, which transmits dengue, *Anopheles stephensi*, which transmits malaria, and *Culexquinquefasciatus*, which is associated with filariasis (Table 1). Therefore, wild ginger may be considered a promising plant-based larvicidal agent for eco-friendly mosquito control programs.

Figure 2: Wild ginger

Table 1: Mechanism of Larvicidal Action of *Zingiber zerumbet*

Mechanism	Brief Description
Neurotoxic effect	Zerumbone and terpenes inhibit acetylcholinesterase, causing nerve overstimulation, paralysis, and larval death.
Cuticle disruption	Essential oils damage the larval cuticle and lipid layer, causing dehydration and osmotic imbalance.
Oxidative stress	Zerumbone increases reactive oxygen species, leading to mitochondrial damage and cell death.
Growth inhibition	Bioactive compounds may disturb moulting hormones, causing delayed development and failure of pupation.

Wild turmeric (*Curcuma aromatica Roxb.*)[7]

A member of the Zingiberaceae family, is another herbal plant with potent larvicidal activity (Figure 3). It has been studied against various mosquito larvae *(Aedes, Anopheles, Culex)* and other insect vectors.

Figure 3: Wild Turmeric

Wild turmeric, botanically known as *Curcuma aromatica* Roxb., belongs to the family Zingiberaceae. It is commonly known as wild turmeric, aromatic turmeric, or Kasturimanjal. The rhizome extract or essential oil of this plant is commonly used for larvicidal studies. Wild turmeric contains several important bioactive phytochemicals, including curcumin, ar-turmerone, α-turmerone, β-turmerone, zingiberene, curcumol, curdione, and 1,8-cineole. These compounds are known to possess insecticidal, antioxidant, and neuroactive properties, which may contribute to the larvicidal activity of the plant. The active constituents may interfere with larval growth, nervous system function, and normal development, leading to larval mortality. *Curcuma aromatica* has been reported to show activity against important mosquito vectors such as *Aedesaegypti*, which transmits dengue, *Anopheles stephensi*, which transmits malaria, and *Culexquinquefasciatus*, which is associated with filariasis (Table 2). Therefore, wild turmeric can be considered a promising plant-based larvicidal agent for eco-friendly mosquito control.

Table 2: Mechanism of Larvicidal Action of *Curcuma aromatica*

Mechanism	Description	Effect on Larvae
Neurotoxic action	Turmerones and curcumin may inhibit acetylcholinesterase enzyme activity, causing accumulation of acetylcholine at nerve junctions.	Continuous nerve stimulation, paralysis, and larval death.
Cuticle disruption	Essential oils can damage the lipid layer of the larval cuticle and increase body surface permeability.	Water loss, dehydration, deformity, and mortality.
Midgut damage	Curcuminoids may produce toxic effects on the larval midgut epithelium.	Cell damage, digestive failure, poor nutrient absorption, and death.
Respiratory and enzyme inhibition	Volatile compounds may interfere with respiratory enzymes and detoxifying enzymes such as oxidases and esterases.	Reduced metabolism, weak survival ability, and larval mortality.
Growth regulation interference	Curcuminoids may disturb ecdysone-related moulting and development.	Incomplete moulting, abnormal development, and failure of pupation.

Mango seeds (*Mangifera indica L.)*[8]

Mango seeds, obtained from *Mangifera indica* L., are known for their larvicidal activity against mosquito larvae such as *Aedesaegypti*, *Anopheles stephensi*, and *Culexquinquefasciatus*. *Mangifera indica*, commonly known as mango or aam, belongs to the family Anacardiaceae (Figure 4). The seed kernel is the main plant part used for larvicidal studies. Mango seed kernel extract contains several bioactive phytochemicals, including mangiferin, a xanthone glycoside, along with phenolic compounds such as gallic acid, catechin, and ellagic acid. It also contains tannins, flavonoids, fatty acids such as stearic acid, palmitic acid, and oleic acid, as well as saponins and alkaloids. These phytochemical constituents are believed to contribute to the toxic effect against mosquito larvae by interfering with their growth, feeding, metabolism, and survival. The phenolic compounds, tannins, and fatty acids present in mango seed kernel extract may damage larval tissues, disturb normal development, and lead to mortality (Table 3). Due to its natural origin, availability, and larvicidal potential, mango seed kernel extract can be considered a promising plant-based larvicidal agent for eco-friendly mosquito control programs.

Figure 4:Mango seeds

Table 3: Mechanism of Action of Mango Seed Extract

Mechanism	Brief Effect
Cell membrane disruption	Damages larval cuticle and membranes, causing leakage, dehydration, and death.
AChE inhibition	Causes nerve overstimulation, convulsions, paralysis, and mortality.
Enzyme inhibition	Reduces respiratory and detoxifying enzyme activity, leading to metabolic failure.
Oxidative stress	Produces ROS, damaging proteins, lipids, and DNA in larval cells.

The tea plant (*Camellia sinensis L.)*[9]

The tea plant, botanically known as *Camellia sinensis* L., belongs to the family Theaceae and is widely known for producing green tea and black tea (Figure 5). Apart from its common use as a beverage plant, *Camellia sinensis* has also been reported to possess larvicidal activity against several mosquito species. The leaves and seeds of the plant are commonly used for preparing aqueous, ethanolic, or methanolic extracts. The larvicidal activity of tea plant extracts is mainly attributed to the presence of bioactive phytochemicals such as catechins, including epigallocatechin gallate, epicatechin, and epicatechin gallate, along with caffeine, theaflavins, thearubigins, tannins, saponins, and flavonoids. These compounds may produce toxic effects on mosquito larvae by interfering with their nervous system, enzyme activity, feeding behaviour, and normal growth. They may also cause structural damage to larval tissues, resulting in reduced survival. *Camellia sinensis* extracts have shown activity against important mosquito vectors such as *Aedesaegypti*, the dengue vector, *Anopheles stephensi*, the malaria vector, and *Culexquinquefasciatus*, the filariasis vector (Table 4). Therefore, the tea plant can be considered a useful natural source of larvicidal, antifeedant, and growth-inhibitory agents for eco-friendly mosquito control.

Figure 5: The tea plant

Table 4: Mechanism of Action of *Camellia sinensis*

Mechanism	Brief Effect
AChE inhibition	Causes nerve overstimulation, paralysis, and death.
Cuticle damage	Damages larval cuticle, causing dehydration and mortality.
Midgut damage	Disrupts gut cells, leading to digestive failure.
Enzyme inhibition	Reduces respiratory and detoxifying enzyme activity.
Oxidative stress	Produces ROS, causing cellular and mitochondrial damage.
Growth interference	Disturbs moulting and pupation, causing abnormal development.

Neem (*Azadirachta indica A. Juss.*)[10]

Neem, botanically known as *Azadirachta indica* A. Juss., belongs to the family Meliaceae. It is commonly known as neem, Indian lilac, or margosa (Figure 5). Neem is one of the most extensively studied medicinal plants for larvicidal activity against mosquito larvae. Different parts of the plant, including leaves, seeds, bark, kernels, and kernel oil, have been used in mosquito control studies. The larvicidal activity of neem is mainly due to the presence of bioactive limonoids and other phytochemicals such as azadirachtin, nimbin, salannin, meliantriol, gedunin, and nimbidin. Among these, azadirachtin is considered the major active compound responsible for insecticidal, antifeedant, and growth-regulating effects. These compounds interfere with larval feeding, growth, moulting, development, and nervous system function, ultimately causing larval mortality. Neem extracts and neem oil have shown activity against important mosquito vectors such as *Aedesaegypti*, the dengue vector, *Anopheles stephensi*, the malaria vector, and *Culexquinquefasciatus*, the filariasis vector (Table 5). Due to its natural origin, biodegradability, and lower environmental risk, neem is considered a promising plant-based larvicidal agent for eco-friendly mosquito control programs.

Figure 6: Neem

Table 5:Mechanism of Action of Neem

Mechanism	Brief Effect
Growth hormone inhibition	Azadirachtin interferes with ecdysone hormone, preventing normal moulting and pupation.
Antifeedant action	Azadirachtin and salannin reduce feeding, causing starvation and weakness.
Neurotoxic effect	Neem compounds disturb nerve transmission and may inhibit AChE, leading to paralysis.
Cuticle disruption	Neem oil and limonoids damage cuticle formation, causing dehydration and death.
Enzyme inhibition	Inhibits detoxifying and metabolic enzymes, reducing larval survival.
Oxidative stress	Produces cellular damage in larval tissues, especially midgut cells.

Materials and Methods Used in Reviewed Larvicidal Studies

Several studies have reported the larvicidal potential of plant-derived extracts against mosquito vectors. In the reviewed experimental procedures, fresh plant materials such as leaves of *Azadirachta indica* Neem, rhizomes of *Curcuma aromatica* Wild turmeric, and leaves of *Camellia sinensis* Tea were collected from local regions. The collected plant materials were first washed thoroughly with water to remove dust, soil, and other impurities. After washing, the materials were shade-dried for about 7–10 days to prevent degradation of heat-sensitive phytoconstituents. The dried plant materials were then ground into fine powder using a mechanical grinder and stored in airtight containers until extraction [11].

For preparation of plant extracts, 100 g of dried plant powder was soaked in 500 mL of methanol for 72 hours at room temperature with intermittent shaking. After extraction, the mixture was filtered through Whatman No. 1 filter paper. The obtained filtrate was concentrated and evaporated to dryness using a rotary evaporator. The dried crude methanolic extracts were then stored at 4°C until further use. This method helped obtain concentrated plant extracts containing bioactive phytochemical constituents responsible for larvicidal activity [11-12].

The prepared crude methanolic extracts were subjected to preliminary phytochemical screening. Standard qualitative procedures were used to detect the presence of major phytoconstituents such as alkaloids, flavonoids, terpenoids, saponins, tannins, and phenolic compounds. These phytochemicals are important because many of them are associated with insecticidal, growth-inhibitory, and larvicidal effects. Screening of plant extracts therefore provides a basic understanding of the possible chemical groups responsible for mosquito larval mortality.

Mosquito larvae used in the reviewed studies included *Aedesaegypti*, *Anopheles stephensi*, and in some studies *Culexquinquefasciatus*. Larvae were collected from stagnant water sources and reared under controlled laboratory conditions following standard mosquito-rearing procedures. Healthy fourth instar larvae were selected for larvicidal bioassay because this stage is commonly used in mosquito susceptibility testing and provides reliable mortality data [12-13].

Larvicidal bioassays were generally carried out using different concentrations of plant extracts. A stock solution of 1000 ppm was prepared by dissolving 100 mg of crude extract in 1 mL of acetone, and the volume was made up to 100 mL with distilled water. From this stock solution, different test concentrations such as 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm were prepared in 200 mL of deionized water. In each test beaker, 25 fourth instar mosquito larvae were released. The larvae were exposed to the test solutions, and mortality was recorded after 24 hours and 48 hours. Each treatment was replicated five times to improve the reliability of results. Control groups were maintained using larvae exposed to 0.1 mL acetone without plant extract. The beakers were maintained in a temperature-controlled room at approximately 28°C ± 2°C during the experiment [13-14].

Larval susceptibility tests were performed according to standard WHO procedures. In these tests, 25 fourth instar larvae of *Anopheles stephensi*, *Aedesaegypti*, and *Culexquinquefasciatus* were introduced into 200 mL of extract solution. Parallel control experiments without plant extract were also maintained. After exposure for 24 and 48 hours, the number of dead larvae was counted, and percentage mortality was calculated from the average of five replicates. Larvae were considered dead when they showed no movement after gentle probing. If control mortality ranged between 5% and 20%, the observed mortality was corrected using Abbott's formula. This correction was necessary to remove the influence of natural mortality in the control group [15-17].

The percentage mortality data obtained from the bioassay were further used to calculate lethal concentration values. LC_{50} and LC_{90} values, representing the concentrations required to kill 50% and 90% of larvae respectively, were calculated by probit analysis following the method of Finney. Regression analysis was used to determine the relationship between extract concentration and larval mortality. These values allowed comparison of larvicidal potency among different plant extracts and mosquito species. Overall, the reviewed methods show that plant extracts are commonly evaluated through standardized larval bioassays involving controlled extract concentrations, fourth instar larvae, replicate testing, mortality recording, Abbott's correction, and probit-based LC_{50} and LC_{90} determination [18–20].

CONCLUSION

Herbal larvicides represent a promising and eco-friendly alternative to synthetic chemical insecticides for mosquito control. The continuous and excessive use of chemical larvicides has raised concerns related to environmental pollution, toxicity to non-target organisms, development of resistance in mosquito populations, and disturbance of ecological balance. In this context, plant-based larvicides offer a safer and more sustainable approach for controlling mosquito larvae at their breeding sites.

Extracts obtained from medicinal plants such as neem, wild turmeric, wild ginger, tea leaves, and mango seeds have shown strong larvicidal potential against different mosquito species. These plants contain bioactive phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, essential oils, and saponins, which may interfere with larval growth, respiration, feeding, moulting, and development. Their natural origin, biodegradability, easy availability, and comparatively low environmental risk make them suitable candidates for mosquito control programs.

The use of herbal larvicides can be especially beneficial in integrated vector management, where multiple control strategies are combined to reduce mosquito populations effectively. Plant-based larvicidal formulations may help minimize dependence on synthetic insecticides and reduce the chances of resistance development. They may also be more acceptable for use in rural and community-based mosquito control programs due to their natural source and local availability.

However, further scientific investigation is required before large-scale application. Future studies should focus on formulation development, stability testing, standardization of active constituents, field trials, safety evaluation, and toxicity studies on non-target organisms. Thus, herbal larvicides have strong potential to become an effective, economical, and environmentally sustainable tool for mosquito vector control.

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