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Research Article

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Another Approach, and Analysis, to Evaluate the Risk of Malaria Transmission in Angola According to Vector Control Method and Duration of Stage: The Birley's Formula

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Abstract

Background:

Three indicators are used to evaluate the entomological intensity of malaria transmission: the “entomological inoculation rate” of Ross; the “reproduction rate” of Macdonald and the “vectorial capacity” of Garrett-Jones. They were successfully used to evaluate the impact of vector control. But they do not consider the duration of stay in malarious area. Therefore, Birley introduced the parameter “t” (in days) in the new formula of the inoculation rate which became $h = 1 - (1 - s)^{ma \cdot t}$, where ma = biting rate; s = infectivity and t the time of exposure.

Objective:

We used the Birley's formula in the framework of the long-term malaria vector control program implemented in eight villages around Balombo (Angola) to compare the efficacy of four methods.

Method:

The method of vector control were: the long-lasting insecticide (deltamethrin) treated nets (“LINS”) PermaNet® 2.0 (P2.0) alone, in two villages; LLINs P2.0 in combination with insecticide (deltamethrin) treated plastic sheeting (“ITPS”) model ZeroFly®, in two villages; ITPS model ZeroVector® (deltamethrin treated) alone, in two villages; and two rounds of lambda-cyhalothrin inside residual spraying (“IRS”) followed by installation of ITPS, in two villages. In the first five years both entomological and parasitological field surveys were done every two months, two years before vector control and three years after. Entomological evaluation was based CDC Light Trap inside houses; the “density per trap” was used as a proxy of the “biting rate” inside the house to get the “risks in the house” before and after vector control. Mosquito were identified and analyzed to get species identification and infectivity.

Results:

Main vectors (MV) were *Anopheles funestus* and *An. gambiae* which were gathered and used as “ ma ”, in the Birley' formula. The risks of being inoculated increased sharply as a logarithmic function of the length of exposure. Without vector control, with an average density of 0.6 “main vectors per trap”, and an infectivity of 4.53% the risks of infections were 2.7% in one day; 17.2% in one week; 55.5% in one week and 99.5% in one year. After vector control, with a density becoming 0.17 main vectors per trap, and an infectivity of 2.59%, the risks were 0.15% in one day; 3.1% in one week; 12.7% in one month and 80.5% in one year; meaning a protection of 94.4% in one day; 82% in one week; 77% in one month and 19% for one year. The trends in the reduction of risks were similar with the four methods of vector control but the level of reduction was different with methods. In the condition of the trial, the best method, in term of efficacy, and long-lasting effect, was the ITPS ZeroVector® alone.

1 Introduction

A WHO Report of Malaria in the World indicated that “an estimated 2.2 billion cases of malaria and 12.7 million deaths have been averted since 2000, but the disease remains a serious global health threat, particularly in the WHO African Region. According to this report, there were an estimated 263 million cases and 597,000 malaria deaths worldwide in 2023. This represents about 11 million more cases in 2023 compared to 2022, and nearly the same number of deaths. Approximately 95% of the deaths occurred in the WHO African Region, where many at risk still lack access to the services they need to prevent, detect and treat the disease” (World Health Organization, 2024).

On the other hand, it was reported “that *Plasmodium falciparum* infection prevalence in endemic Africa halved and the incidence of clinical disease fell by 40% between 2000 and 2015. We estimate that interventions have averted 663 (542-753 credible interval) million clinical cases since 2000. Insecticide-treated nets, the most widespread intervention, were by far the largest contributor (68% of cases averted)” (Bhatt *et al.*, 2015). Combining the data of WHO on malaria morbidity and estimation of Bhatt *et al.* (*loc.cit.*), it could be assumed that some 1,36 billion of clinical cases were averted thanks to large scale distribution of insecticide treated mosquito nets “ITNs have had by far the largest effect, but have also been generally present for longer and at higher levels of coverage. IRS and ACTs have both made important contributions to reducing prevalence and incidence where they have been implemented at scale (although it is important to note that the primary role of ACTs is in averting severe disease and death rather than reducing transmission and uncomplicated cases” (Bhatt *et al.*, *loc.cit.*).

Reducing transmission is the role of vector control which requires a sound entomological knowledge for its evaluation. The entomological malaria parasite transmission could be described by two factors: rhythm (permanent, seasonal regular long, regular seasonal short, episodic (Boyd, 1949) and intensity. Three main formulae were developed to estimate the amount of entomological transmission in an area or period (dry-rainy season), and its evolution with implementation of vector control. The first notion of «risk with days» seems to be found in the book “Essential Malariology” chapter “Quantitative Epidemiology of Malaria”: “many aspects of malaria now require some quantitative data, the collection and interpretation of which depends on the elementary mathematical principles. In answering the usual epidemiological question “Who? When? Why?” some degree of precision is needed. There is no tropical communicable disease in which mathematical approach has been applied more widely and thoroughly more than malaria.” (Bruce-Chwatt, 1985).

The first formulating the quantitative laws of epidemiology of malaria, and its transmission and control, was made by Ronald Ross at the beginning of the XXth century (Ross, 1911). In his book “Theory of Happening” (word from which is issue the famous “h” parameter) Ross developed the concept of Entomological Inoculation Rate (“EIR”) defined as the relative proportion of the human population receiving an infective bite in unit of time, from the average number of *Anopheles* found in a room or in a hut. It can be estimated by multiplying the anopheles’ density per person per day, by the sporozoite rate. It combined the density of bite received in one night (“ma” for “man-biting rate”) and the infectivity (“s” for “sporozoite index”) of the vector to present the now, famous, and largely used first formula: $h = ma \cdot s$ which represents the number of infective bites of *Anopheles* vector received in one night (unity of time) by one human being. Thus, “if an average of six *Anopheles* were found every day in a room where three persons slept and where the sporozoite rate was 5%, then the supposed daily inoculation rate would be $(6/3) \times 0.050 = 0.1$ infective bite per human being per night.

However, not all anopheles found in the room would have fed on that night. Assuming a gonotrophic cycle of two days, only half of anopheles would be involved. In view of this, the previous figure of 0.1 must be halved and the postulated figure would be 0.05. Thus, every person in this room would receive an infective bite every 20 days” (Bruce-Chwatt, 1985). It seems that this is the first time that duration of stay in risky area was taken into consideration.

Then, was developed the concept of reproduction rate “z” (Macdonald, 1957). In the course of its time of infectivity ($1/r$) a case will be bitten each day by ma mosquitoes of which “b” is the proportion having sporozoites

in their salivary glands which are actually infective. The probability of a mosquito surviving one day is called “p”. The proportion of mosquitoes surviving for “n” days is p^n and their subsequent expectation of life is $1/(-\log_e p)$. The expectation of life after surviving through n days is $p^n/(-\log_e p)$. During this time, they will bite “a” time each day (with “a” is defined as “the average number of men bitten by one mosquito in one day”), and b the proportion of these bites will be infective (meaning potentially inducing malaria infection, *i.e.* without considering human immunity). Thus, the reproduction rate of the parasite by anopheles’ vectors is estimated by the famous formula:

$$z = ma^2bp^n/(-\log_e p)$$

The reproduction rate corresponds to the total number of new (“secondary”) infections which could be distributed by anopheline population from a single human “primary” infection, in the absence of immunity. The aims of reducing transmission are to decrease this “z” below 1, with entomological action against density of bite (ma), longevity (p), anthropophily (a).

But, considering the issue of evaluation of parameters “r” (called “recovery rate”) and “b” (for actual infectivity of sporozoites) it was developed the concept of vectorial capacity (Garrett-Jones, 1964a) with the simplified formula:

$$CV = ma^2p^n/(-\log_e p)$$

The vectorial capacity is defined as “the average number of inoculations with a specified parasite, originating from one case of malaria in unit time, that a vector population would distribute to man if all the vector females biting the case became infected” (Garrett-Jones and Grab, 1964). The vectorial capacity is thus a density-and longevity dependent attribute of the mosquito and was used for “The Assessment of Insecticidal Impact on the Malaria Mosquito's Vectorial Capacity, from Data on the Proportion of Parous Females” (Garrett-Jones and Grab, *loc.cit.*). Vectorial capacity is an interesting indicator in epidemiological entomology, for example as “prognosis for interruption of malaria parasite transmission (Garrett-Jones, 1964b) or to evaluate the house spraying with DDT (Garrett-Jones and Shidrawi, 1969) where it was compared to reproduction rate.

Reproduction rate is the total new infections which could occurred from one human infection (with infective gametocytes) while the vectorial capacity is new infections in a unit of time (Smith *et al.*, 2007).

The vectorial capacity is easy to evaluate with the biting rate (ma) and the parous rate (Detinova, 1962) which allows an estimation of the longevity of vectors and the “assessment of insecticidal impact” (Molineaux *et al.*, 1979).

The main parameters used in entomological evaluation of malaria parasite transmission are the inoculation rate and the vectorial capacity.

But, in both formulae, one parameter is not taken into consideration; “t” (in days) of length of stay in malarious area, to evaluate the evolution of the risk of being bitten by infective vector according to the times spent on this place. Dealing with this issue Martin Birley developed a new formula of the risk “ h_r ” of receiving an infective bite according to the density of bite (ma), infectivity of vector (s) and the time, duration of exposure, “t”: and the formula is now $h_r = 1 - (1-s)^{ma \cdot t}$

The formula was already used to describe other entomological situation (Gazin *et al.*, 1985) and it is used in Angola to evaluate, and compare, four methods of vector control implemented in Balombo program considering the length of stay of human being in house before, and after, implementation of vector control.

2 Materials and Method

In 2007 a long-term (11 years) comprehensive malaria vector control program was implemented in eight villages around the town of Balombo, (Benguela Province, Angola). The goal was to compare, in natural situation, the efficacy of four methods of vector control: classical long-lasting deltamethrin insecticide treated nets (“LLINs”) PermaNet 2.0®, installed in two villages; LLINs in combination with insecticide (deltamethrin) treated plastic sheeting (“ITPS”) model ZeroFly® in two villages; deltamethrin treated ITPS model ZeroVector® in two villages,

and inside residual spraying (“IRS”) in two villages: two rounds house sprays with lambdacyhalothrin followed by installation of ITPS. The first five years evaluation involved entomology, parasitology and immunology. The entomological evaluation included two steps: two years before vector control implementation and three years after. Entomological evaluation was based upon regular use, every two months, of classical CDC Light Trap (“CDC LT”) in ten inhabited houses (one trap per house, always the same). CDC LT were recently used to estimation of entomological inoculation rate in Tanzania (Mboera, 2005) and elsewhere (Service, 1970; Service, 1977).

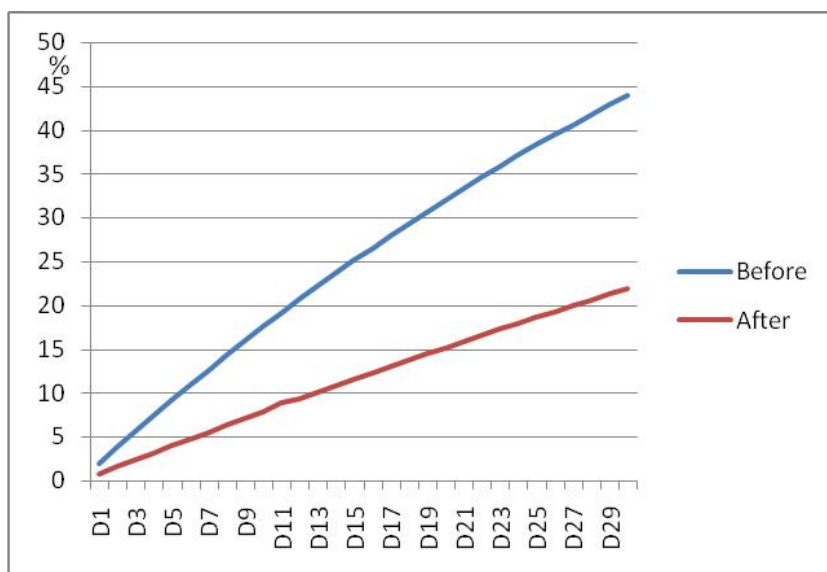
Anopheles were isolated from the samples of the CDC LT; determined at species level *in situ* then further biological analysis were performed in OCEAC Organization (Yaoundé, Cameroon) to precise species of the *Anopheles gambiae* complex and Elisa tests were performed to precise the infectivity of species caught (Beier *et al.*, 1987; Wirtz *et al.*, 1987). The biotope, methods, first entomological parasitological results were already published (Carnevale *et al.*, 2024), and first immunological data were already published (Brosseau *et al.*, 2012). Bruce-Chwatt (1985), considered that sampling inside house will give information of the entomological inoculation rate “at house level”. Thus, was decided to use the Birley’s formula $h = 1 - (1-s)^{ma.t}$ to estimate the evolution of risks in houses of the villages according to the duration of stay (in days) and each of the four method of vector control.

3 Results

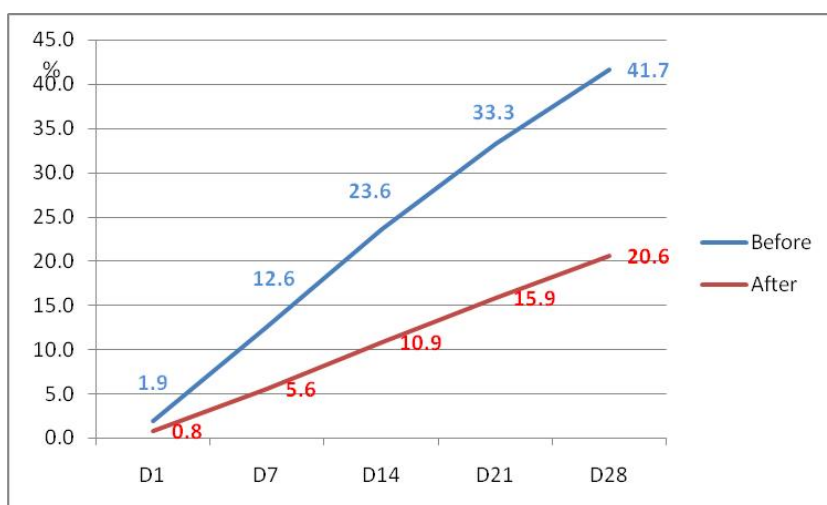
A total of 202 catching sessions with CDC Light Traps were conducted in the eight villages, representing 1,880 trap-nights, which caught 1,153 *Anopheles* specimens, belonging to 11 taxa including nine species and one species complex, *Gambiae*. *An. gambiae* and *An. funestus* only were considered as the main vectors (“MV”) for further analysis.

3.1 Evolution of risks before, and after, LLINs full coverage

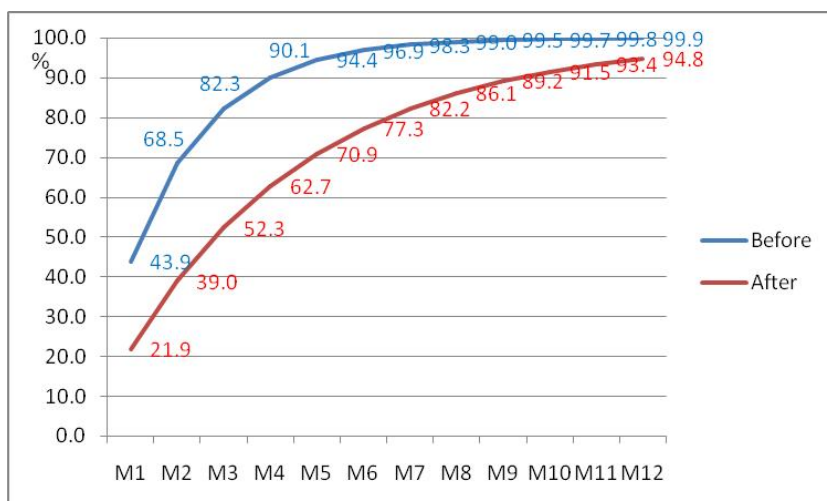
With full coverage in LLINs the number of main vectors per trap decreased from 0.67 to 0.26 and the sporozoite index slightly changed from 2.83% to 3.08%. With these data in the Birley’s formula it is possible to calculate the risk of receiving an infective bite in sleeping one day, one week, one month (Graph 1a and 1b), one year (Graph 1c) in a house without, then with LLINs.



Graph 1a Evolution of the daily risk, in one month, of receiving an infective bite of the main vector of malaria in a house before and after installation of LLINs. (D= number of days)



Graph 1b Evolution of the weekly risks of receiving, in one month, an infective bite of the main vector of malaria in a house before, and after, installation of LLINs. (D= number of days)



Graph 1c Evolution of the monthly risks of receiving, in one year, an infective bite of the main vector of malaria, in a house before, and after, installation of LLINs. (M= number of months)

3.1.1 Evolution of the weekly risk in one month

In a house without LLIN the weekly risk sharply increased, 2% in one day; 13% in one week; 24% in two weeks; 33% in three weeks and 42% in four weeks. With installation of LLINs they were respectively reduced at < 1%; 6%; 11%; 16% and 21% meaning that LLIN conferred always a >50% reduction of risks during the month (Table 1a).

Table 1a Evolution of the weekly risks, in one month, of getting an infective bite before, and after, full coverage in LLINs; with the difference of risks induced

	Before	After	Diff.
D1	1.9	0.8	-57.1%
D7	12.6	5.6	-55.6%
D14	23.6	10.9	-54.0%
D21	33,3	15.9	-52.3%
D28	41.7	20.6	-50.6%

3.1.2 Evolution of the monthly risks in one year

Before LLINs installation the risk of being infected was 44% in one month; reached 90% in four months; 97% in

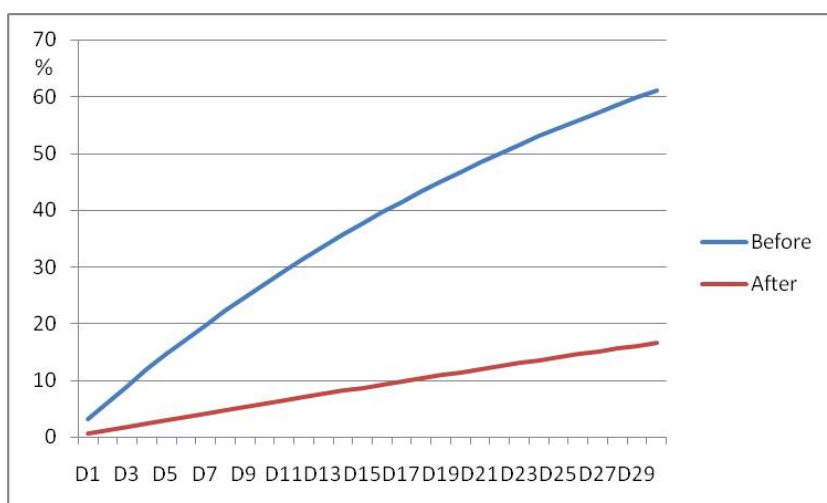
six months and 99% in eight months; being #100% in one year. After LLINs installation these risks were respectively 22%; 63%; 77%; 86% and 95% (Table 1b). The reduction of risks with vector control based on LLINs sharply decreased with time; from 50% in one month to 5% in one year. It is of great concern that, even with full coverage in LLINs, the risk of receiving one infective bite in one year could > 90%.

Table 1b Evolution of the monthly risks of receiving, in one year an infective bite of the main vectors of malaria, in a house before, and after, installation of LLINs, with the difference of risks

	Before	After	Diff.
M1	43.9	21.9	-50.2%
M2	68.5	39.0	-43.1%
M3	82.3	52.3	-36.5%
M4	90.1	62.7	-30.4%
M5	94.4	70.9	-24.9%
M6	96.9	77.3	-20.3%
M7	98.3	82.2	-16.3%
M8	99.0	86.1	-13.0%
M9	99.5	89.2	-10.3%
M10	99.7	91.5	-8.2%
M11	99.8	93.4	-6.5%
M12	99.9	94.8	-5.1%

3.2 Evolution of risks before and after LLINs + ITPS Model ZeroFly®

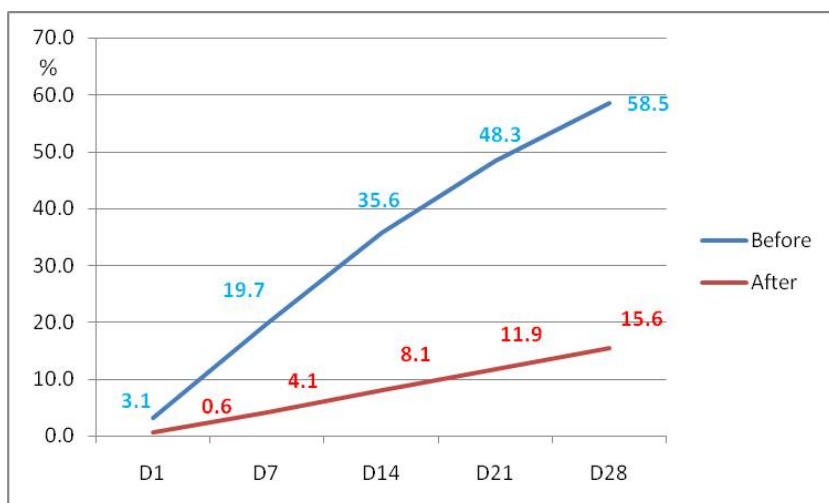
With full coverage in LLINs, in combination of ZF the number of main vectors per trap decreased from 0.73 to 0.23 and the sporozoite index decreased from 4.20% to 2.59%. With these data in the Birley's formula it is possible to calculate the risk of receiving an infective bite in sleeping one day, one week, one month (Graph 2a and 2b), one year (Graph 2c) in a house without, then with LLINs + ZF.



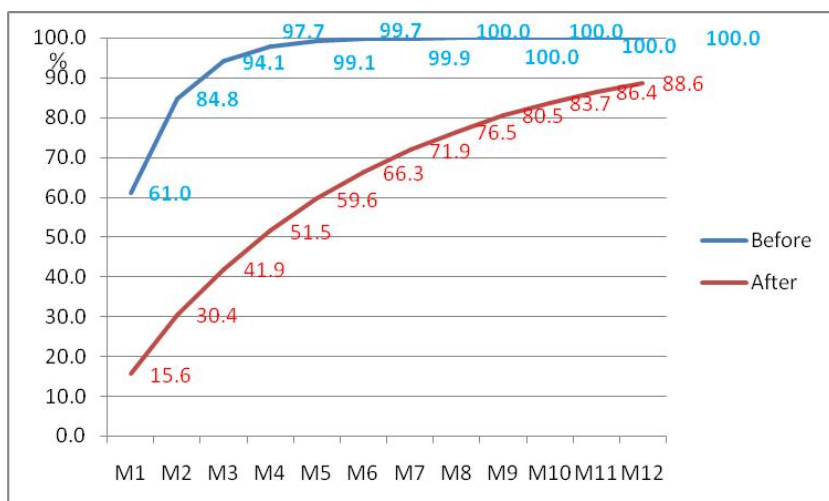
Graph 2a Evolution of the daily risks of receiving, in one month, an infective bite of the main vector of malaria in a house before, and after, installation of LLINs+ ZF. (D= number of days)

3.2.1 Evolution of the weekly risk in one month

In a house without vector control the weekly risk sharply increased, 3% in one day; 20% in one week; 36% in two weeks; 48% in three weeks and >58% in four weeks. With installation of LLINs + ZF they were respectively reduced at < 1%; 4%; 8%; 12% and 16% meaning that LLIN+ZF conferred a >70% reduction of risks during one month (Table 2a).



Graph 2b Evolution of the weekly risks of receiving, in one month, an infective bite of the main vector of malaria in a house before, and after, installation of LLINs+ ZF. (D= number of days)



Graph 2c Evolution of the monthly risks of receiving, in one year, an infective bite of the main vector of malaria in a house before, and after, installation of LLINs+ZF. (M= number of months)

Table 2a Evolution of the weekly risks, in one month, of getting an infective bite before, and after, full coverage in LLINs+ ZF, with the difference of risks induced

LLIN+ZF	Before	After	Diff.
D1	3.1	0.6	-80.6%
D7	19.7	4.1	-79.0%
D14	35.6	8.1	-77.2%
D21	48.3	11.9	-75.4%
D28	58.5	15.5	-73.4%

3.2.2 Evolution of the monthly risks in one year

Before LLINs+ ZF installation the risk of being infected reached 61% in one month; 85% in two months; > 90% in three months and > 99% in six months; being #100% in one year. After LLINs + ZF installation these risks were respectively 16%; 30%; 55%, 66% and 89% (Table 2b) meaning a worthy reduction of risks. This reduction of risks: was higher with the combination LLIN+ZF than LLIN alone; decreased with time (Graph 2c); from 75% in one month to 11% in one year.

Table 2b Evolution of the monthly risks of receiving an infective bite of the main vector of malaria in a house before, and after, installation of LLINs + ZF; with the difference of risks

LLIN+ZF	Before	After	Diff.
M1	61.0	15.6	-74.5%
M2	84.8	30.4	-64.2%
M3	94.1	41.9	-55.5%
M4	97.7	51.5	-47.3%
M5	99.1	59.6	-39.9%
M6	99.7	66.3	-33.5%
M7	99.9	71.9	-28.0%
M8	100.0	76.5	-23.5%
M9	100.0	80.5	-19.5%
M10	100.0	83.7	-16.3%
M11	100.0	86.4	-13.7%
M12	100.0	88.6	-11.4%

Comparing the reduction of monthly risks conferred by the combination LLN + ZF (Table 2b; column 4) and of LLINs alone (Table 1b; column 4) it appeared that adding ITPS on the wall to nets on sleeping units (Table 2c) exponentially increased the reduction of risks (Table 2c) by some 40% in six months and even >50% in one year (Graph 2d).

Table 2c Difference of risks in house with LLINs in combination of ITPS versus houses with LLIN alone

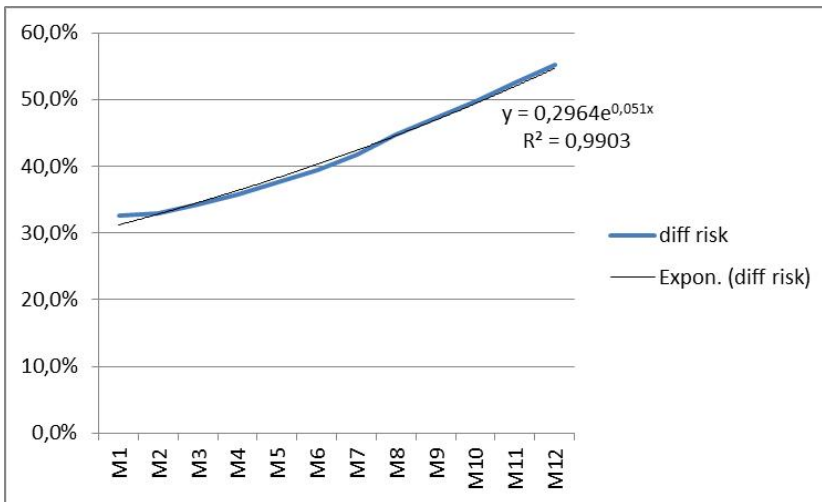
Months	diff risks
M1	-32.6%
M2	-32.9%
M3	-34.2%
M4	-35.7%
M5	-37.6%
M6	-39.4%
M7	-41.8%
M8	-44.7%
M9	-47.2%
M10	-49.7%
M11	-52.6%
M12	-55.3%

3.3 Evolution of risks before and after ITPS alone model ZeroVector®

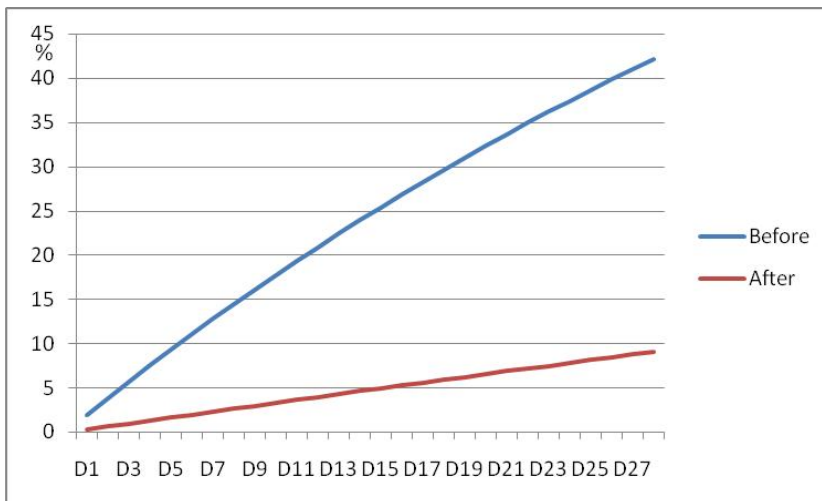
With full coverage in ITPS ZV only, the number of main vectors per trap decreased from 0.42 to 0.07 and the sporozoite index remained almost the same: 4.54% then 4.76%. With these data in the Birley's formula it is possible to calculate the risks of receiving an infective bite in sleeping one day, one week, one month (Graph 3a and 3b), one year (Graph 3c) in a house before, and after, installation of ITPS ZV alone.

3.3.1 Evolution of the weekly risk in one month

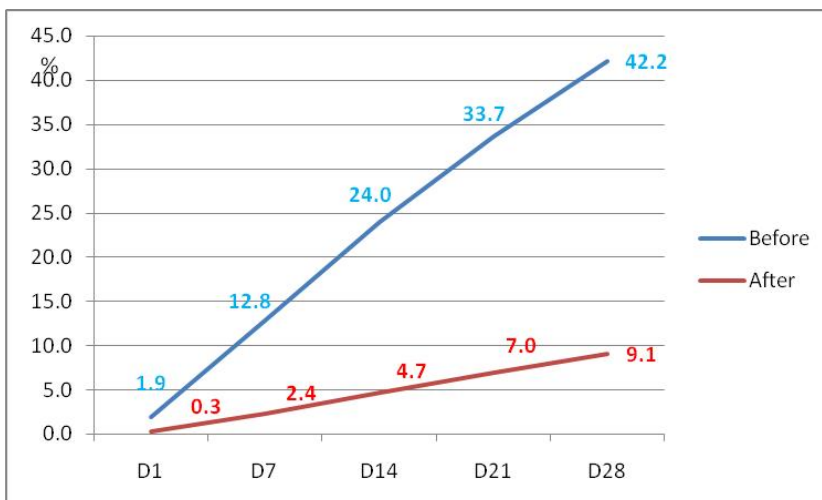
In a house without vector control, the weekly risk sharply increased, 2% in one day; 13% in one week; 24% in two weeks; 34% in three weeks and 42% in four weeks (Graph 3b). With installation of ITPS ZV® alone they were respectively reduced to 0.3%; 2.4%; 5%; 7% and 9%; meaning that ITPS alone conferred some 80% reduction of risks the first month (Table 3a).



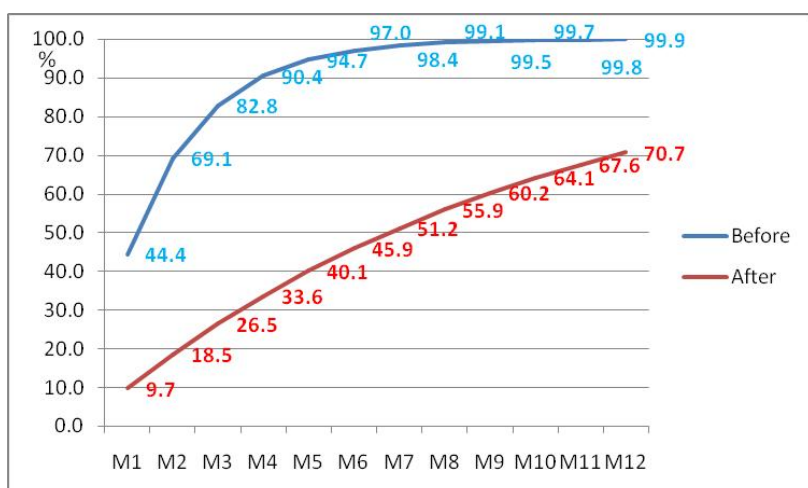
Graph 2d Increasing protection, with time, by adding ITPS to treated mosquito nets versus mosquito nets alone



Graph 3a Evolution of the daily risks, in one month, of receiving an infective bite of the main vector of malaria in a house before and after installation of ITPS ZF alone. (D= number of days)



Graph 3b Evolution of the weekly risk of receiving, in one month, an infective bite of the main vector of malaria, in a house before, and after, installation of ITPS ZV. (D= number of days)



Graph 3c Evolution of the monthly risks of receiving, in one year, an infective bite of a main vector of malaria in a house before and after installation of ITPS ZV alone. (M= number of months)

Table 3a Evolution of the weekly risks, in one month, of getting an infective bite before and after full coverage in ITPS ZV alone with the difference of risks induced

ITPS ZV	Before	After	Diff.
D1	1.9	0.3	-82.5%
D7	12.8	2.4	-81.6%
D14	23.9	4.7	-80.5%
D21	33.7	7.0	-79.3%
D28	42.2	9.1	-78.4%

3.3.2 Evolution of the monthly risk in one year

Without vector control the risks were high with 44% in one month; 83% in three months; 97% in six months. After installation of ITPS ZV® the risks dropped respectively at 10%; 26%; and 46% (Table 3b). Insecticide treated plastic, model ZeroVector® alone, conferred a relatively more efficient and longer protection than LLINs alone, or in combination with ITPS ZF; with 50% till 7th month and even a 30% reduction of risk after one year (Graph 3c).

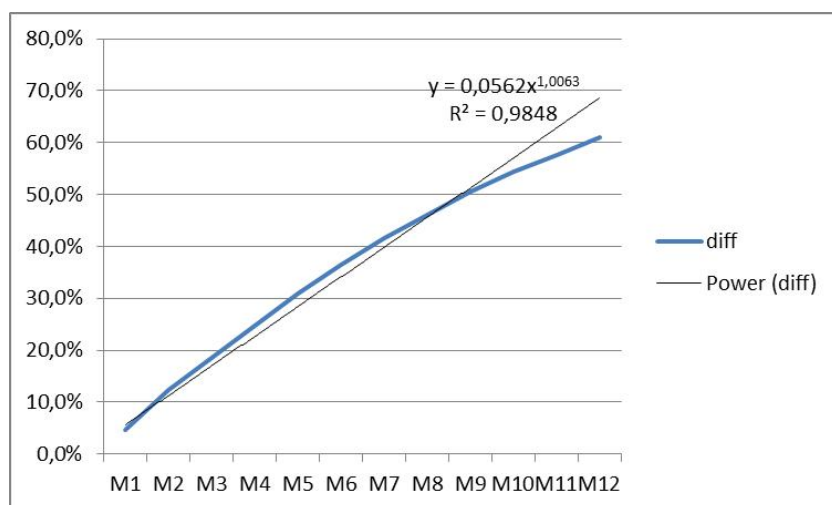
Table 3b Evolution of the monthly risk of receiving an infective bite of main vector of malaria in a house before and after installation of ITPS ZeroVector® alone, and the difference of risks

ITPS ZV	Before	After	Diff.
M1	44.4	9.7	-78.1%
M2	69.1	18.5	-73.2%
M3	82.8	26.5	-68.1%
M4	90.4	33.6	-62.8%
M5	94.7	40.1	-57.7%
M6	97.0	45.9	-52.7%
M7	98.4	51.2	-48.0%
M8	99.1	55.9	-43.6%
M9	99.5	60.2	-39.5%
M10	99.7	64.1	-35.7%
M11	99.8	67.6	-32.3%
M12	99.9	70.7	-29.2%

In term of risk, at house level, ITPS ZV alone appeared a promising efficient tool with its longer, and higher, efficacy, compared to LLINs alone. Comparing the reduction of risks by ITPS ZV alone (Table 3b) versus combination LLIN + ZF (Table 2b) it appeared (Table 3c) that the protection was better and longer with ITPS ZV alone than adding nets and wall lining (Graph 3d).

Table 3c Increasing protection with time conferred by ITPS ZV alone versus combination LLIN+ ITPS ZF (diff= difference)

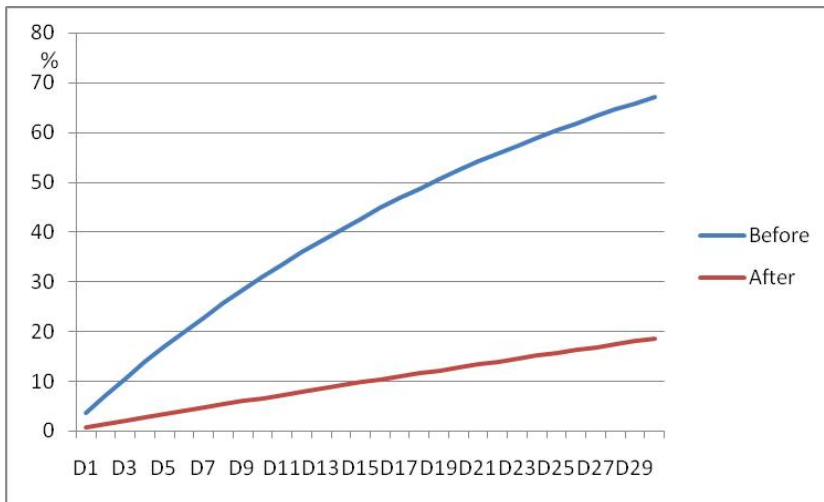
Months	Diff.
M1	4.6%
M2	12.3%
M3	18.5%
M4	24.7%
M5	30.8%
M6	36.4%
M7	41.7%
M8	46.1%
M9	50.6%
M10	54.3%
M11	57.6%
M12	61.0%



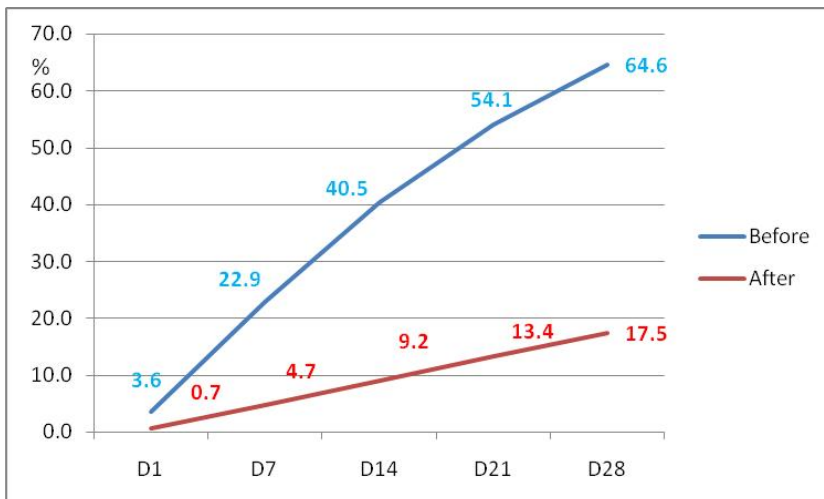
Graph 3d Evolution, with time, of the reduction of risks comparing ITPS alone versus association LLIN+ ITPS

3.4 Evolution of risk before and after IRS then ITPS

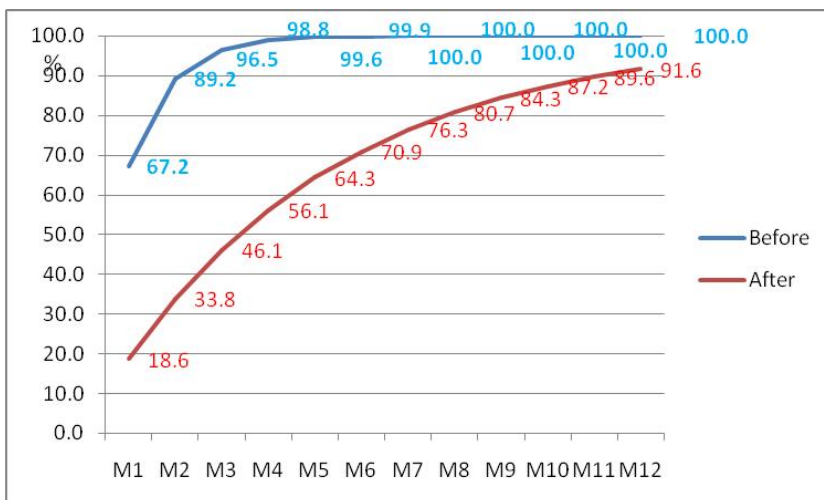
With two rounds of inside house spraying with lambdacyhalothrin, then installation of ITPS (deltamethrin), the number of main vectors per trap decreased from 0.50 to 0.13 and the sporozoite index dropped from 7.14% to 5.26%. With these data in the Birley's formula it is possible to calculate the risk of receiving an infective bite in sleeping one day, one week, one month (Graph 4a and 4b), one year (Graph 4c) in house before and after IRS then ITPS.



Graph 4a Evolution of the daily risks, in one month, of receiving an infective bite of main vector of malaria in a house before and after installation of ITPS ZF alone. (D= number of days)



Graph 4b Evolution of the weekly risks of receiving, in one month, an infective bite of a main vector of malaria in a house before and after inside house spraying then ITPS installation. (D= number of days)



Graph 4c Evolution of the monthly risk of receiving, in one year, an infective bite of a main vector of malaria in a house before, and after, two rounds of IRS followed by ITPS installation. (M= number of months)

3.4.1 Evolution of the weekly risk in one month

Before house spraying the risks quickly increased with time, with 4% in one day; 23% in one week; > 50% in three weeks and 65% in one month. (Graph 4b). With vector control these risks decreased < 1% in one day; 5% in one week; 13% in three weeks and 17% in one month meaning a 75%-80% reduction of risks in one month (Table 4a).

Table 4a Evolution of the weekly risks, in one month, of getting an infective bite before, and after, inside house spraying followed by ITPS installation, with the difference of risks induced

IRS ITPS	Before	After	Diff.
D1	3.6	0.7	-81.3%
D7	22.9	4.7	-79.5%
D14	40.5	9.2	-77.4%
D21	54.1	13.4	-75.2%
D28	64.6	17.5	-72.9%

3.4.2 Evolution of the monthly risk in one year

Before house spraying the risks were very high with nearly 70% in one month; nearly 90 in two months; 99% in four months (Table 4b). The risks decreased respectively < 20% in one month; 34% in two months; 56% in four months but reached 90% the 11th-12th month. The reduction of risks came from 72% to 10% in one year, reaching 50% in three months; 30% in six months; still 20% in eight months and almost 10% in one year (Graph 4c).

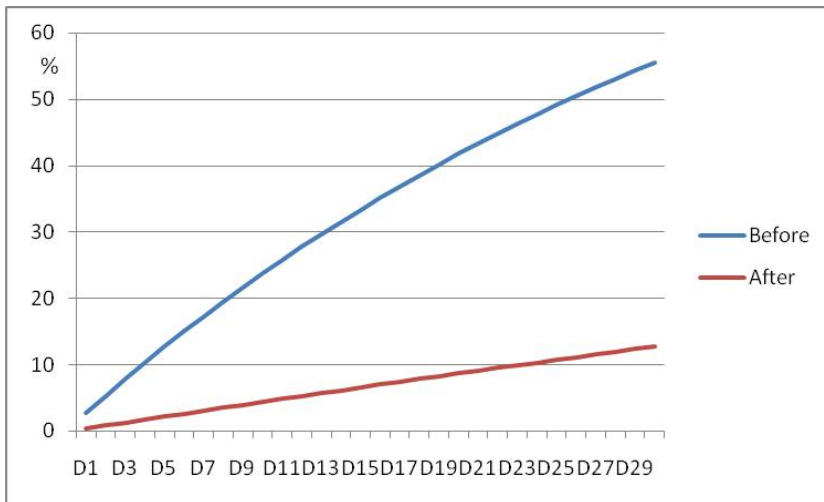
Table 4b Evolution of the monthly risks of receiving an infective bite of a main vector of malaria in a house before and after house spraying followed by ITPS installation, and the difference of risks

IRS then ITPS	Before	After	Diff.
M1	67.2	18.6	-72.3%
M2	89.2	33.7	-62.2%
M3	96.5	46.1	-52.2%
M4	98.8	56.1	-43.2%
M5	99.6	64.3	-35.5%
M6	99.8	70.9	-29.0%
M7	99.9	76.3	-23.6%
M8	99.9	80.7	-19.3%
M9	100	84.3	-15.7%
M10	100	87.2	-12.8%
M11	100	89.6	-10.4%
M12	100	91.6	-8.5%

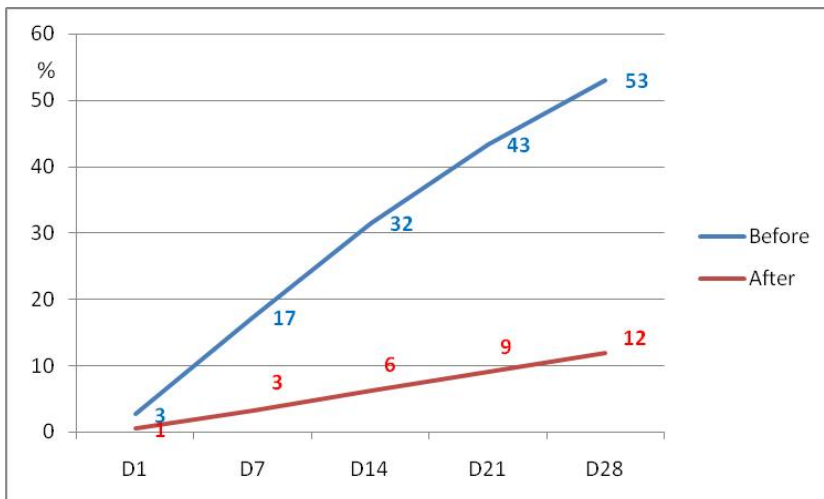
3.5 Average evolution of risks before and after vector control

Following vector control the average number of main vectors per trap decreased from 0.58 to 0.17 and the sporozoite index dropped from 4.53% to 2.59%. With these data in the Birley's formula it is possible to calculate the risks of receiving an infective bite in sleeping one day, one week, one month (Graph 5a and 5b), one year (Graph 5c) in house before, and after, IRS then ITPS.

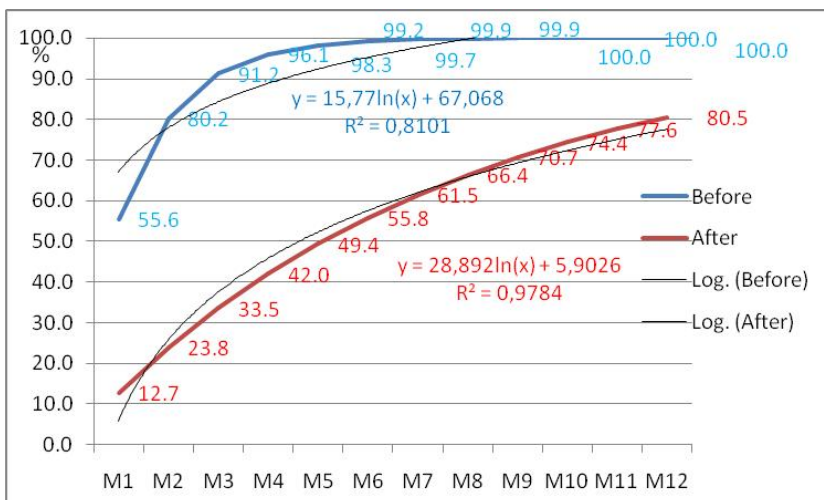
Before vector control the risk of being infected reached 50% the 26th day; in one month the risk was 55.5% without vector control and 12.7 after vector control meaning a 77% reduction of risks conferred by vector control the first month.



Graph 5a Evolution of the daily risks, in one month, of receiving an infective bite of main vector of malaria in a house before and after implementation of vector control. (D= number of days)



Graph 5b Evolution of the weekly risk of receiving, in one month, an infective bite of a main vector of malaria in a house, before, and after, inside house spraying then ITPS installation. (D= number of days)



Graph 5c Evolution of the monthly risks of receiving, in one year, an infective bite of a main vector of malaria in a house before, and after, vector control. (M= number of months)

3.5.1 Evolution of the weekly risk in one month

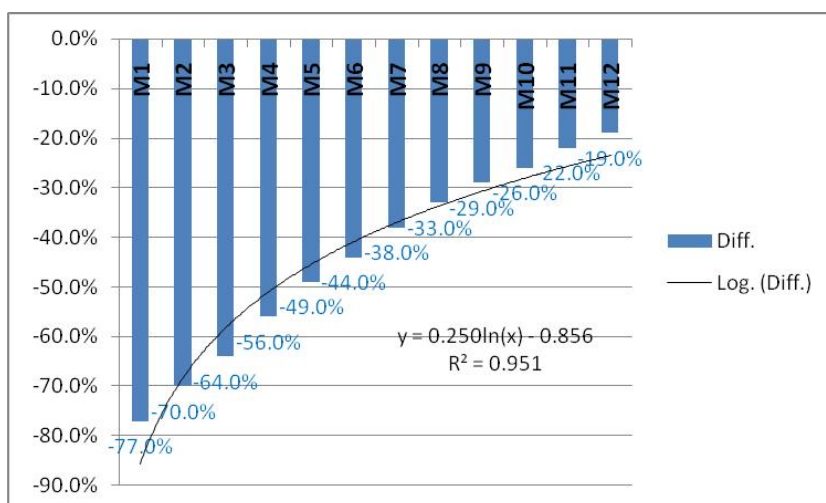
Before vector control the risks quickly increased with time, with 3% in one day; 17% in one week; > 40% in three weeks and > 50% in one month (Table 5a). After vector control the risks dropped respectively to < 1% in one day 3%; in one week; < 10% in three weeks and 12% in one month meaning an 80% reduction during one month.

Table 5a Evolution of the weekly risks, in one month, of getting an infective bite before and after vector control, with the difference of risks induced

	Before	After	Diff
D1	2.7	0.5	-83.1%
D7	17.2	3.1	-81.8%
D14	31.5	6.2	-80.4%
D21	43.3	9.1	-79.0%
D28	53.1	11.9	-77.5%

3.5.2 Evolution of the monthly risk in one year

Without vector control the risk of being infected was > 50% in one month; reached > 90% in three months and >99% in six months. With vector control implementation, the risks were reduced by 77% in one month; >60% in three months and > 40% in six months. Even in one year, vector control reduced transmission by 20% (Table 5b). In one year, the difference of risk remained spectacular after implementation of vector control (Graph. 5c). The risks increased as logarithmic function of time (Graph 5c) and, correlatively, the reduction of risks induced by vector control decreased as logarithmic function of time (Graph 6).



Graph 6 Evolution of the reduction of risks conferred by vector control according to the duration of stay. (M= number of months)

With vector control 80 to 20 % of new inoculations could be averted. The 20% reduction observed even after one year of exposure is noteworthy at community level and for programming vector control operations.

3.6 Synthesis

Gathering the level increasing risks with time and reduction of risks, conferred by each one of the four methods of vector control (Table 6) showed that, excepted LLIN, the level of protection was about 70% the first month with an average of 77%; a level which appeared well in line with the 80% protection usually considered for vector control. Then the protection decreased with time but could still be around 30% in one year with insecticide treated plastic sheeting model ZeroVector® alone. With implementation of vector control the average reduction was about 20% which is greatly appreciable at community level.

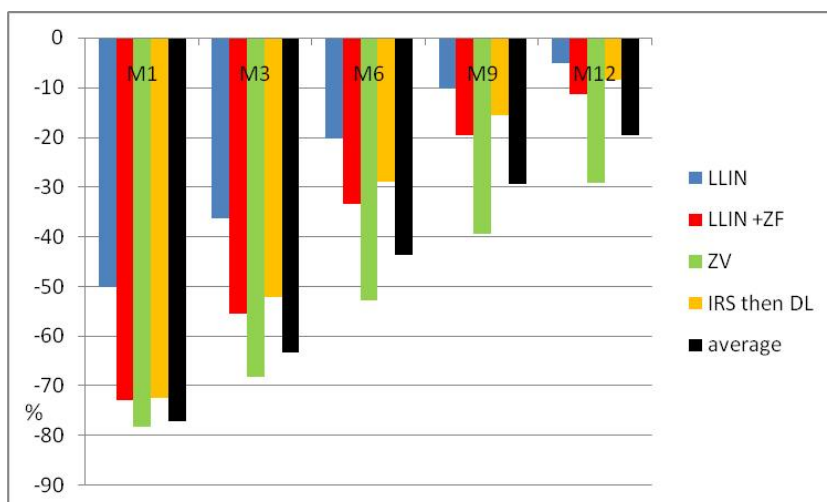
Table 5b Evolution of the monthly risks of receiving an infective bite of a main vector of malaria in a house before, and after, vector control, and the difference of risks.

	Before	After	Diff.
M1	55.6	12.7	-77.1%
M2	80.2	23.8	-70.3%
M3	91.2	33.5	-63.2%
M4	96.1	42.0	-56.3%
M5	98.3	49.4	-49.7%
M6	99.2	55.8	-43.7%
M7	99.7	61.5	-38.3%
M8	99.9	66.4	-33.5%
M9	99.9	70.7	-29.3%
M10	100.0	74.4	-25.6%
M11	100.0	77.6	-22.4%
M12	100.0	80.5	-19.5%

Table 6 Evolution, with time, of the protection afforded by each method of vector control. (M= number of months)

Diff (%)	LLIN	LLIN +ZF	ZV	IRS then DL	Average
M1	-50.2	-72.9	-78.1	-72.3	-77.1
M3	-36.4	-55.5	-68.1	-52.2	-63.2
M6	-20.3	-33.5	-52.7	-29.0	-43.7
M9	-10.3	-19.6	-39.5	-15.7	-29.3
M12	-5.1	-11.4	-29.2	-8.5	-19.5

The evolution of level of protection with duration of exposure was different according to the method of vector control (Graph 6). With time: six months “M6”; nine months “M9”, one year “M12”, the reduction of risks conferred by ITPS ZeroVector® (“ZV”) alone appeared clearly higher than the other methods (Graph 7).



Graph 7 Reduction of risk according to the method of vector control and duration of exposure. (M= number of months)

4 Discussion-Conclusion

The notion of “risks” appeared the article “Malaria Vectorial Capacity of a Population of *Anopheles gambiae*. An Exercise in Epidemiological Entomology” (Garrett-Jones and Shidrawi, 1969). “The index of inoculation risk was derived from the infective density by taking into account the mosquito's supposed biting-frequency and the

average number of people per house. It may be noted that a similar "risk of infection index", but based on the incidence of infective mosquitos caught biting, has recently been proposed. The need for a fresh quantitative approach to the interacting entomological variables that play a part in determining the malaria inoculation rate became evident in the years immediately following the widespread introduction of DDT.

Boyd (1949) pointed out that malariologists had largely ignored Ross (Ross, 1911) mathematical expression of the various factors involved in the perpetuation of a malaria situation; and in concluding his full review of the entomological factors (pp. 608-697) he wrote: "It is to be regretted that even now quantitative values for Ross's factors are lacking. Observed data enable us to assign values to many of the factors on the human side of the equation, but not those pertaining to the vector. "

Another landmark was the concept of vectorial capacity (Garrett-Jones, 1964a) which is density-dependent, the parameter of density to be measured is the man-biting rate ("ma") with the issue of sampling (Service, 1970; Service, 1977), the longevity ("p") of vectors involved (Detinova, 1962) and the length of the sporogonic development of the *Plasmodium* species involved ("n"), but not the infectivity "s" (with the issues of ELISA versus salivary glands classical examination) (Fontenille *et al.*, 2001; Bassene *et al.*, 2009).

The changes of vectorial capacity induced by vector control operations targeting adults' vectors was largely used. DDT house spraying in Northern Nigeria "reduced the malaria vectorial capacity of *Anopheles gambiae* sp B (the main vector of *Plasmodium falciparum* in the area) by an over-all factor of about 23 times." (Garrett-Jones and Shidrawi, 1969). Vectorial capacity was also used to evaluate the impact of inside residual spraying with propoxur during three years (with one year as control) during the Garki Project (N Nigeria) and the mathematical model developed from data obtained (Dietz *et al.*, 1974; Molineaux *et al.*, 1978). Evolution of vectorial capacity was also evaluated with data collected in Kisumu (Kenya) over a period of three years including 20 months during which the insides of houses were sprayed with fenitrothion (Molineaux *et al.*, 1978).

The Balombo Project adopted the same protocol as Garki project with two years control and three years following vector control implementation. The entomological inoculation rate (EIR) is easier to evaluate as involving only two parameters: biting rate ("ma") and infectivity (s) and was largely used recently (Hay *et al.*, 2000; Elissa *et al.*, 2003; Kelly-Hope and McKenzie, 2009; Mboera *et al.*, 2010; Shaukat *et al.*, 2010; Das *et al.*, 2017; Amoah *et al.*, 2021; Doumbe-Belisse *et al.*, 2021; Ukawuba and Shaman, 2022; Degefa *et al.*, 2024).

But as presented, the EIR, such as vectorial capacity, seems to increase as a linear function of time while in the Birley's formula the risk is log function of density, infectivity and times of exposure. This is well in line with the analysis of "31 sites throughout Africa to establish fundamental relationship between annual EIRs and the prevalence of *Plasmodium falciparum* malaria infection. The majority of sites fitted a linear relationship ($r^2 = 0.71$) between malaria prevalence and the logarithm of the annual EIR" (Beier *et al.*, 1999). It was considered that "the analysis also highlights that the EIR is a more direct measure of transmission intensity than traditional measures of malaria prevalence or hospital-based measures of infection or disease incidence. Therefore, malaria field programs need to consider both entomologic and clinical assessments of the efficacy of transmission control measures." This approach was actually implemented in the Balombo project with parasitological surveys systematically done two weeks after each entomological survey (Carnevale *et al.*, 2024) such as the protocol already implemented in Northern Cote d'Ivoire to evaluate the efficacy of lambda cyhalothrin treated nets in an area where the main vector, *Anopheles gambiae* has a kdr based resistance to pyrethroid (Henry *et al.*, 2005).

Such comprehensive approach was already carried out, in Burkina Faso, several decades ago, to evaluate the efficacy of house spraying in the Pilot zone of Bobo-Dioulasso (Hamon *et al.*, 1959); and, more recently, in Peru where parasitological observations were made along with entomological one for an epidemiological evaluation of vector control (Rosas-Aguirre *et al.*, 2021).

The village scale Balombo vector control project was planned to follow the simultaneous changes of entomological and parasitological index during five years, then parasitological surveys were done for six years

more to check for an eventual “rebound effect” long time after cessation of vector control. Entomological data were used to evaluate, with the Birley’s formula, the evolution of risks of being infected, before and after vector control, but also with the time spent in these conditions. Before vector control the risks increased sharply, as a log function of time, reaching 3% in one day; almost 20% in one week; more than 50% in one month and more than 90% in six months. This means that communities, permanently living in such context, will, likely, be malaria infected and vector control is an absolutely need. Actually, risks were greatly reduced, for several months, after vector control, the best having been obtained after installation of insecticide treated plastic sheeting on the walls, inside the houses.

But, even with such a successful vector control, the risks of being infected in one year remained high. This induced the need for regularly repeated vector control operations, in a comprehensive program including, among other measures, improved diagnosis and case management at the most peripheral level. The long-lasting nets must keep their entomological efficacy for three, and more, years but it appeared that, in one year in the field, the risks became as high as before vector control, rising concern to program managers, and this could participate to explain the stalling of malaria situation reported by WHO.

The longer lasting efficacy of insecticide treated plastic sheeting open a new field of research, for example fitting then with treated nets, combining different insecticide to deal with insecticide resistance (Ngufor *et al.*, 2014). To know the risks in living, more or less long time, in malarious area (or period), and their reduction with vector control, (Molineaux and Hempel, 1989; Baudon and Martet, 1997; Batchelor and Gherardin, 2007; Pistone *et al.*, 2010; Wendt *et al.*, 2021), should be important for temporary workers, or displaced population, or tourists as well as for communities living in these areas.

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Conflicts of Interest

Authors confirmed there is no conflict-of-interest. The document was prepared without any a.i.

Authors

NC was involved in data analysis and writing, GC was involved in data analysis, writing, editing English. BO was involved in writing, discussion of data and English editing PC was involved in field surveys, data collection and analysis, writing the document They read and approved the document. No need of ethical clearance as this work dealt only with mosquitoes.

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Research Report

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***In Vitro* Toxicity Levels of *Urtica massaica* Mildbr (Family: *Urticaceae*) on *Anopheles gambiae* Giles (Diptera: Culicidae) Mosquitoes**Yugi J.O.¹ ✉, Khatoro R.T.², Aketch C.O.³, Gitonga N.M.⁴¹ School of Science and Technology, University of Kabianga, P. O Box 2030 - 20200. Kericho, Kenya² School of Environmental Studies, University of Eldoret, P. O. Box 1125 - 30100, Eldoret, Kenya³ Kenya National Examination Council, P. O. Box 73598-00200, City Square, Nairobi-Kenya⁴ School of Biological Sciences, Karatina University, P. O. Box 1957 - 10100 Karatina, Kenya✉ Corresponding email: yugijared@gmail.comJournal of Mosquito Research, 2026, Vol.16, No.1 doi: [10.5376/jmr.2026.16.0002](https://doi.org/10.5376/jmr.2026.16.0002)

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Abstract Botanicals are targets for green insecticides and alternatives to synthetic insecticides. In this study, a randomized experimental design with control was used to evaluate *in vitro* toxicity level (LC₅₀ and LC₉₀) of crude methanol and hexane *Urtica massaica* leaf, stem and root extracts on immature stages of *Anopheles gambiae*. 100 eggs, larvae or pupae were exposed to doses of 80 mg/100mls (e/w), 40 mg/100mls (e/w), 20 mg/100mls (e/w), 10 mg/100mls (e/w), 5 mg/100mls (e/w), 2.5 mg/100mls (e/w) of the extracts in clear plastic containers measuring 6 cm × 5.7 cm × 3.5 cm. Each container held 33 mls of a dose and either 33 eggs, larvae or pupae. The experiments were replicated four times. The set ups were left to stand overnight except that of eggs that stood for 48hrs. Mortality was assessed at the end of the period. It was found that methanol extracts were more toxic than hexane and leaf and root extracts were more toxic than stem extracts. Dose and solvent of extraction significantly influenced mortality ($p < 0.05$) of all stages for methane and hexane extracts except for hexane root extracts ($p > 0.05$) used against L3s. Since calculated goodness of fit was greater than the critical value ($\chi^2 = 22.4$; $df = 22$; $p < 0.05$) for all cases, the null hypothesis was rejected and the conclusion that *U. massaica* crude extracts was toxic to immatures of *An. gambiae* in vitro was adopted. It is concluded that *U. massaica* crude extracts are toxic against immatures of *An. gambiae* in vitro.

Keywords *Urtica massaica*; Methanol; Hexane; Lethal effect; *Anopheles gambiae*

1 Background

Mosquitoes are vectors of global public health importance as the mosquito borne infections (MBI) (WHO, 2020) for which they are famous are of global public health concern (WHO, 2022). Indeed, the infections threaten more than 40% of the world's population (Franklinos et al., 2019), with malaria accounting for the highest reported cases of morbidity and mortality (Maharaj et al., 2019; WHO, 2021; Oladipo et al., 2022; Li et al., 2024). Most cases of malaria infections occur in sub-Saharan Africa (WHO, 2019; WHO, 2020). Intervention against malaria is largely through vector management due to lack of effective medication and vaccination and though RTS, S/AS01 vaccine has been endorsed against malaria (WHO, 2021, Ogieuhi et al., 2024), it is faced with a myriad of challenges (Sallam et al., 2025). Additionally, the vaccine is mainly meant for children and regions with moderate to high *Plasmodium falciparum* malaria transmission (WHO, 2021).

The first line mitigative measure against malaria vector over the years has been the use of synthetic insecticides on various platforms (WHO, 2018; WHO, 2019). These have successfully managed the vector densities (Derua et al., 2018; Derua et al., 2019), interfered with their host-seeking behaviour, reduced their contacts with humans and reduced malaria disease transmission (Cibulskis et al., 2016, Govindarajan et al., 2016). However, their continued indiscriminate use has led to resistance in mosquito populations (Oduola et al., 2019; WHO, 2020; Peng et al., 2022) in addition to unwarranted environmental toxicity (Deng et al., 2019; Semenza et al., 2022; Wafula et al., 2023). These challenges are expected to escalate with emerging issue with climate change, alien vector spp. and the ever-growing threat of resistance to antimalarial drugs and insecticides (Mordecai et al., 2020; Li et al., 2024). Envisaged solution is bringing on board new inventions and strategies (Richards et al., 2020).

In the recent years, the use of natural products from plants has witnessed a lot of attention as the derived biopesticides also known as secondary metabolites, have shown abilities of killing or repelling mosquitoes (Youmsi et al., 2017; Ali et al., 2023). It has also been found to be target specific, non-toxic to valuable natural enemies, fully biodegradable (Vivekanandhan et al., 2018; Vivekanandhan et al., 2020), of broad spectrum (Ebadollahi et al., 2020), and a promising alternative to synthetic insecticides (Yohana et al., 2022). Their use is envisaged to be sustainable as they are readily regenerative, of low cost and environmentally safe (Borges, 2016; WHO, 2020). The use of extracts of *U. massaica*, though proven as botanicals with promising biopesticide potential (Khatoro et al., 2021; Owiti et al., 2025), the demonstration has not been exhaustive. This study therefore demonstrates in vitro toxicity levels of *Urtica massaica* on *Anopheles gambiae* mosquitoes.

2 Results

It was found that methanol extracts were required in smaller doses as compared to hexane extracts of stem or roots for the LC₅₀ regardless of plant parts or immature stage exposed. However, the trend for the LC₉₀ was different. Dose did not influence ($p > 0.05$) mortality of exposed aquatic stages (Table 1). For hexane solvent, extracts from the roots were more toxic than those of stem and leaves for LC₅₀ regardless of immature stage exposed. No singular trend was observed for the LC₉₀. Dose and solvent of extraction significantly influenced mortality ($p < 0.05$) of all exposed aquatic stages except for L3s exposed to root extract ($p > 0.05$) (Table 2). However, because all observed calculated goodness of fit were greater than the critical value ($\chi^2 = 22.4$; $df = 22$; $p < 0.05$) for all cases, the null hypothesis was rejected and the alternative adopted.

Table 1 Lethal concentration (LC₅₀ & LC₉₀) of methanol extracts of *U. massaica* plant parts against different aquatic stages of *An. gambiae* mosquitoes

Life stage	Plant part	LC ₅₀			LC ₉₀			Chi-Square Tests		
		Estimate	95% confidence limit for		Estimate	95% confidence limit for		χ^2	df	p
			log ₁₀ (Concentration)			log ₁₀ (Concentration)				
			Lower	Upper		Lower	Upper			
Eggs	Leaves	4.86 ^a	0.42	0.86	48.39 ^a	1.46	2.10	269.13	22	0.000
	Stem	4.56 ^a	0.60	0.71	19.48 ^a	1.22	1.37	49.28	22	0.001
	Roots	11.91 ^a	0.92	1.22	50.69 ^a	2.33	3.37	98.54	22	0.000
L3	Leaves	1.86 ^a	2.20	0.57	9.05 ^a	0.68	2.39	901.19	22	0.000
	Stem	-	-	-	-	-	-	-	-	-
	Roots	3.06 ^a	0.45	0.52	5.64 ^a	0.66	0.75	46.96	22	0.001
Pupae	Leaves	5.22 ^a	0.68	0.76	8.61 ^a	0.88	1.02	129.74	22	0.000
	Stem	6.12 ^a	0.69	0.91	9.33 ^a	0.86	1.24	704.08	22	0.000
	Root	12.46 ^a	0.46	1.61	21.02 ^a	2.93	13.50	175.40	22	0.000

Notes: df = degree of freedom; χ^2 = the chi-square factor; P = probability for the level of significance. P was taken as significant at $p < 0.05$; LC = refers to lethal concentration, LC₅₀ & LC₉₀ concentration that kills 50% & 90% of exposed experimental aquatic stage; L3 = third larval instar. Rows having LC estimates superscripted with letter "a" denotes no significant influence of dose on exposed *An. gambiae* aquatic stages

3 Discussion

In the study herein, it was demonstrated that leaf and root extracts of methanol and hexane extracts respectively were required in smaller amounts and therefore more toxic than extracts of the other parts of *U. massaica* regardless of aquatic stage exposed or dose administered. This could be explained by differential concentration of bioactives in different parts of a plant. Indeed, secondary metabolites also known as botanicals are distributed differently in different plant parts. That is some plant parts have higher and others lower concentrations. This could be judged from the activity of extracts from different parts of a plant in a bioassay. The amount therein being directly proportional to activity. The more the concentration, the more potent the extracts (Yugi and Kiplimo, 2017). It is assumed therefore that the leaves and roots for methanol and hexane extracts contained the highest concentrations of botanicals respectively, a finding that was consistent with those of Anupam et al., (2012) and Yugi and Kiplimo, (2017), for methanol extracts but inconsistent with that of Thouri et al., (2017) for hexane extracts.

Table 2 Lethal concentration (LC₅₀ & LC₉₀) of hexane extracts of *U. massaica* plant parts against different aquatic stages of *An. gambiae* mosquitoes

Life stage	Plant part	LC ₅₀			LC ₉₀			Chi-Square Tests		
		Estimate	95% confidence limit for log ₁₀ (Concentration)		Estimate	95% confidence limit for log ₁₀ (Concentration)		χ ²	df	p
			Lower	Upper		Lower	Upper			
Egg	Leaves	103.30 ^a	1.73	2.62	39.11 ^a	2.88	5.266	179.96	22	0.000
	Stem	7.81 ^a	0.80	0.98	41.87 ^a	1.49	1.799	133.24	22	0.000
	Roots	6.89 ^a	0.69	0.96	61.19 ^a	1.60	2.079	167.86	22	0.000
L3	Leaves	8.53 ^a	0.85	1.02	17.99 ^a	1.15	1.430	314.57	22	0.000
	Stem	3.88 ^a	0.54	0.64	7.88 ^a	0.83	0.994	113.84	22	0.000
	Roots	1.05 ^b	-0.30	0.16	2.67 ^b	0.35	0.486	33.79	22	0.052
Pupae	Leaves	70.05 ^a	1.77	1.96	13.91 ^a	1.99	2.383	295.15	22	0.000
	Stem	4.94 ^a	0.63	0.76	9.69 ^a	0.91	1.108	193.38	22	0.000
	Roots	31.34 ^a	1.25	1.92	56.28 ^a	2.90	6.056	138.86	22	0.000

Notes: As described (Table 1) except for “b” to denote significant influence of dose on the aquatic stages

In this study methanol extracts were more potent than hexane extracts. Methanol is more polar than hexane and according to the findings of Thouri et al. (2017), Borges et al. (2020) and Nguyen et al., (2021) possess optimal extraction ability as well as capacity to conserve the stability of the chemical structure of desired compounds. This finding was similar to others that demonstrated the influence of solvent type on extracted bioactives as well as larviciding potency (Anupam et al., 2012). However, it is noted that there is not a single standard solvent for optimal bioactive extraction as different solvents react differently for different plant matrices (Ngo et al., 2017).

Mosquitoes are a very important group of arthropods based on their role in the transmission and impact of mosquito borne infection (MBI) to humanity (WHO, 2020) and thus have been under constant human surveillance. Such has today yielded the best possible approaches of mosquito attack some of which include oviciding (Khatoro et al., 2021), larviciding (Yohana et al., 2022), pupiciding (Khatoro et al., 2021) and adulticiding (Muhammed et al., 2022) targeting the life stages of the vector. But again, the success observable today in managing malaria vector population is only possible because the ontogeny of the vector is predictable. As the adage goes, ‘a chain is as strong as its weakest link’. The vector’s aquatic stages (eggs, larvae and pupae) are the weakest link in the chain (life cycle). This is because their movement is restricted to the breeding ground (stagnant water) and are unable to escape to avoid “invasion or attack” by natural enemies (predators) or anthropogenic neutralization (through insecticides). It follows therefore that programmes that target mosquito immature stage are highly impactful (Chung et al., 2009; Conti et al., 2010). Indeed, larviciding has been the most preferred malaria vector control tool (Thomas, 2018) as the statistics on reduced malaria incidence and mortality due to reduced larval and adult mosquito abundance (Afrane et al., 2016; Ingabire et al., 2017) has largely been due to targeting the larvae (William et al., 2018; Zhou et al., 2020). When the larvicide is a biopesticides as is in this study, the programme transforms into the use of green biopesticide and biodiversity conservancy. This is because the biopesticide is not only lethal but since extract is a composition of varied acting bioactives, the vectors don’t get to mount effective resistance against them. Additionally, since plants from which the biopesticides are derived are easily accessible and regenerative, the programme is not only sustainable and safe (Govindarajan et al., 2016), it also leads to conservation of the plant resources. It is therefore a solution to insecticide resistance mosquitoes, sustainable use and conservation of resources (Rahimi et al., 2019; Rahimi et al., 2020).

3.1 Conclusion

It is concluded that leaf, stem and root methanol and hexane crude extracts of *U. massaica* are required in small amount to kill immature *An. gambiae* mosquitoes. Their toxicity levels are promising as candidates for natural mosquito control strategies.

4 Materials and Methods

4.1 Study area, experimental mosquitoes, study design and laboratory conditions

This was an *in vitro* designed study conducted at the Centre for Global Health Research (CGHR) entomology laboratory where immature stages (Eggs, third larval instars (L3s) and pupae) of *An. gambiae* were sourced. The experimental design used was as described (Kothari, 2004; Yugi and Kiplimo, 2017). Briefly, a completely randomized informal ‘after-only with control’ experimental design was used with the solvent, dose and biopesticide extracts taken as independent while mortalities as dependent variables. Distilled water was taken as negative control. The laboratory temperatures and humidity were 28 °C~30 °C and 70%~80% respectively. Photoperiod was 12 hrs light (06.30~18.30 hrs) and 12 hrs darkness (18.30~0630 hrs).

4.2 *Urtica massaica* plant parts source, extraction and stocks solution preparation

U. massaica plant (leaves, stem and roots), voucher specimen number JOY2017/001 were sourced from 35°16’ 46’’ E, 0°31’ 41’’ N in Eldoret, Kenya. The extraction and processing of the biopesticide was done as described by Khatoro et al., (2021). Briefly, two grams of crude biopesticide stock’s extracts was dissolved in 200 millilitres (mls) of dimethyl sulfoxide (DMSO). 160 mls (with 160 mls (v/v) of extract) of this solution was obtained and topped up with 40 mls of distilled water to make 200 mL (with 160 mls (v/v) of extract). This solution was then aliquoted in two beakers of equal capacity (100 mL) each to give a concentration of 80 mL/100mL (s/w). One of this was picked and 100mL distilled water added to top it up to 200 mls and then aliquoted in equal units of 100 mls to give a concentration of 40 mL/100 mL (s/w). This procedure was repeated until serial dilution of 80 mL/100 mL (s/w), 40 mL/100 mL (s/w), 20 mL/100 mL (s/w), 10 mL/100 mL (s/w), 5 mL/100 mL (s/w), 2.5 mL/100 mL (s/w) were obtained for the leaf, stem and root extracts.

4.3 Toxicity bioassay

Toxicity bioassays was conducted as described by Khatoro et al., (2021) and insecticidal potency of the biopesticide determined following the WHO, (2005) procedures. Briefly, 100 freshly transformed third larval instars (L3) were transferred by means of a dropper to plastic containers measuring 6 cm × 5.7 cm × 3.5 cm and left exposed for 24 hours after which the experiment was stopped. The experiments were replicated four times. This procedure was repeated for eggs and pupae for both methanol and hexane extracts. Eggs were however exposed for 48 hours. Mortality was calculated (i) and corrected (ii) using Abbot’s (1925) formula for mortality of 5 % larvae in the control.

$$\% \text{ Mortality} = \frac{\text{Number of dead aquatic stage}}{\text{Total number of aquatic stage introduced}} \times 100 \dots\dots\dots(i)$$

$$\% \text{ Corrected mortality} = \frac{\text{Percent mortality in test} - \text{Percent mortality in control}}{100 - \text{Percent in control}} \times 100 \dots\dots(ii)$$

4.4 Statistical Analysis

Data was organized in excel spreadsheets and analysed using regression (probit) statistics to determine levels of toxicity (LC₅₀ and LC₉₀) for the 50% and 90% respectively for dose and solvent of extraction. Levels of significance were adopted at 95% confident interval (CI) (that is at $p \leq 0.05$). Calculated goodness of fit was compared with critical value ($\chi^2 = 22.4$) at the same CI to inform on the relationship with the hypothesis of no relation. All statistical analysis was performed using statistical package for social scientists (SPSS) version 22.

Authors’ contributions

Conceptualization, data analysis, and writing of the original draft done by JOY, supervision, methodology, investigation, data collection, review and editing done by all authors.

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Competing interests

None

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Research Insight

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Evaluation of Plant-Based Extracts for the Control of *Anopheles gambiae* Mosquitoes under Laboratory Conditions

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Abstract *Anopheles gambiae* is one of the principal vectors of malaria and remains a major target in mosquito control programs. However, the widespread development of insecticide resistance has reduced the effectiveness of conventional chemical interventions, creating an urgent need for alternative control agents. This review summarizes laboratory-based evaluations of plant extracts for the control of *A. gambiae*, with emphasis on their larvicidal, adulticidal, and repellent activities. It also examines the chemical composition of these extracts, including the identification of major bioactive compounds, and discusses their possible mechanisms of action at physiological, enzymatic, and cellular levels. In addition, the review considers safety issues, such as toxicity to non-target organisms and environmental persistence, and compares plant-derived products with synthetic insecticides. Overall, plant extracts show considerable promise as eco-friendly and sustainable tools for mosquito management, although further studies are needed to standardize formulations and validate their field efficacy.

Keywords *Anopheles gambiae*; Plant extracts; Mosquito control; Larvicidal activity; Insecticide resistance

1 Introduction

Anopheles gambiae mosquitoes are the primary vectors responsible for malaria transmission in sub-Saharan Africa, where the disease remains a major public health challenge. Their capacity to transmit *Plasmodium* parasites is influenced by factors such as their abundance, biting behavior, and longevity, which enable effective parasite development and spread among human populations. Understanding the biology and ecology of *An. gambiae* is crucial for developing targeted interventions to reduce malaria incidence, as these mosquitoes predominantly feed indoors at night and breed in aquatic habitats, making them accessible to control measures (Takken et al., 2024; Simoni et al., 2025).

Current mosquito control strategies largely rely on synthetic insecticides applied through insecticide-treated nets (ITNs) and indoor residual spraying (IRS). While these methods have significantly reduced malaria burden, their effectiveness is increasingly compromised by the rapid emergence of insecticide resistance in *An. gambiae* populations, as well as behavioral adaptations that reduce mosquito contact with treated surfaces. Additionally, concerns about environmental toxicity and sustainability highlight the limitations of chemical-based approaches, necessitating alternative or complementary vector control tools that can overcome resistance and minimize ecological impact (Paton et al., 2022; Simoni et al., 2025).

Plant-based extracts have gained attention as promising alternative agents for controlling *An. gambiae* due to their eco-friendly nature and diverse bioactive compounds with repellent, larvicidal, and adulticidal properties. Various studies have demonstrated that essential oils and phytochemicals from plants such as *Croton macrostachyus*, *Cymbopogon* species, *Azadirachta indica*, and *Ocimum* spp. exhibit significant efficacy against different life stages of *An. gambiae* under laboratory conditions. These natural products offer potential advantages including reduced risk of resistance development and lower environmental toxicity, positioning them as valuable candidates for integrated malaria vector management strategies (Lamuntani et al., 2025; Tadesse et al., 2025).

2 Materials and Methods

2.1 Source of plant materials and extraction procedures

Plant materials used for this study were collected from local sources, ensuring freshness and proper identification before extraction. The extraction process involved selecting appropriate solvents based on the polarity of target bioactive compounds, commonly including polar solvents such as methanol or ethanol, which are effective in extracting a wide range of phytochemicals. Extraction methods such as maceration and Soxhlet extraction were employed to maximize yield; maceration involves soaking plant material in solvent at room temperature for extended periods, while Soxhlet extraction uses continuous solvent reflux to enhance compound recovery. These methods have been widely used in mosquito control research due to their efficiency in isolating larvicidal and adulticidal compounds from plants (Figure 1) (Ravi et al., 2018; Abubakar and Haque, 2020).



Figure 1 Morphological midgut content induced by *Azolla pinnata* plant extract from soxhlet extraction method in larvae of *Ae. Aegypti* (Adopted from Ravi et al., 2018)

Image caption: (A) Control test for midgut content view in early 4th instar larvae of *Ae. Aegypti* (B) *A. pinnata* crude extract for midgut content view in larvae of *Ae. Aegypti*. Arrows indicating the plant extracts (greenish colour), GC: gut content (after 24hours) (Adopted from Ravi et al., 2018)

Following extraction, crude extracts were concentrated under reduced pressure using rotary evaporation to remove solvents without degrading active constituents. The chemical composition of extracts was characterized using chromatographic techniques like gas chromatography-mass spectrometry (GC-MS) to identify major phytochemicals responsible for insecticidal activity. Such characterization is essential for understanding the bioactive profile and guiding further bioassays. Quality control measures included standardizing extract concentrations and storing samples at low temperatures to preserve stability prior to testing (Abutaha and Al-Mekhlafi, 2020; Hafsi et al., 2022).

2.2 Origin of experimental mosquitoes and laboratory rearing conditions

Experimental *Anopheles gambiae* mosquitoes were obtained from established laboratory colonies maintained under controlled environmental conditions to ensure uniformity in age, physiological status, and genetic background. Colonies were typically reared at temperatures between 25-28°C with relative humidity around 70-80%, simulating natural tropical environments conducive for mosquito development. Photoperiods were maintained on a 12:12 hour light-dark cycle to regulate circadian rhythms affecting mosquito behavior and physiology (Ahamd et al., 2023; Dutta and Dey, 2023).

Larvae were fed standardized diets such as finely ground fish food or yeast suspensions to promote consistent growth rates, while adult mosquitoes were provided with sugar solutions for sustenance and periodic blood meals when necessary for egg production. Rearing containers were regularly cleaned to prevent microbial contamination that could affect mosquito health or experimental outcomes. These controlled rearing protocols ensured that test mosquitoes exhibited normal development and behavior suitable for reliable bioassay results (Priya and Jones, 2021; Dutta and Dey, 2023).

2.3 Bioassay methods and experimental design

Bioassays followed standardized World Health Organization (WHO) protocols adapted for evaluating larvicidal and adulticidal effects of plant extracts against *Anopheles gambiae*. Larval bioassays involved exposing early fourth instar larvae to various concentrations of plant extracts diluted in water, with mortality recorded after 24 hours of exposure. Control groups received solvent only to account for any non-specific effects. Concentration-mortality data were analyzed using probit analysis to determine lethal concentration values (LC50 and LC90), indicating the potency of each extract (Ravi et al., 2018; Pavela et al., 2019).

Adulticidal assays entailed topical application or exposure of adult mosquitoes to treated surfaces impregnated with plant extracts, monitoring knockdown rates and mortality over specified time intervals. Experimental designs incorporated replicates per treatment concentration alongside controls to ensure statistical robustness. Additionally, sub-lethal effects such as behavioral changes or reproductive impairments were observed where applicable. This comprehensive approach allowed assessment not only of acute toxicity but also potential impacts on mosquito population dynamics relevant for vector control strategies (Hafsi et al., 2022; Dutta and Dey, 2023).

3 Chemical Composition Analysis of Plant Extracts

3.1 Determination of physicochemical properties of extracts

The physicochemical properties of plant extracts are fundamental for understanding their stability, solubility, and bioactivity, which influence their efficacy as mosquito control agents. Parameters such as pH, viscosity, density, and refractive index are commonly measured to characterize the extracts and ensure consistency across batches. These properties can affect the interaction of bioactive compounds with mosquito targets and their formulation into usable products. For example, pH can influence the ionization state of active molecules, altering their penetration or binding affinity (Godlewska et al., 2023; Zhang et al., 2023).

Additionally, preliminary qualitative tests such as colorimetric assays help identify classes of phytochemicals present in the extracts, including phenols, flavonoids, alkaloids, tannins, and saponins. These compounds often contribute to insecticidal activity through various mechanisms like enzyme inhibition or membrane disruption. Rapid screening methods provide an initial profile that guides more detailed chemical analyses and bioassays. Maintaining standardized physicochemical characteristics is essential for reproducibility in experimental evaluations and potential field applications (Heinrich et al., 2022; Godlewska et al., 2023).

3.2 Identification of active components

Gas chromatography-mass spectrometry (GC-MS) is a widely used analytical technique for identifying volatile and semi-volatile compounds in plant extracts. It separates complex mixtures into individual constituents based on their retention times and mass spectra, allowing precise identification by comparison with spectral libraries. GC-MS analysis typically reveals a diverse array of bioactive molecules such as terpenes, hydrocarbons, alcohols, esters, and fatty acids that may contribute to larvicidal or adulticidal effects against *Anopheles gambiae* (Khdera and Saad, 2024; Dhanaraj et al., 2025).

High-performance liquid chromatography (HPLC) complements GC-MS by enabling the separation and quantification of non-volatile or thermally labile compounds like polyphenols, flavonoids, and alkaloids. Coupled with detectors such as UV-Vis or mass spectrometry (LC-MS), HPLC provides detailed profiles of major phytochemicals responsible for biological activity. Combining these chromatographic techniques ensures comprehensive characterization of plant extracts' chemical composition critical for correlating specific compounds with observed insecticidal properties (Figure 2) (Heinrich et al., 2022; Hodoşan et al., 2025).

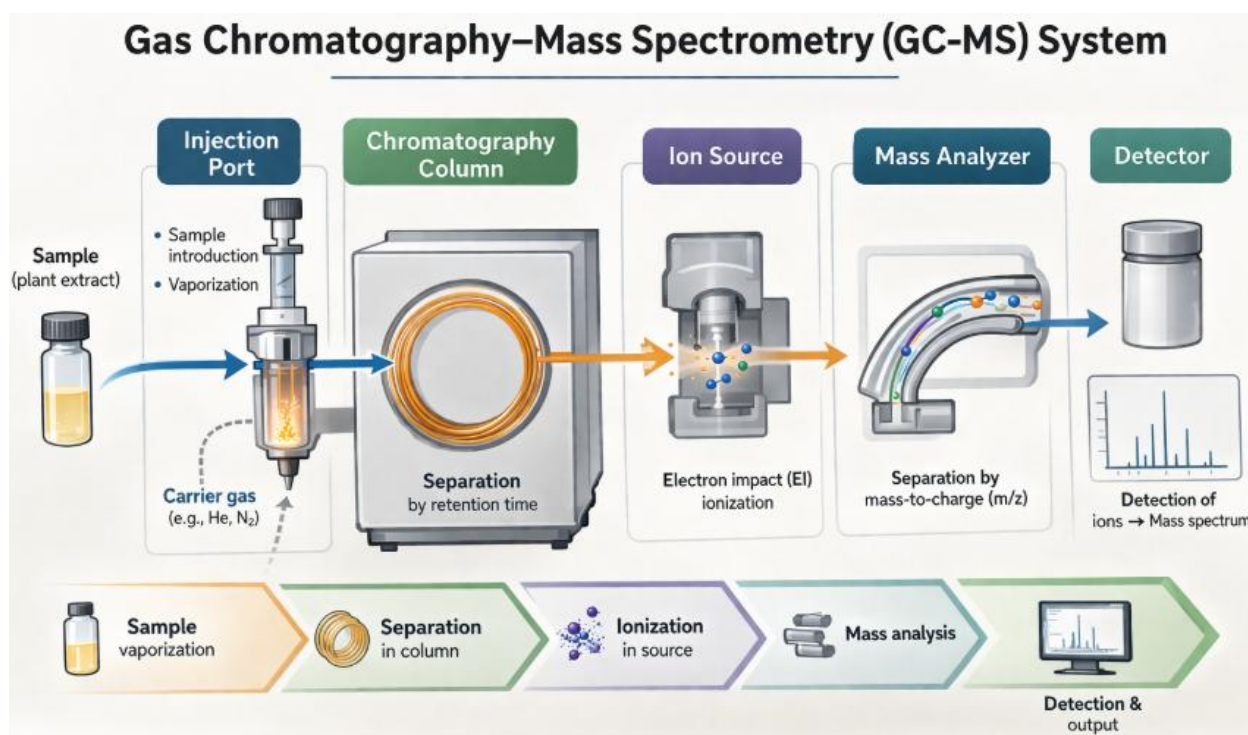


Figure 2 Schematic diagram of the gas chromatography-mass spectrometry (GC-MS) system illustrating sample injection, chromatographic separation, and mass spectral detection. Adapted from general analytical instrumentation principles

3.3 Quantitative analysis of major active compounds

Quantitative determination of key bioactive constituents is essential to standardize plant extracts and evaluate dose-dependent effects on mosquito populations. Techniques such as calibration curve-based HPLC quantification allow accurate measurement of major phenolic acids, flavonoids, or terpenoids known for insecticidal or repellent activities. This quantitative data supports reproducibility in bioassays and facilitates comparisons between different plant species or extraction methods (Dhanaraj et al., 2025; Hodoşan et al., 2025).

Furthermore, advanced mass spectrometry methods including high-resolution MS enable precise quantification even in complex mixtures at low concentrations. Such analyses help identify marker compounds that can serve as quality control indicators during extract production. Understanding the concentration ranges of active components also informs formulation strategies to optimize efficacy while minimizing potential toxicity to non-target organisms or the environment (Heinrich et al., 2022; Kapadia et al., 2022).

4 Larvicidal Activity of Plant Extracts against *Anopheles gambiae*

4.1 Mortality rates under different concentration treatments

The larvicidal efficacy of various plant extracts against *Anopheles gambiae* larvae generally exhibits a clear dose-dependent relationship, with higher concentrations producing increased mortality rates. For instance, methanol crude extracts of *Croton macrostachyus* achieved 100% mortality at 400 ppm, while lower concentrations showed proportionally reduced effects, demonstrating strong larvicidal potential (Tadesse et al., 2025). Similarly, ethanol leaf extracts from *Azadirachta indica*, *Carica papaya*, and *Annona muricata* caused significant larval mortality, with complete mortality observed at concentrations above 10% for *A. indica* and *C. papaya* and above 5% for *A. muricata* (Ekpo et al., 2025).

Other studies confirm this trend across different plant species and extraction methods; for example, methanol leaf extracts of *Ocimum gratissimum* and *Cymbopogon citratus* showed high larvicidal activity with mortality rates reaching up to 100% at the highest tested concentrations (1 000 ppm) after 72 hours exposure (Opoggen et al., 2019). These findings underscore the importance of optimizing extract concentration to maximize larvicidal effects while considering practical application limits in vector control programs (Okbatinsae and Haile, 2017).

4.2 Determination of lethal concentrations (LC50, LC90)

Lethal concentration values such as LC50 and LC90 are critical metrics for quantifying the potency of plant extracts against mosquito larvae. The ethyl acetate fraction of *Croton macrostachyus* exhibited an LC50 of 38.60 ppm and an LC90 of approximately 100 ppm against *Anopheles gambiae* larvae, indicating high efficacy compared to other tested plants (Tadesse et al., 2025). Hexane extracts from *Abutilon bidentatum* also demonstrated potent larvicidal activity with an LC50 value of 40.77 ppm and an LC90 value near 100.54 ppm under semi-field conditions (Muhammed et al., 2024).

In addition, hexane extracts of *Lantana camara* showed remarkable toxicity with an LC50 value as low as 20.19 ppm and an LC90 around 49.29 ppm against local strains of *Anopheles gambiae* larvae (Wangrawa et al., 2016). These quantitative assessments provide essential benchmarks for comparing bioactivity across different plant species and extraction solvents, guiding selection for further development as biopesticides.

4.3 Analysis of time-dose response relationships

Time-dose response studies reveal that both exposure duration and extract concentration significantly influence larval mortality outcomes. For example, methanol extracts from *Momordica foetida*, *Gnidia glauca*, and *Vepris soyauxii* showed increasing mortality rates over time, with LC50 values decreasing after 48 hours compared to 24-hour exposures, indicating enhanced toxicity with prolonged contact (Njuabe et al., 2022). This temporal effect is important for understanding how quickly plant-based larvicides act in practical settings.

Similarly, ethanol extracts from several plants demonstrated higher larval mortality after extended exposure periods; *Jatropha curcas* and *Ricinus communis* ethanol extracts reached near-complete mortality only after 48 hours at high concentrations (1000 ppm) (Okbatinsae and Haile, 2017). These findings highlight the necessity to consider both dose and exposure time when evaluating the effectiveness of plant-derived larvicides to optimize their use in integrated vector management strategies.

5 Effects of Plant Extracts on Adult Mosquitoes

5.1 Evaluation of contact toxicity and repellency

Plant extracts have demonstrated significant contact toxicity against adult *Anopheles gambiae* mosquitoes, with some extracts achieving high mortality rates at relatively low concentrations. For example, methanol crude extracts of *Croton macrostachyus* showed strong adulticidal activity, reaching 100% mortality at 400 ppm and exhibiting LC50 and LC90 values of 55.32 ppm and 86.77 ppm, respectively (Tadesse et al., 2025). Similarly, essential oils from plants such as *Cymbopogon winterianus*, *Cinnamomum zeylanicum*, and *Thymus vulgaris* exhibited combined repellent, irritant, and toxic effects on adult mosquitoes, suggesting multiple modes of action that could reduce mosquito-human contact effectively (Delétré et al., 2013).

Repellency studies further support the potential of plant extracts as alternatives to synthetic insecticides. Extracts from *Eclipta alba* and *Andrographis paniculata* provided dose-dependent protection against mosquito bites without causing allergic reactions in human subjects, indicating their suitability for topical application (Govindarajan and Sivakumar, 2011). Additionally, ethanolic extracts of *Cymbopogon citratus* and *Ocimum basilicum* leaves showed effective repellency against *Culex quinquefasciatus* adults, with *Cymbopogon citratus* demonstrating superior efficacy (Aïzoun et al., 2025). These findings highlight the dual utility of plant extracts in both killing and deterring adult mosquitoes.

5.2 Effects on adult survival rate and longevity

Exposure to certain plant extracts significantly reduces the survival rate and longevity of adult *Anopheles* mosquitoes. For instance, aqueous leaf extracts of *Zehneria scabra* caused notable adult mortality with an LC50 value of 176.20 ppm against *Anopheles stephensi* adults after 24 hours exposure (Muhammed et al., 2022). Similarly, methanol extracts of *Azadirachta indica* achieved 75% adult mortality at 300 ppm against *Anopheles arabiensis*, indicating substantial impact on adult survival (Ejeta et al., 2021). These reductions in lifespan can decrease the likelihood of disease transmission by shortening the period during which mosquitoes remain infectious.

Histopathological studies reveal that some plant-derived compounds induce physiological damage in adult mosquitoes that likely contributes to reduced longevity. Essential oils from *Ageratum conyzoides* caused degeneration in compound eyes, muscular tissues, gut epithelium, *Malpighian tubules*, and ovarian cells in *Aedes aegypti* adults (Pintong et al., 2020). Such tissue damage impairs vital functions including feeding and reproduction, thereby limiting mosquito population growth over time. This evidence supports the use of botanical insecticides not only for immediate toxicity but also for long-term vector control through lifespan reduction (Figure 3).

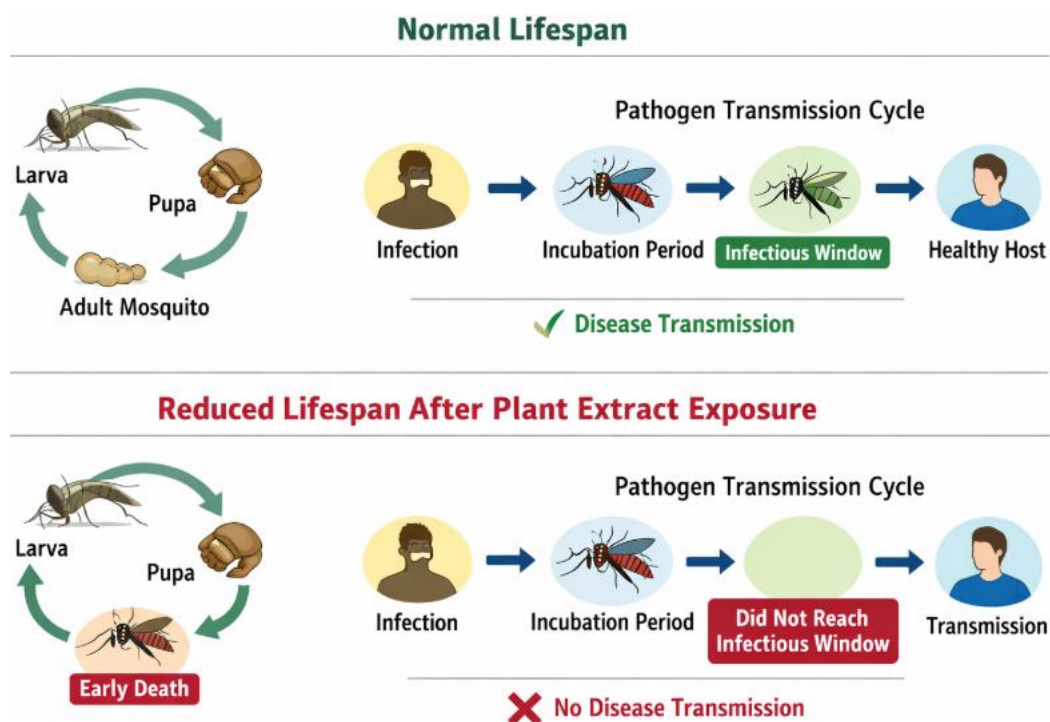


Figure 3 Conceptual diagram illustrating how reduced adult mosquito lifespan limits the transmission potential of malaria parasites by shortening the infectious period

5.3 Effects on blood-feeding behavior and reproductive capacity

Plant extracts can alter blood-feeding behavior in adult mosquitoes by acting as repellents or irritants that reduce host-seeking activity. The irritant and repellent effects observed with essential oils from *Cymbopogon winterianus* and *Thymus vulgaris* suggest disruption of normal feeding patterns in *Anopheles gambiae* adults (Delétré et al., 2013). By decreasing successful blood meals, these extracts may lower pathogen transmission rates indirectly through behavioral modification rather than direct toxicity alone.

Moreover, some botanical compounds negatively affect reproductive capacity by causing ovarian degeneration or reducing egg production. Histopathological alterations induced by *Ageratum conyzoides* essential oils included ovarian cell degeneration in *Aedes aegypti* females (Pintong et al., 2020), which likely translates into decreased fecundity. Although detailed quantitative data on reproductive inhibition are limited for *Anopheles* species specifically, these findings indicate that plant-based products may impair mosquito population growth through multiple physiological pathways beyond immediate lethality.

6 Mechanism of Action Analysis

6.1 Effects on the nervous system

Plant extracts exert significant neurotoxic effects on mosquitoes, primarily targeting their nervous system to disrupt normal physiological functions. Many secondary metabolites found in these extracts, such as alkaloids, flavonoids, and terpenoids, interfere with nerve impulse transmission by affecting ion channels and neurotransmitter receptors, leading to paralysis and death of mosquito larvae and adults (Pavela et al., 2019;

Senthil-Nathan, 2020). Essential oils from various plants have been shown to cause neuroexcitation or inhibition by modulating acetylcholine receptors and other neural targets, which impairs mosquito motor functions and feeding behavior (Duque et al., 2023).

In addition to direct neurotoxicity, some plant compounds disrupt mitochondrial function within nerve cells by inhibiting electron transport chain enzymes, thereby reducing ATP production essential for nerve signal propagation (Duque et al., 2023). This dual action on both synaptic transmission and cellular energy metabolism enhances the overall insecticidal efficacy of botanical extracts. Such neurotoxic mechanisms are crucial for developing plant-based insecticides that can overcome resistance issues associated with synthetic chemicals.

6.2 Inhibition of key enzyme activities

A major mode of action for many plant-derived insecticides involves the inhibition of acetylcholinesterase (AChE), an enzyme critical for terminating nerve impulses by hydrolyzing the neurotransmitter acetylcholine. Several studies have demonstrated that essential oils and phytochemicals bind to AChE active sites, preventing its function and causing accumulation of acetylcholine at synapses, which results in continuous nerve stimulation and eventual paralysis of mosquitoes (Duque et al., 2023; Montaña-Campaz et al., 2025). Molecular docking analyses support these findings by showing stable interactions between plant compounds such as γ -elemene and germacrene D with mosquito AChE enzymes (Montaña-Campaz et al., 2025).

Beyond AChE inhibition, other enzymes involved in detoxification processes like glutathione S-transferase and cytochrome P450 monooxygenases are also targeted by certain plant extracts, impairing the mosquito's ability to metabolize toxic substances (Pavela et al., 2019; Senthil-Nathan, 2020). This multi-enzyme inhibition not only increases toxicity but may also reduce the development of resistance by attacking several biochemical pathways simultaneously.

6.3 Toxicological effects at cellular and tissue levels

At the cellular level, plant extracts induce a range of toxic effects including disruption of cell membranes, oxidative stress, and damage to vital organelles such as mitochondria. These effects compromise cell integrity and function in mosquito larvae and adults (Pavela et al., 2019; Baz et al., 2024). Histopathological examinations reveal degeneration in tissues critical for survival such as midgut epithelium, *Malpighian tubules*, muscles, and reproductive organs following exposure to botanical insecticides (Baz et al., 2024).

Such tissue-level damage impairs digestion, excretion, locomotion, and reproduction in mosquitoes, contributing to reduced population growth beyond immediate mortality effects. For example, essential oils from *Ageratum conyzoides* caused ovarian cell degeneration in adult mosquitoes alongside gut epithelial damage (Baz et al., 2024), indicating that botanical extracts can exert prolonged sublethal impacts that affect vector capacity. These multifaceted toxicological actions highlight the potential of plant-based products as effective tools for integrated mosquito management.

7 Safety and Environmental Impact Assessment

7.1 Toxicity evaluation on non-target organisms

Plant-based extracts used for mosquito control generally exhibit lower toxicity to non-target organisms compared to synthetic insecticides, making them safer alternatives in integrated vector management. Studies have shown that many botanical insecticides, including neem oil and various essential oils, are target-specific and biodegradable, causing minimal harm to beneficial insects, aquatic organisms, and mammals (Demirak and Canpolat, 2022; Chatterjee et al., 2023). For example, methanolic extracts from certain plants demonstrated larvicidal activity against mosquitoes while showing weak or no toxicity in zebrafish embryos, a common model for assessing environmental safety (Figure 4) (Alqurashi et al., 2025). This selective toxicity is attributed to the complex mixtures of bioactive compounds in plant extracts that often act on specific mosquito physiological pathways without broadly affecting other species.

Despite their relative safety, some plant extracts can still pose risks if used improperly or at high concentrations. Therefore, thorough ecotoxicological assessments are necessary before large-scale application to ensure non-target species such as pollinators, aquatic invertebrates, and vertebrates remain unharmed (Pavela et al., 2019; Hillary et al., 2024). The rapid biodegradability of many plant compounds also reduces the risk of bioaccumulation and long-term environmental persistence. Overall, current evidence supports the use of plant-based products as environmentally friendly mosquito control agents with a favorable safety profile for non-target organisms.

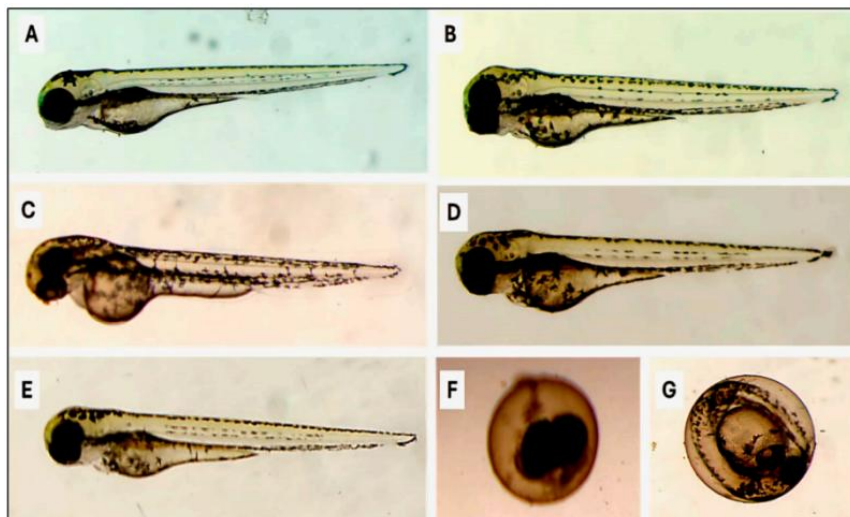


Figure 4 Biosafety evaluation of *H. erectus* in the zebrafish embryos. Representative micrographs of the zebrafish embryos at 3 days post-fertilization were treated with the crude methanolic extract of the marine sponge *H. erectus* and its various fractions. The embryos treated with the crude methanolic extract (B), as well as the n-hexane (<25 μ L) (C), chloroform (D), and n-butanol (E) fractions, developed normally with no observable differences in morphology or growth compared to the control group (A). However, the embryos exposed to a higher concentration of n-hexane (>25 μ L) showed complete lethality (F). Additionally, unhatched embryos were also observed in some treatments (G) (Adopted from Alqurashi et al., 2025)

7.2 Environmental degradation and residue characteristics of plant extracts

Plant-derived insecticidal compounds typically degrade more rapidly in the environment than conventional chemical insecticides, resulting in lower residual toxicity and reduced ecological impact. Many phytochemicals such as terpenoids, flavonoids, and alkaloids are naturally biodegradable under sunlight and microbial action, which limits their persistence in soil and water bodies (Pavela et al., 2019; Hillary et al., 2024). This rapid degradation helps prevent contamination of aquatic ecosystems and reduces the likelihood of adverse effects on non-target organisms over time.

However, some formulations like neem oil may face challenges related to stability under atmospheric conditions; direct application can lead to quick disintegration reducing efficacy (Chatterjee et al., 2023). Advances in formulation technologies such as nanoemulsions or encapsulation have been developed to enhance stability and prolong residual activity while maintaining environmental safety. These improved formulations balance effective mosquito control with minimal environmental footprint by controlling release rates and protecting active ingredients from rapid degradation.

7.3 Comparison with conventional chemical insecticides

Compared to synthetic chemical insecticides like organophosphates and pyrethroids, plant-based extracts offer several advantages including reduced toxicity to humans and wildlife, lower environmental persistence, and decreased risk of resistance development in mosquito populations (Demirak and Canpolat, 2022; Hillary et al., 2024). Synthetic chemicals often cause pollution through bio-magnification and have been linked to health issues such as neurotoxicity and skin irritation in humans (Hillary et al., 2024). In contrast, botanical insecticides tend to be safer due to their natural origin and complex mixtures of active compounds that reduce the chance of mosquitoes developing resistance.

Nevertheless, plant extracts sometimes exhibit lower immediate potency or shorter duration of action than conventional insecticides, which can limit their standalone effectiveness in some settings (Pavela et al., 2019; Ahamd et al., 2023). Combining botanical products with other control methods or improving formulations can help overcome these limitations while preserving their eco-friendly benefits. Overall, plant-based mosquito control agents represent promising alternatives or complements to chemical insecticides by offering effective vector management with fewer environmental and health risks.

8 Discussion and Conclusion

The experimental evaluation of plant-based extracts against *Anopheles gambiae* mosquitoes demonstrated significant larvicidal and adulticidal activities, confirming the potential of botanical compounds as effective mosquito control agents. Extracts from plants such as *Parthenium hysterophorus* and *Nicotiana tabacum* showed high toxicity with low LC50 values, indicating strong potency even at low concentrations. These findings align with broader literature reporting that secondary metabolites like alkaloids, terpenoids, and flavonoids disrupt mosquito physiology through multiple mechanisms including neurotoxicity and enzyme inhibition. The observed synergistic effects between different plant extracts or when combined with conventional insecticides further enhance their efficacy, suggesting opportunities for integrated approaches to overcome resistance issues. However, while laboratory results are promising, the translation to field conditions remains limited due to factors such as environmental degradation of active compounds and variability in mosquito populations. Stability challenges of some plant extracts under natural conditions can reduce residual activity, necessitating improved formulations or delivery systems to maintain effectiveness over time. Additionally, sublethal effects on mosquito development and reproduction observed in some studies indicate that botanical extracts may contribute to population suppression beyond immediate mortality, which is important for sustainable vector control strategies.

Plant-based extracts offer a valuable alternative or complement to synthetic insecticides due to their biodegradability, lower toxicity to non-target organisms, and reduced risk of resistance development in mosquitoes. Their broad-spectrum activity against various mosquito life stages-including larvae, pupae, and adults-makes them versatile tools for integrated vector management programs. Moreover, the availability of many effective plants locally supports cost-effective production and use in endemic regions where resources are limited. Advances in formulation technologies such as nanoencapsulation and synergistic blends have improved stability and potency, enhancing field applicability. Despite these advantages, challenges remain for large-scale implementation including standardization of extract composition, regulatory approval processes, and ensuring consistent efficacy across diverse ecological settings. Field validation studies are urgently needed to assess epidemiological impacts and optimize application protocols. Combining plant-based products with other control measures like insecticide-treated nets or biological agents could maximize overall effectiveness while minimizing environmental impact. Thus, plant extracts hold considerable promise as eco-friendly components of sustainable mosquito control strategies.

This study's primary limitation lies in its laboratory-based design which may not fully capture the complexities encountered under field conditions such as environmental variability and interactions with other biotic factors. The stability and persistence of active compounds in natural habitats require further investigation to ensure practical utility. Additionally, the potential development of resistance against botanical insecticides has not been extensively studied and warrants long-term monitoring. Future research should focus on large-scale field trials to validate laboratory findings and evaluate the epidemiological benefits of plant-based interventions. Exploring synergistic combinations among different plant extracts or with conventional insecticides could improve efficacy while reducing doses required. Advances in formulation science aimed at enhancing stability, controlled release, and target specificity will be critical for successful deployment. Finally, comprehensive safety assessments on non-target organisms and environmental fate studies must accompany efficacy evaluations to support regulatory approval and public acceptance.

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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.

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Review Article

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Seasonal Dynamics and Habitat Characteristics of Mosquito Populations in a Tropical Environment

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Abstract Mosquito populations in tropical environments exhibit marked seasonal dynamics shaped by climatic variability, habitat availability, and human disturbance. This paper examines how temperature, rainfall, humidity, and ecological conditions influence temporal changes in mosquito abundance and distribution, with particular attention to the relationship between seasonal patterns and habitat characteristics. Both natural habitats, such as wetlands, ponds, and vegetated areas, and artificial habitats, including urban containers, drainage systems, and domestic water storage sites, are considered in order to understand their roles in sustaining mosquito breeding and survival. The study also explores species-specific responses to environmental drivers, showing that different mosquito taxa vary in their seasonal peaks and habitat preferences. In addition, the paper reviews commonly used field sampling methods, statistical analyses, and spatial tools such as geographic information systems and remote sensing for monitoring mosquito populations and identifying high-risk habitats. The findings highlight that seasonal mosquito dynamics in tropical regions are closely linked to habitat heterogeneity and environmental change, which together affect the risk of vector-borne disease transmission. A better understanding of these interactions can support more targeted habitat management and seasonally optimized mosquito control strategies for improving public health outcomes in tropical settings.

Keywords Mosquito populations; Tropical environments; Seasonal dynamics; Habitat characteristics; Vector control

1 Introduction

Mosquitoes play a critical role in tropical regions as vectors of numerous infectious diseases that significantly impact public health worldwide. Tropical climates, characterized by warm temperatures and high humidity, provide ideal conditions for mosquito breeding and survival, facilitating the transmission of pathogens such as dengue, malaria, Zika, and chikungunya viruses. The burden of these vector-borne diseases is particularly severe in tropical areas due to the year-round presence of mosquitoes and the complex interactions between environmental factors and mosquito biology. Understanding mosquito population dynamics in these regions is essential for developing effective control strategies to reduce disease transmission and protect vulnerable populations (Blanco-Sierra et al., 2024; García-Suárez et al., 2024).

Research on mosquito ecology in tropical environments has advanced considerably, focusing on how seasonal changes and habitat characteristics influence mosquito abundance and species composition. Studies have demonstrated that climatic variables such as temperature, rainfall, and humidity are major drivers of mosquito life cycles, affecting their reproduction, development, and survival rates. Additionally, anthropogenic factors like urbanization alter habitat availability and quality, influencing mosquito community structure and vector potential. Recent modeling efforts have integrated environmental data with mosquito life history traits to predict spatiotemporal population dynamics across diverse tropical landscapes. These approaches highlight the importance of local-scale ecological studies to capture the variability in mosquito populations driven by both natural seasonality and human-induced habitat changes (García-Suárez et al., 2024; Rakotoarison et al., 2025).

This study aims to elucidate the seasonal dynamics and habitat characteristics of mosquito populations within a tropical environment to inform vector control efforts. By characterizing how environmental variables and habitat types affect mosquito abundance and diversity over time, this research seeks to identify key factors that govern

vector population fluctuations. The findings will contribute to optimizing surveillance programs and tailoring control interventions according to seasonal patterns and habitat preferences of different mosquito species. Ultimately, understanding these ecological relationships is vital for mitigating the public health risks posed by mosquitoes in tropical regions under changing climatic conditions (Hinne et al., 2021; Blanco-Sierra et al., 2024).

2 Study Area and Ecological Background

2.1 Tropical climate characteristics and seasonal classification

Tropical regions are characterized by consistently high temperatures and significant humidity levels, creating an environment conducive to mosquito survival and reproduction. These climates typically exhibit distinct wet and dry seasons rather than the four-season pattern seen in temperate zones. The wet season, marked by heavy rainfall, increases the availability of aquatic habitats necessary for mosquito larval development, while the dry season often limits breeding sites but may still support mosquito populations in permanent water bodies or human-made containers. Temperature fluctuations within tropical climates also influence mosquito physiology and behavior, with warmer conditions generally accelerating development rates and increasing biting frequency (De Mello et al., 2022; Mazarire et al., 2024). Microclimatic variations caused by factors such as vegetation cover can further modulate local temperature and humidity, thereby affecting mosquito abundance and species distribution within tropical landscapes (Figure 1) (Abdullah et al., 2025).

Seasonal classification in tropical environments is often based on precipitation patterns, with the rainy season promoting peak mosquito activity due to increased habitat availability. However, temperature and humidity also play critical roles in shaping seasonal dynamics. For example, studies have shown that relative humidity above certain thresholds enhances adult mosquito survival, while temperature influences both larval development time and adult longevity. These climatic variables interact complexly to produce temporal fluctuations in mosquito populations that vary across different tropical ecosystems. Understanding these seasonal patterns is essential for predicting periods of heightened vector-borne disease risk and optimizing timing for control interventions (Chaiphongpachara et al., 2024; Arisanti et al., 2025).



Figure 1 Aerial view of non-dengue and dengue hotspots with GOS trap placement. The image presents an aerial view of the designated non-dengue hotspot (A) and dengue hotspot (B) within the study area. GOS (Gravid Oviposition Sticky) traps are strategically positioned across both sites to monitor *Aedes* mosquito populations. The spatial arrangement of traps provides comprehensive coverage, facilitating the study of mosquito activity and distribution patterns relative to environmental characteristics. This layout supports comparisons of *Aedes* species distribution and dengue virus prevalence between the two distinct ecological settings (Adopted from Abdullah et al., 2025)

2.2 Types of typical ecosystems

Tropical regions encompass a variety of ecosystems that provide diverse habitats for mosquitoes, including dense forests, wetlands, agricultural landscapes, and urban areas. Forested environments offer shaded breeding sites such as tree holes and leaf axils, supporting species adapted to sylvatic habitats. Wetlands and rice paddies create extensive aquatic habitats favorable for larval development of several vector species like *Anopheles* mosquitoes associated with malaria transmission. Urban environments introduce artificial containers and water storage systems that serve as prolific breeding grounds for *Aedes aegypti* and *Aedes albopictus*, vectors responsible for dengue and other arboviral diseases (García-Suárez et al., 2024; Nayak et al., 2025). The heterogeneity of these ecosystems influences mosquito community composition by providing niches suited to different species' ecological preferences.

Land use changes such as urbanization significantly alter habitat availability and microclimatic conditions within tropical landscapes. Urban areas often experience higher temperatures (urban heat island effect) and reduced vegetation cover compared to natural habitats, which can favor certain mosquito species over others. Fragmented urban landscapes with mixed residential and cropland areas have been identified as hotspots for *Aedes* breeding due to abundant artificial containers combined with suitable climatic conditions. Conversely, natural wetlands maintain stable populations of other vector species like *Mansonia* mosquitoes that rely on aquatic vegetation during their immature stages. Thus, ecosystem type strongly shapes the spatial distribution patterns of mosquitoes by influencing habitat suitability at local scales (Bennett et al., 2021; García-Suárez et al., 2024).

2.3 Major mosquito species and their distribution patterns

Several key mosquito species dominate tropical environments due to their adaptability to diverse habitats and climatic conditions. Among these are *Aedes aegypti* and *Aedes albopictus*, which are primary vectors of dengue virus; their distributions often overlap but show microhabitat preferences influenced by vegetation cover and urbanization gradients. For instance, *Ae. albopictus* tends to be more abundant in vegetated suburban or rural areas with higher humidity levels, whereas *Ae. aegypti* thrives in densely populated urban settings with warmer microclimates (Bennett et al., 2021; Abdullah et al., 2025). Both species exhibit seasonal fluctuations linked to rainfall patterns that create breeding sites in artificial containers.

Anopheline mosquitoes responsible for malaria transmission also display distinct spatial distributions shaped by ecological factors such as water quality parameters (pH, salinity), illumination levels in breeding sites, temperature ranges, and predator presence. Species like *Anopheles vagus* dominate rice-growing agroecosystems where flooded fields provide ideal larval habitats. Climatic variables including temperature peaks around 23°C~24°C combined with high relative humidity optimize biting rates for several *Anopheles* species (Arisanti et al., 2025). Additionally, *Culex* mosquitoes are widespread across various tropical habitats due to their tolerance of diverse environmental conditions but show abundance patterns influenced by diurnal temperature ranges (García-Suárez et al., 2024). Understanding these species-specific distribution patterns is crucial for targeted vector surveillance and control strategies tailored to local ecological contexts.

3 Seasonal Dynamics of Mosquito Populations

3.1 Variations in mosquito population density across seasons

Mosquito populations in tropical environments exhibit pronounced seasonal fluctuations, often characterized by peaks in abundance during or shortly after the rainy season when breeding habitats are most abundant. Studies monitoring mosquito activity have documented bimodal or unimodal seasonal patterns depending on local climatic conditions and species composition. For example, research conducted in Mediterranean botanical gardens showed that adult mosquito abundance followed a seasonal pattern influenced by temperature but with two distinct peaks linked to cumulative rainfall events, highlighting the complex interplay between climate and mosquito life cycles (Blanco-Sierra et al., 2024). Similarly, long-term surveillance across urbanization gradients in Yucatan, Mexico, revealed that mosquito densities varied significantly over the year, with higher captures during wetter months and lower numbers during dry periods, reflecting the dependence of larval habitats on precipitation (García-Suárez et al., 2024).

Seasonal variations are not uniform across all tropical regions or mosquito species; some areas experience extended periods of high mosquito abundance due to persistent favorable conditions. Modeling studies incorporating temperature and rainfall data from multiple African sites demonstrated that mosquito populations could peak once to multiple times annually depending on regional climate patterns. In locations with less pronounced dry seasons, such as parts of Ghana, mosquitoes maintain relatively high densities year-round, whereas other regions show prolonged low-abundance periods lasting several months (Baafi and Hurford, 2025). These findings emphasize the importance of local climatic context in shaping temporal population dynamics and suggest that vector control programs must be tailored to regional seasonal profiles for maximum effectiveness.

3.2 Effects of rainfall, temperature, and humidity on population fluctuations

Rainfall is a primary driver of mosquito population dynamics as it creates and replenishes aquatic habitats necessary for larval development. Accumulated precipitation in the weeks preceding sampling has been consistently identified as a significant predictor of adult mosquito abundance across diverse tropical settings. For instance, studies in Yucatan found that accumulated rainfall four weeks prior strongly correlated with increased captures of multiple species including *Culex quinquefasciatus* and *Aedes aegypti* (García-Suárez et al., 2024). However, excessive rainfall can sometimes flush out larvae or reduce habitat stability, indicating a nonlinear relationship between precipitation and mosquito populations.

Temperature influences multiple aspects of mosquito biology including development rate, survival, and biting frequency. Warmer temperatures generally accelerate larval maturation and increase adult activity but may also elevate mortality if exceeding optimal thresholds. Research from central Thailand coconut plantations showed that while meteorological variables did not always have statistically significant effects on *Culex* abundance individually, seasonal temperature variation was linked to changes in wing morphology indicative of phenotypic plasticity (Laojun et al., 2025). Relative humidity further modulates adult survival; higher humidity levels enhance longevity and feeding activity. Studies from temperate regions also highlight species-specific responses to weather variables such as diurnal temperature range affecting *Culex* species differently (Figure 2) (Baril et al., 2023). Together, these climatic factors interact dynamically to produce complex seasonal fluctuations in mosquito populations.

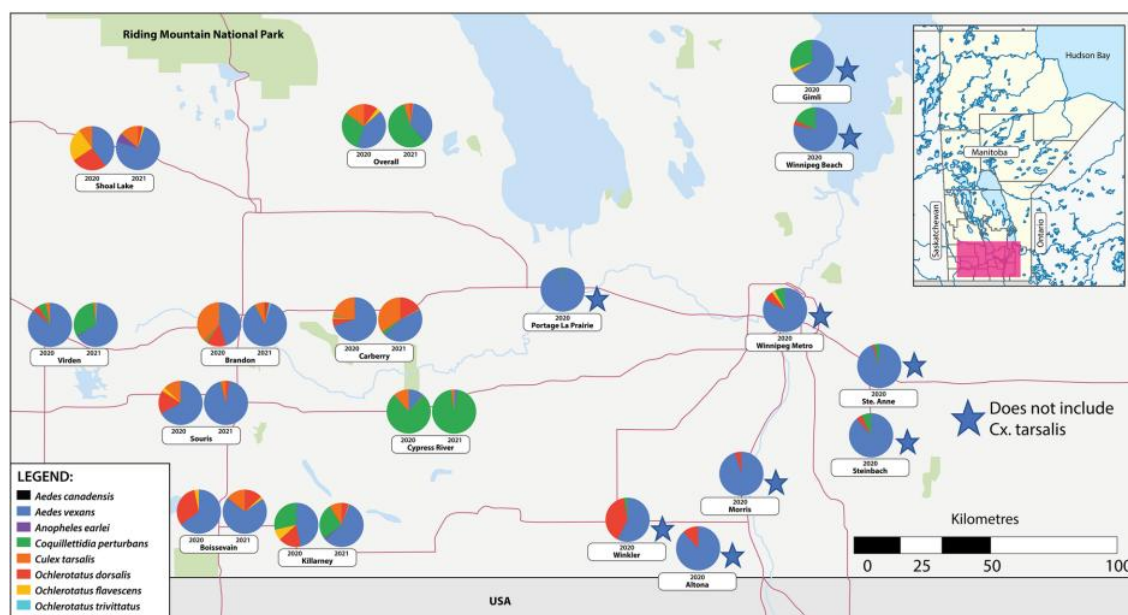


Figure 2 Relative trap counts for the eight most commonly found mosquito species in 2020 and 2021. Mosquitoes were captured on a weekly basis (May to September) from 17 sampling sites throughout Manitoba, Canada. *Culex tarsalis* counts are not included for all locations in the eastern part of the region (denoted with an asterisk*). *Ae. canadensis*, *An. earlei*, *Oc. trivittatus*, and *Oc. triseriatus* were not surveyed in 2020. We collected one *Oc. triseriatus* in 2021, which was not included on the figure (Adopted from Baril et al., 2023)

3.3 Seasonal differences among mosquito species

Different mosquito species exhibit distinct seasonal patterns driven by their ecological adaptations and habitat preferences. *Aedes aegypti* typically peaks during warm wet seasons when artificial containers fill with water in urban environments, whereas *Ae. albopictus* may show greater abundance in more vegetated suburban or rural areas with higher humidity (Blanco-Sierra et al., 2024; García-Suárez et al., 2024). Anopheline mosquitoes responsible for malaria transmission often display seasonality closely tied to agricultural practices and natural water availability; for example, *Anopheles vagus* thrives in flooded rice paddies during rainy seasons (Rakotoarison et al., 2025). Species like *Culex sitiens* and *Culex gelidus* show differing peak abundances within the same region one peaking early in the rainy season and another later reflecting niche partitioning (Laojun et al., 2025).

Seasonal timing also affects life stage distributions within species populations. Stage-structured models reveal that eggs, larvae, pupae, and adults respond differently to environmental cues such as temperature and rainfall throughout the year. This results in shifts not only in total abundance but also in population age structure which can influence disease transmission potential (Baafi and Hurford, 2025). Moreover, urbanization gradients modify these seasonal dynamics by altering habitat availability and microclimate conditions favoring some species over others (García-Suárez et al., 2024; Whittaker et al., 2022). Understanding these interspecific differences is critical for designing targeted vector control strategies that consider both temporal windows of peak risk and species-specific ecology.

4 Types and Characteristics of Mosquito Habitats

4.1 Natural habitats

Natural mosquito habitats in tropical environments primarily include standing water bodies such as ponds, swamps, slow-moving streams, and phytotelmata water-holding structures in plants like bromeliads and bamboo internodes. These habitats provide essential aquatic environments for mosquito larvae development. For example, *Anopheles funestus*, a major malaria vector in southeastern Tanzania, predominantly breeds in small spring-fed pools, natural ponds that retain water most of the year, and slow-moving river tributaries with clear water and emergent vegetation (Nambunga et al., 2020). Similarly, phytotelmata in tropical forests support diverse mosquito communities; bamboo internodes have been shown to harbor high species richness and abundance due to favorable microhabitat conditions such as temperature and pH (De Almeida et al., 2025). Vegetated areas adjacent to water bodies often offer shaded breeding sites that protect larvae from predators and extreme environmental fluctuations.

The physicochemical characteristics of these natural habitats influence mosquito species composition and larval productivity. Studies in Iran found significant differences among mosquito species in their preferences for chloride content and water temperature but not for pH or turbidity (Amini et al., 2020). In addition, dissolved oxygen levels, alkalinity, and emergent plant coverage have been linked to larval abundance for various species including *Anopheles vagus* and *Culex quinquefasciatus* (Bashar et al., 2016). The permanence of water bodies also plays a role; permanent or semi-permanent habitats tend to support stable populations of certain vectors by providing consistent breeding sites throughout the year (Dida et al., 2018; Nambunga et al., 2020). Understanding these natural habitat features is critical for identifying key breeding sites for vector control efforts.

4.2 Artificial habitats

Urbanization creates numerous artificial aquatic habitats that serve as prolific breeding grounds for mosquitoes, especially container-breeding species like *Aedes aegypti*. Common urban habitats include buckets, flower pots, ornamental bromeliads, discarded tires, drainage systems, and water storage containers. Research conducted in Miami-Dade County demonstrated that *Ae. aegypti* was highly concentrated in specific neighborhoods where such artificial containers were abundant (Wilke et al., 2019). These man-made habitats often lack natural predators and provide stable microclimates favorable for larval development. The spatial clustering of these breeding sites within urban landscapes highlights the importance of targeted source reduction strategies.

Artificial habitats vary widely in their physical characteristics but generally share features such as small volume, intermittent water availability, and proximity to human dwellings. Studies from semi-urban Dhaka revealed that chemical oxygen demand and dissolved oxygen levels influenced the abundance of *Culex* larvae in drainage ditches and other urban water bodies (Bashar et al., 2016). Additionally, urban microclimates characterized by higher temperatures (urban heat islands) can accelerate mosquito development rates compared to rural settings (Wilke et al., 2019). The diversity of artificial habitats supports multiple mosquito species simultaneously; however, *Ae. aegypti* tends to dominate container habitats while *Culex* species are more common in larger drainage systems or polluted waters (Wilke et al., 2019; Bashar et al., 2016). Effective control requires understanding the heterogeneity of these anthropogenic environments.

4.3 Physicochemical properties of habitats

The physicochemical environment within mosquito larval habitats significantly affects species presence, abundance, and developmental success. Key parameters include water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), turbidity, dissolved oxygen (DO), alkalinity, chloride content, and light exposure. For instance, studies from West Azerbaijan Province in Iran reported significant interspecific differences in chloride content and temperature preferences among mosquito larvae but no significant variation regarding pH or turbidity (Amini et al., 2020). Similarly, research along the Mara River basin found strong correlations between larval abundance and DO levels as well as temperature and turbidity (Dida et al., 2018).

Light exposure influences habitat suitability by affecting algal growth and predator presence; shaded or partially shaded sites often harbor different mosquito assemblages than open sunlit pools. Water temperature typically ranges between 25°C to 29°C in productive tropical habitats such as those used by *Anopheles funestus* (Nambunga et al., 2020), with warmer temperatures generally accelerating larval development but potentially increasing mortality if too high. Electrical conductivity and TDS reflect mineral content which can affect larval survival differently across species; some *Culex* mosquitoes tolerate higher salinity levels while others prefer fresher waters (Wang et al., 2020; Martínez-Barciela et al., 2025). Overall, the complex interplay of these physicochemical factors shapes habitat quality and determines which mosquito species can successfully exploit particular aquatic environments (Figure 3).

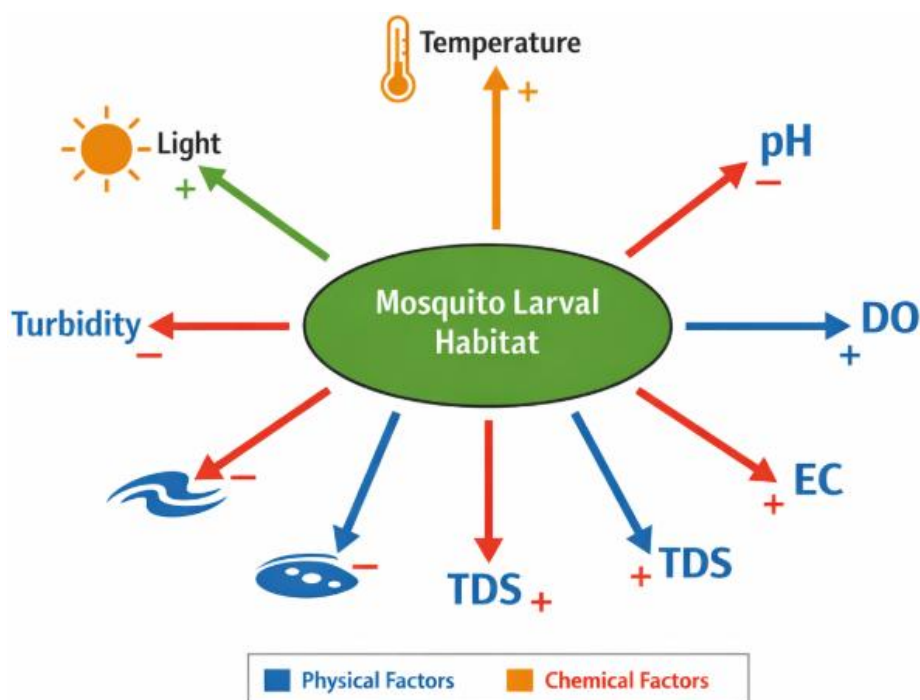


Figure 3 Conceptual diagram illustrating the influence of physicochemical parameters on mosquito larval habitat suitability and species distribution. Arrows indicate the direction and relative strength of influence of each environmental factor

5 Driving Mechanisms of Environmental Factors on Populations and Habitats

5.1 Relationships between climatic factors and mosquito life cycles

Climatic factors such as temperature, rainfall, humidity, and photoperiod play critical roles in regulating mosquito life cycles by influencing development rates, survival, reproduction, and behavior. Temperature affects the speed of larval development and adult activity; warmer conditions generally accelerate growth but can increase mortality if exceeding species-specific thresholds. For example, accumulated rainfall in the weeks preceding sampling strongly correlates with increased mosquito abundance by creating suitable aquatic habitats for larvae, while diurnal temperature range influences species like *Culex quinquefasciatus* and *Aedes aegypti* differently (García-Suárez et al., 2024). Additionally, humidity modulates host-seeking and oviposition behaviors, with higher humidity enhancing adult survival and feeding activity (Meuti, 2025). Photoperiod cues induce diapause or arrested development in temperate mosquitoes to survive unfavorable seasons, though tropical species may respond differently due to less pronounced seasonal changes (Meuti, 2025).

Interactions among these climatic variables create complex temporal patterns in mosquito populations. Nonlinear relationships have been observed where extremely high temperatures reduce abundance despite otherwise favorable conditions (Ferraguti et al., 2024). Rainfall not only provides breeding sites but also interacts with urban environmental factors to influence local mosquito distributions (Wouters et al., 2024). Seasonal peaks in mosquito populations often coincide with periods of optimal temperature and sufficient precipitation, but these patterns vary by species and location. Understanding these multifactorial climatic influences is essential for predicting population dynamics under changing climate scenarios and for timing vector control interventions effectively (Blanco-Sierra et al., 2024; García-Suárez et al., 2024).

5.2 Impacts of human activities on habitat formation

Human activities profoundly shape mosquito habitats by altering land use, water management, and urban infrastructure. Urbanization creates artificial breeding sites such as water containers, drainage systems, and abandoned infrastructure that support container-breeding species like *Aedes aegypti* and *Aedes albopictus* (Little et al., 2017; Wouters et al., 2024). Studies show that infrastructural decay combined with vegetation presence increases mosquito abundance in urban neighborhoods; abandoned blocks with more vegetation harbor larger populations due to increased habitat availability (Little et al., 2017). Moreover, impervious surfaces associated with urbanization influence microclimates by increasing temperatures (urban heat islands) which can accelerate mosquito development rates (García-Suárez et al., 2024; Vandergiesen et al., 2025).

Anthropogenic stressors such as eutrophication and salinization of water bodies also affect mosquito population parameters by modifying survival rates, development times, and reproductive behaviors. Experiments demonstrate that increased nutrient pollution enhances larval survival and egg-laying behavior while salinity negatively impacts survival at higher temperatures (Boerlijst et al., 2022). These human-induced environmental changes often interact synergistically with climatic factors to promote mosquito proliferation. Consequently, human-driven habitat modifications are key drivers of spatial heterogeneity in mosquito populations and must be integrated into vector control strategies to address disease risks effectively (Little et al., 2017; Boerlijst et al., 2022).

5.3 Biological factors

Biological interactions including predation and interspecific competition significantly influence mosquito population dynamics within their habitats. Predators such as fish, aquatic insects, and other invertebrates regulate larval densities by consuming immature stages, thereby affecting overall population size and species composition. The presence or absence of natural predators varies across habitat types; artificial containers often lack predators compared to natural water bodies, allowing some species like *Aedes aegypti* to thrive in urban environments (Wouters et al., 2024). Interspecific competition among co-occurring mosquito species can lead to niche partitioning or competitive exclusion depending on resource availability and environmental conditions.

These biological factors interact with environmental variables to shape community structure; for instance, habitat heterogeneity created by human activities may alter predator-prey dynamics or competitive relationships among

mosquitoes (Vandergiesen et al., 2025). Sugar feeding behavior influenced by vegetation availability also affects adult fitness and population persistence across landscapes differing in ecological complexity (Vandergiesen et al., 2025). Understanding these biotic mechanisms alongside abiotic drivers is crucial for developing comprehensive models of mosquito ecology that inform targeted control measures addressing both environmental management and biological regulation.

6 Research Methods and Data Analysis Techniques

6.1 Mosquito sampling and monitoring methods

Mosquito sampling methods vary widely depending on the target life stage and species behavior, with adult trapping and larval surveys being the most common approaches. Adult mosquito collection often employs traps such as BG-Sentinel II (BGS), BG Gravid Traps (GAT), and sweep netting, each with distinct advantages. A multi-country study in Pacific Island nations found BGS traps to be the most effective in capturing a higher number of mosquitoes without significant species bias, making them suitable for routine surveillance in diverse tropical settings (Craig et al., 2025). Larval surveys complement adult trapping by identifying breeding sites through dipping or direct collection from aquatic habitats, providing critical data on immature stages that inform control strategies (Becker et al., 2010). Combining multiple sampling methods targeting different mosquito behaviors enhances surveillance comprehensiveness and reduces bias inherent in single-method approaches (Van De Straat et al., 2021).

Operational feasibility is a key consideration in selecting sampling techniques, especially in resource-limited or remote tropical environments. Simpler, durable tools that require minimal maintenance are preferred for sustained monitoring programs (Craig et al., 2025). Passive surveillance methods, including community-based reporting and citizen science initiatives, have gained traction as cost-effective supplements to active trapping by expanding spatial coverage and enabling early detection of invasive species (Kampen et al., 2015). Additionally, behavioral monitoring technologies such as high-resolution video tracking and AI-driven analysis offer promising avenues for detailed studies of mosquito activity patterns but remain underutilized in field surveillance (Javed et al., 2024). Overall, integrating diverse sampling tools tailored to local ecological contexts improves data quality for vector management.

6.2 Statistical and modeling approaches for data analysis

Analyzing mosquito population data requires robust statistical and modeling techniques to accurately estimate abundance, species composition, and temporal trends while managing large sample sizes efficiently. Subsampling methods have been developed to reduce labor-intensive processing of large mosquito collections without compromising accuracy. For instance, area-based subsampling of 20% of specimens provides reliable estimates of total numbers and dominant species proportions with acceptable error margins (~12% for specimen counts) (Jaworski et al., 2019). Image processing software like ImageJ has also demonstrated resilience and precision in digitized optical counting across varying sample conditions, offering an efficient alternative to manual enumeration (Faraji et al., 2025). These approaches enable timely decision-making critical for vector control interventions.

Modeling frameworks often incorporate generalized linear mixed models (GLMMs) to account for spatial-temporal variability and hierarchical data structures inherent in mosquito surveillance datasets (Craig et al., 2025). Multimethod sampling designs facilitate more comprehensive models by capturing diverse vector behaviors influencing disease transmission risk (Van De Straat et al., 2021). Advanced statistical analyses also support molecular xenomonitoring efforts by evaluating how different collection strategies affect parasite detection rates within mosquito populations (Reimer and Pryce, 2023). Integrating these quantitative tools with ecological knowledge enhances predictive capacity regarding population dynamics under changing environmental conditions. Thus, combining subsampling efficiency with sophisticated modeling strengthens entomological research outcomes.

6.3 Applications of remote sensing and GIS in habitat analysis

Remote sensing (RS) and geographic information systems (GIS) have revolutionized habitat analysis by enabling large-scale mapping and monitoring of environmental variables relevant to mosquito ecology. High-resolution satellite imagery facilitates identification of potential breeding sites such as water bodies, vegetation cover, and urban infrastructure features that influence habitat suitability (Javed et al., 2024). GIS platforms integrate spatial data layers including climate variables, land use patterns, and vector occurrence records to model habitat distribution and predict hotspots for targeted interventions. These technologies enhance understanding of landscape-level drivers shaping mosquito populations beyond localized field surveys.

Emerging applications combine RS/GIS with behavioral monitoring tools to provide dynamic assessments of vector activity patterns over time (Javed et al., 2024). This integration supports adaptive management by allowing real-time evaluation of control measures' effectiveness across heterogeneous environments. Moreover, spatial analyses assist in stratifying risk areas based on environmental correlates derived from remotely sensed data, improving resource allocation efficiency (Kampen et al., 2015). Despite their potential, challenges remain regarding data resolution limitations and the need for ground-truthing to validate remote observations. Nonetheless, RS and GIS represent indispensable components of modern entomological research frameworks aimed at controlling mosquito-borne diseases effectively.

7 Results and Discussion

7.1 Correlation analysis between seasonal dynamics and environmental variables

Seasonal dynamics of mosquito populations show strong correlations with environmental variables such as temperature, precipitation, and habitat type, which collectively influence population fluctuations over time. Studies on other taxa in aquatic and terrestrial ecosystems reveal that population densities often peak during seasons with optimal climatic conditions; for example, bird populations increased by 65% in spring compared to fall, linked to habitat composition and seasonal factors (Azizoğlu et al., 2023). Similarly, scorpion foraging activity and microhabitat colonization vary seasonally, reflecting climatic impacts on population dynamics through resource availability and refuge use (Lira et al., 2018). These patterns suggest that mosquito populations likely respond similarly to seasonal environmental changes, with temperature and rainfall driving breeding site availability and survival rates (Figure 4).

Temporal variation in habitat quality also shapes species distribution and abundance by altering resource availability across seasons. Research on waterbirds demonstrated that seasonal deterioration of habitat quality leads to weakened distribution-abundance relationships later in the summer due to habitat homogenization (Charalambous et al., 2024). Migratory species exhibit seasonal shifts in habitat selection aligned with life-history stages, indicating that spatial distribution is dynamic and influenced by both local and landscape-scale environmental heterogeneity (Stanley et al., 2021). These findings underscore the importance of incorporating multiple environmental variables and temporal scales when analyzing mosquito seasonal dynamics to capture complex ecological responses accurately.

7.2 Relationships between habitat characteristics and population distribution

Habitat characteristics such as vegetation structure, water availability, and human disturbance strongly influence mosquito population distribution by affecting breeding site suitability and resource access. Studies on diverse taxa highlight that species richness and functional diversity differ between wet and dry habitats due to microhabitat preferences; for instance, scorpions showed higher functional richness in wet forests compared to semiarid areas, reflecting habitat-driven spatial segregation (Lira et al., 2018). Similarly, waterfowl populations varied significantly among habitats like open water surfaces versus reed beds, indicating that specific habitat types support different population densities (Azizoğlu et al., 2023). These patterns imply that mosquito species distributions are closely tied to fine-scale habitat features that determine larval development success and adult survival.

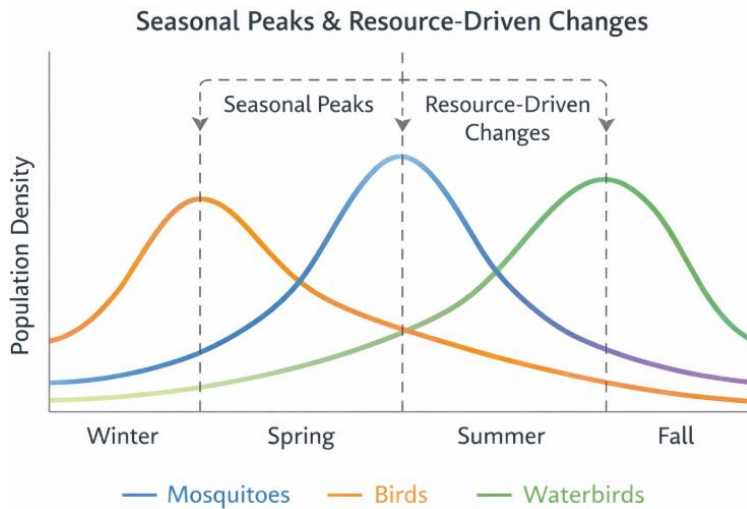


Figure 4 Comparative illustration of seasonal population dynamics across taxa, highlighting shared responses to environmental variability and resource availability

Human-modified landscapes further complicate these relationships by creating novel or altered habitats that can either promote or restrict mosquito proliferation. For example, urbanization often increases container habitats favorable for *Aedes* mosquitoes but may reduce natural breeding sites preferred by other species (Xu et al., 2024). Habitat fragmentation influences migratory bird distributions seasonally by modifying landscape composition, which may analogously affect mosquito dispersal and local abundance (Stanley et al., 2021). Understanding how natural habitat heterogeneity interacts with anthropogenic changes is essential for predicting spatial patterns of mosquito populations across tropical environments.

7.3 Comparison with existing studies and interpretation of differences

Comparisons with existing ecological studies reveal both consistencies and divergences in how seasonal dynamics relate to environmental drivers across taxa. While many species exhibit clear seasonal peaks linked to climatic variables such as temperature and precipitation (Azizoğlu et al., 2023; Lira et al., 2018), the magnitude and timing of these peaks vary depending on life-history traits and regional conditions. For instance, migratory birds show complex habitat selection patterns influenced by breeding stage and landscape fragmentation not always paralleled in resident insect populations (Stanley et al., 2021). Differences may also arise from methodological approaches; some studies emphasize classical regression models while others advocate for more descriptive or predictive analytical methods to capture nonlinear or interactive effects better (Azizoğlu et al., 2023).

Discrepancies in observed patterns can result from varying spatial scales, taxonomic focus, or environmental contexts. For example, waterbird assemblages demonstrated a weakening of distribution-abundance relationships due to seasonal habitat degradation not universally reported in other groups (Charalambous et al., 2024). Additionally, climate change projections indicate potential shifts in species distributions over time that may alter established seasonal dynamics (Xu et al., 2024). These nuances highlight the need for integrative frameworks combining field data with advanced modeling techniques to interpret complex ecological processes governing mosquito populations effectively.

8 Control Strategies and Public Health Implications

Optimizing the timing of mosquito control interventions by aligning them with seasonal population dynamics can significantly enhance their effectiveness. Seasonal fluctuations in vector abundance, driven by environmental factors such as temperature and rainfall, influence disease transmission risk and thus the optimal periods for intervention. For example, modeling studies on vector-borne diseases demonstrate that applying control measures during peak vector growth phases or just before population surges can reduce disease burden more effectively than untimed efforts. However, reliance solely on rainfall as a predictor for timing may be insufficient, as some vectors show peak activity patterns poorly correlated with precipitation but more closely linked to temperature and land

use. Therefore, integrating local entomological surveillance data with environmental monitoring is critical to identify precise windows for intervention. Mathematical models incorporating seasonality and time-dependent control parameters have been used to determine optimal strategies combining insecticide spraying and environmental decontamination. These studies reveal that while insecticide application alone may not fully eliminate vector populations, combining it with habitat management yields significantly better outcomes. Similarly, pest management research highlights that targeting specific life stages or age groups during vulnerable seasonal periods maximizes population suppression. Early-season interventions often reduce reproductive potential and seedbank densities in invasive species control, suggesting analogous benefits in mosquito management by preempting population buildup. Overall, adaptive timing of control efforts informed by seasonal dynamics enhances resource efficiency and public health impact.

Habitat management through environmental modification plays a crucial role in reducing mosquito breeding sites and interrupting transmission cycles. Environmental interventions such as removal of standing water, vegetation management, and sanitation reduce larval habitats and adult resting sites, thereby lowering vector densities. Studies emphasize that environmental decontamination can be more effective than insecticide spraying alone when integrated into control programs. Additionally, managing invasive plant species or modifying landscape features influences habitat suitability for mosquitoes by altering microclimatic conditions and resource availability. Such habitat-focused strategies complement chemical controls by addressing underlying ecological drivers of mosquito proliferation. The success of habitat management depends on understanding spatial heterogeneity and temporal changes in breeding site distribution. Remote sensing and GIS tools facilitate identification of high-risk habitats for targeted interventions, while community engagement enhances sustainability of environmental measures. Moreover, combining biological controls such as natural predators or parasitoids with habitat modification offers promising integrated pest management approaches that reduce reliance on chemicals. However, challenges remain in balancing effective habitat alteration with conservation goals and minimizing impacts on non-target species. Thus, environmentally based interventions require careful planning tailored to local ecological contexts to optimize public health benefits.

Effective mosquito control informed by seasonal dynamics and habitat characteristics has direct implications for reducing the burden of mosquito-borne diseases in tropical regions where transmission is often intense year-round but fluctuates seasonally. Timing interventions to coincide with periods preceding population peaks can lower infection rates more efficiently than random or calendar-based schedules (Huang et al., 2020; Morreale et al., 2024). This approach is particularly important in urbanizing tropical areas where land use changes alter vector ecology unpredictably. Integrating entomological surveillance data into disease models improves prediction accuracy for outbreak risk and guides resource allocation. Furthermore, combining chemical controls with environmental management addresses both adult vectors and immature stages, enhancing overall program efficacy while mitigating insecticide resistance development. Public health strategies must also consider socio-economic factors influencing community participation in habitat reduction efforts to ensure sustained impact. Advances in modeling optimal control strategies incorporating human behavior and vector ecology provide frameworks for designing cost-effective interventions adapted to local conditions. Ultimately, leveraging knowledge of seasonal mosquito dynamics alongside habitat management strengthens integrated vector management programs critical for controlling tropical diseases such as malaria, dengue, chikungunya, and Zika.

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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.

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Feature Review

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Integrated Mosquito Vector Management Strategies for Reducing Malaria Transmission Risk

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Abstract This review examines integrated mosquito vector management (IVM) strategies for reducing malaria transmission risk. Malaria remains a major global public health burden, with transmission driven by Anopheles mosquitoes whose biological and ecological characteristics are shaped by environmental factors. The complex interactions among mosquito vectors, *Plasmodium* parasites, and human hosts, together with climate change and human activities, influence transmission dynamics. Conventional control methods, including insecticide-treated nets and indoor residual spraying, have achieved significant success but face limitations such as insecticide resistance and behavioral adaptation. Emerging approaches, including plant-based insecticides, genetic technologies, and nanotechnology-based delivery systems, provide promising alternatives. IVM integrates multiple strategies, emphasizing ecological sustainability, community participation, and policy support. Evaluating safety, environmental impact, and cost-effectiveness is essential for long-term success. Future efforts should address resistance management, climate challenges, and implementation barriers to enhance malaria control outcomes.

Keywords Malaria; Mosquito vectors; Integrated vector management; Insecticide resistance; Control strategies

1 Introduction

Malaria remains a major global public health challenge, particularly in tropical and subtropical regions where it causes significant morbidity and mortality. Despite decades of control efforts, the disease continues to impose a heavy burden, with sub-Saharan Africa bearing the highest incidence and prevalence rates worldwide. Vulnerable populations such as children under five years old and pregnant women are disproportionately affected, contributing to substantial socio-economic impacts in endemic countries. The persistence of malaria is driven by complex interactions among biological, environmental, and socio-economic factors that sustain transmission cycles and complicate eradication efforts (Akowe et al., 2025; Kombate et al., 2025).

Central to malaria transmission are mosquito vectors, primarily Anopheles species, which serve as the biological agents facilitating parasite spread between humans. Vector behavior, ecology, and population dynamics critically influence transmission intensity and patterns. Traditional vector control methods such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have significantly reduced malaria incidence but face challenges including insecticide resistance, outdoor biting behaviors, and ecological variability among vector populations. These factors limit the effectiveness of single interventions and underscore the need for comprehensive approaches that address both indoor and outdoor transmission risks while adapting to evolving vector behaviors (Benelli and Beier, 2017; Sougoufara et al., 2020).

Integrated Vector Management (IVM) has emerged as a strategic framework that combines multiple vector control tools tailored to local contexts to enhance malaria control outcomes sustainably. IVM integrates traditional methods like ITNs and IRS with novel interventions such as larviciding, environmental management, house screening, community education, and emerging biotechnologies including Wolbachia-based strategies. Evidence from diverse settings demonstrates that integrated approaches achieve greater reductions in malaria transmission indicators compared to single interventions alone. Moreover, IVM promotes multisectoral collaboration, continuous surveillance, and adaptive management to overcome challenges like insecticide resistance and residual

transmission. This holistic approach is critical for advancing toward malaria elimination goals globally (Musoke et al., 2023; Otolorin et al., 2025).

2 Biological and Ecological Characteristics of Malaria Vectors

2.1 Major vector species and their distribution

The primary malaria vectors in sub-Saharan Africa include *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles funestus*, and *Anopheles coluzzii*, with *An. funestus* dominating transmission in many parts of east and southern Africa. This species is notable for its preference for permanent and semi-permanent aquatic habitats such as river streams, ponds, swamps, and spring-fed pools, enabling it to sustain populations year-round and mediate over 85% of malaria transmission events in some regions despite insecticide resistance challenges (Kahamba et al., 2022). In addition to these major vectors, secondary vectors like *Anopheles merus* along the East and Southern African coast are increasingly recognized for their role in residual malaria transmission due to their exophilic behavior and insecticide resistance; these species have expanded their geographical range and vectorial capacity over time (Bartilol et al., 2021).

In India, the major malaria vectors include species complexes such as *Anopheles culicifacies* and *Anopheles fluviatilis*, which exhibit distinct biological traits across diverse ecosystems. These vectors show varying resting behaviors-*An. culicifacies* is mainly endophilic except in some regions where behavioral shifts are observed-and display widespread insecticide resistance that complicates control efforts. Understanding the distribution patterns of sibling species within these complexes is critical for tailoring effective vector control strategies aligned with India's malaria elimination goals (Figure 1) (Subbarao et al., 2019; Rahi et al., 2022).

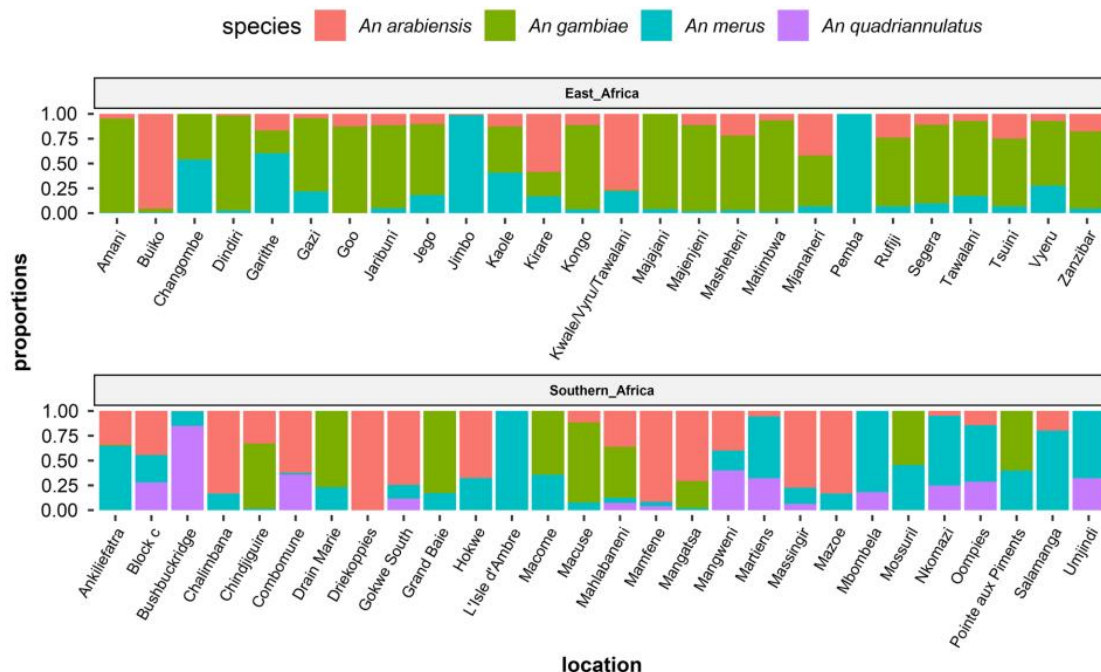


Figure 1 Proportions of *An. Gambiae* complex members: *An. Gambiae* (s.s.), *An. merus*, *An. quadriannulatus* and *An. arabiensis* in the different collection sites along the East and Southern African Coast. However, in Changombe, Drain Marie, Grand Baie, L'Isle d'Ambre, Pemba and Pointe aux Piments, *An. gambiae* (s.l.) were identified using a salt tolerance test and classified as saltwater *An. Gambiae* represented as *An. merus* in the figure or non-saltwater *An. Gambiae* (*An. arabiensis*, *An. quadriannulatus*, *An. Gambiae*), which are represented as *An. Gambiae* (Adopted from Subbarao et al., 2019)

2.2 Life cycle, feeding behavior, and reproductive traits

Malaria vectors undergo a complex life cycle involving aquatic larval stages followed by adult emergence; the duration and success of each stage are influenced by ecological conditions. For example, *An. funestus* females predominantly feed indoors on humans but can exhibit zoophagy in areas with abundant livestock; both males and females rest indoors, which makes them susceptible to indoor interventions despite some reports of outdoor biting

behavior (Kahamba et al., 2022). Similarly, Indian vectors like *An. culicifacies* show variations in feeding preferences with human blood indices ranging widely across regions; reproductive traits such as proportions of gravid mosquitoes also vary geographically, affecting transmission dynamics (Rahi et al., 2022).

Secondary vectors such as *Anopheles squamosus* demonstrate behavioral plasticity that allows them to evade conventional control measures by shifting biting times or locations. Their biology remains understudied but suggests they occupy similar ecological niches as primary vectors in certain African regions, highlighting the need for further research into their life history traits to inform control strategies (Nguyen et al., 2025). Feeding behavior diversity among vector species underscores the importance of integrated approaches that consider both indoor and outdoor transmission risks.

2.3 Influence of environmental factors on vector population dynamics

Environmental factors including land cover, climate variables (temperature, precipitation), topography, and human population density significantly influence the habitat suitability and population dynamics of malaria vectors. Studies using ecological niche modeling have shown that these factors can either facilitate or restrict the occurrence of key vector species such as *An. Gambiae* s.s., *An. coluzzii*, and *An. funestus* s.s., thereby affecting malaria transmission patterns even under intervention pressure like long-lasting insecticidal nets (LLINs) (Talbot et al., 2025). Seasonal changes also impact vector densities; for instance, saltwater-tolerant species like *Anopheles merus* peak during dry seasons when freshwater mosquito populations decline (Bartilol et al., 2021).

Moreover, environmental determinants shape the bionomics of zoonotic malaria vectors in Southeast Asia by influencing abundance and survival through temperature fluctuations, humidity levels, elevation gradients, precipitation patterns, land use changes, and seasonality. These complex interactions necessitate a One Health approach integrating human health with animal reservoirs and environmental management to effectively address malaria transmission risks (Masse et al., 2025). Continuous monitoring of environmental changes is essential for adapting vector control strategies to evolving ecological contexts.

3 Mechanisms and Determinants of Malaria Transmission

3.1 Development of Plasmodium within mosquito hosts

The development of *Plasmodium* parasites within mosquito vectors is a complex process essential for malaria transmission. After an infected female *Anopheles* mosquito takes a blood meal, *Plasmodium gametocytes* ingested from the human host undergo sexual reproduction in the mosquito's midgut, forming zygotes that develop into motile ookinetes. These ookinetes penetrate the midgut wall and form oocysts, where sporozoites mature over a period typically ranging from 7 to 30 days depending on the *Plasmodium* species and ambient temperature. Mature sporozoites migrate to the salivary glands, enabling the mosquito to infect new human hosts during subsequent blood meals (Rossati et al., 2016; Sato, 2021).

Temperature plays a critical role in modulating the rate of parasite development within mosquitoes, influencing transmission potential. Higher temperatures generally accelerate parasite maturation but may reduce mosquito lifespan, creating a trade-off that affects overall vectorial capacity. Different *Plasmodium* species exhibit variable thermal thresholds for development; for example, *P. falciparum* and *P. vivax* have distinct optimal temperature ranges that impact their geographic distribution and seasonality of transmission (Villena et al., 2022; Suh et al., 2024).

3.2 Interactions among host, vector, and pathogen

Malaria transmission depends on intricate interactions between the human host, mosquito vector, and *Plasmodium* parasite. Host factors such as immune responses and genetic traits influence gametocyte production and infectivity to mosquitoes, thereby affecting transmission efficiency. Conversely, mosquitoes possess immune mechanisms and genetic variations that determine their susceptibility to infection and ability to support parasite development. These dynamic interactions shape parasite sexual differentiation rates and vector competence, which are critical determinants of malaria epidemiology (Sollelis et al., 2024; Li et al., 2025).

The coevolutionary relationship between *Plasmodium parasites* and their hosts has led to remarkable plasticity in parasite traits that facilitate adaptation to changing environments and vector species shifts. This adaptability complicates control efforts by enabling parasites to evade interventions through altered transmission dynamics or resistance development. Understanding these biological determinants is vital for designing effective transmission-blocking strategies targeting both parasite stages in humans and vectors (Mukamurera, 2024; Sollelis et al., 2024).

3.3 Effects of climate change and human activities on transmission risk

Climate change significantly influences malaria transmission by altering environmental conditions that affect both mosquito vectors and *Plasmodium parasites*. Rising temperatures can accelerate mosquito development rates, increase biting frequency, extend lifespan under certain humidity conditions, and shorten parasite incubation periods within vectors—all factors that enhance transmission potential. Changes in rainfall patterns create new breeding habitats or eliminate existing ones, while extreme weather events such as floods or droughts can either amplify or suppress vector populations regionally (Idani et al., 2025; Megersa and Luo, 2025).

Human activities including land use changes, urbanization, migration, and political instability further modify ecosystems in ways that impact malaria risk. Deforestation or agricultural expansion can increase vector habitats or bring humans into closer contact with vectors. Additionally, reduced funding for vector control programs due to socioeconomic factors exacerbates vulnerability to outbreaks despite climatic suitability for transmission. Integrated approaches combining climate-informed surveillance with sustainable public health interventions are essential to mitigate these evolving risks (Rossati et al., 2016; Megersa and Luo, 2025).

4 Conventional Mosquito Control Strategies and Their Limitations

4.1 Chemical control methods (insecticide-treated nets, indoor residual spraying)

Chemical control remains the cornerstone of malaria vector management, primarily through the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS). These interventions have significantly reduced malaria incidence by targeting mosquitoes that feed and rest indoors, thereby interrupting transmission cycles. However, their effectiveness is increasingly compromised by the widespread emergence of insecticide resistance among *Anopheles* vectors, which diminishes mortality rates and reduces the protective efficacy of these tools (Benelli and Beier, 2017; Namias et al., 2021). Additionally, ITNs and IRS mainly target indoor-biting mosquitoes, leaving outdoor and early-evening biting vectors less affected, which sustains residual transmission despite high coverage (Benelli and Beier, 2017).

Innovations such as insecticidal paints are being explored to enhance chemical control by providing longer-lasting residual effects and easier application compared to conventional spraying. These paints may improve cost-effectiveness and acceptability in endemic regions like India but still face challenges related to resistance development and environmental safety (Singh et al., 2024). Despite these advances, reliance on chemical methods alone is insufficient for sustainable malaria control due to ecological complexities and evolving vector behaviors that reduce contact with treated surfaces (Benelli and Beier, 2017).

4.2 Environmental management and biological control approaches

Environmental management strategies aim to reduce mosquito breeding sites through habitat modification or manipulation, such as drainage of stagnant water or improved water management practices. These approaches can be effective in limiting vector populations but often require sustained community engagement and infrastructure support, which may be challenging in resource-limited settings (Benelli and Beier, 2017). Biological control methods offer eco-friendly alternatives by utilizing natural predators like larvivorous fish, entomopathogenic fungi, bacteria (e.g., *Bacillus thuringiensis israelensis*), or genetically modified mosquitoes to suppress vector populations without chemical insecticides (Benelli et al., 2016; Hamed et al., 2022).

Biocontrol strategies are gaining attention due to their potential sustainability and reduced risk of resistance development. However, their implementation faces limitations including variable efficacy under field conditions,

species-specificity constraints, and logistical challenges in large-scale deployment (Figure 2) (Benelli et al., 2016; Dahmana and Mediannikov, 2020). Integrating biological controls with environmental management within an integrated vector management framework can enhance overall effectiveness but requires careful evaluation of ecological impacts and operational feasibility (Hamed et al., 2022).

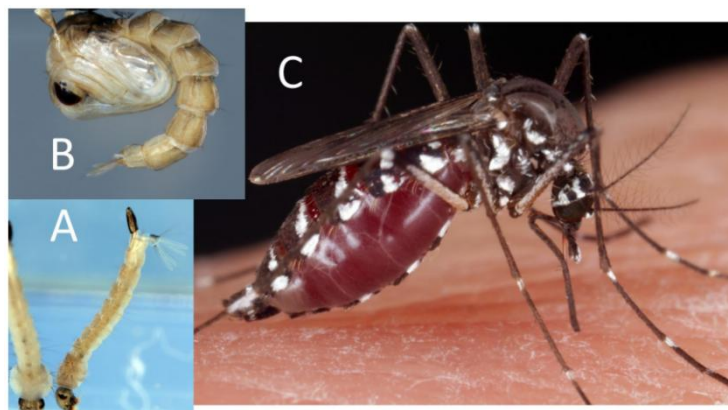


Figure 2 *Aedes albopictus* strain: (A) larvae, (B) pupa, and (C) adult (Adopted from Benelli et al., 2016)

4.3 Insecticide resistance and behavioral adaptations

The rapid evolution of insecticide resistance in mosquito populations poses a major threat to the continued success of chemical-based interventions. Resistance mechanisms include metabolic detoxification, target site mutations, and cuticular changes that reduce insecticide penetration or binding. Importantly, standard laboratory assays often fail to predict the practical impact of resistance on field efficacy due to differences in mosquito age, behavior, and environmental exposure (Namias et al., 2021). This discordance complicates resistance monitoring and necessitates improved guidelines that reflect real-world conditions for better programmatic decision-making.

Beyond physiological resistance, mosquitoes exhibit behavioral adaptations such as altered feeding times, increased outdoor biting, or avoidance of treated surfaces that reduce contact with insecticides. These plastic or constitutive behavioral changes undermine indoor interventions like IRS and ITNs by enabling vectors to evade lethal exposure (Benelli and Beier, 2017; Carrasco et al., 2019). Addressing both physiological resistance and behavioral shifts requires diversified control strategies incorporating novel tools alongside existing methods to sustain malaria transmission reduction efforts effectively (Figure 3) (Benelli and Beier, 2017; Carrasco et al., 2019).

5 Novel and Alternative Mosquito Control Technologies

5.1 Plant-based insecticides and natural product applications

Plant-based insecticides have emerged as promising eco-friendly alternatives to synthetic chemicals for mosquito control. These natural products, derived from various plant extracts and essential oils, exhibit larvicidal, adulticidal, and repellent properties that target multiple mosquito life stages. Their complex chemical compositions reduce the likelihood of resistance development in mosquito populations, making them valuable tools in integrated vector management. For example, neem oil contains bioactive compounds such as azadirachtin that disrupt mosquito development and behavior, demonstrating efficacy as ovicides, larvicides, and repellents while being environmentally safe (Chatterjee et al., 2023; Hillary et al., 2024).

Advances in green nanotechnology have further enhanced the potential of plant-based insecticides by enabling the synthesis of metallic nanoparticles using plant extracts. These nanoparticles exhibit broad-spectrum mosquitocidal activity with improved stability and targeted delivery compared to conventional formulations. Such green-synthesized nanoparticles offer biodegradable, non-toxic options that minimize environmental impact and can be tailored for specific vector species. However, challenges remain in scaling up production and ensuring consistent field efficacy under diverse ecological conditions (Kumar et al., 2020; Onen et al., 2023).

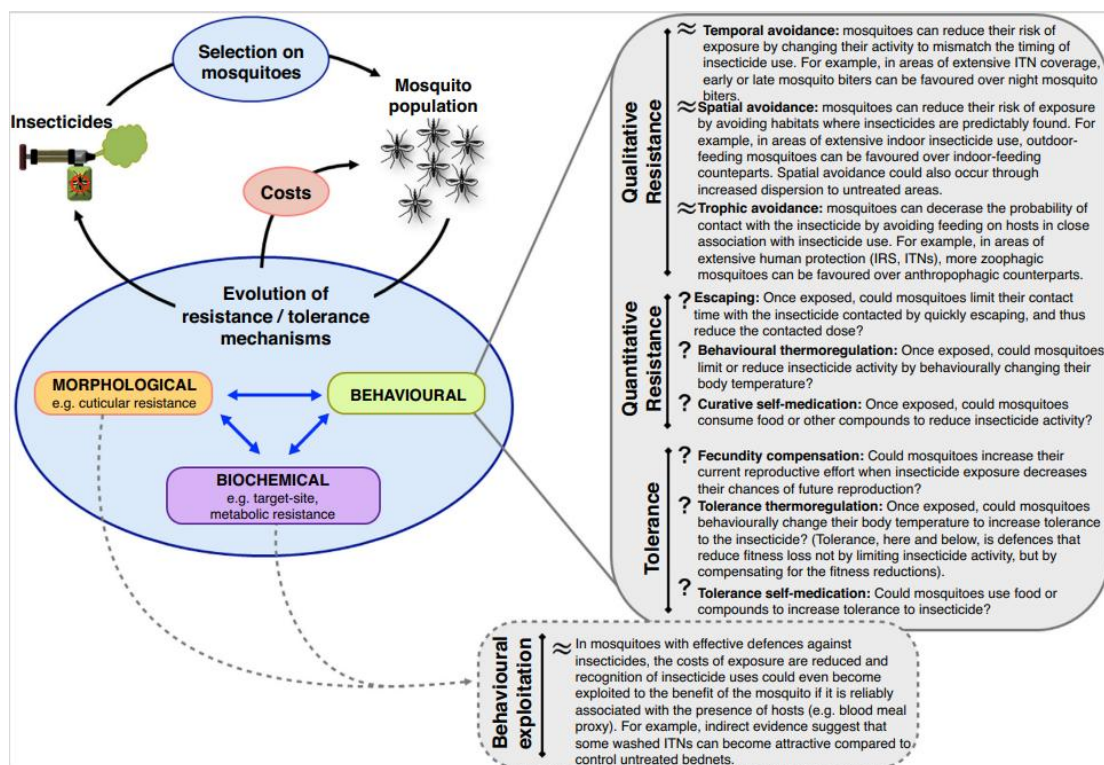


Figure 3 Expanding the field of mosquito insecticide resistance (Adopted from Benelli and Beier, 2017)

5.2 Biotechnological approaches

Biotechnological innovations are revolutionizing mosquito control by targeting vector populations at the genetic level. Gene drive systems use engineered genetic elements to spread traits through mosquito populations rapidly, such as reducing fertility or blocking pathogen transmission. This approach holds promise for sustainable suppression or modification of vector populations but requires careful assessment of ecological risks and ethical considerations before widespread deployment (Jones et al., 2020; Wang et al., 2021). Complementing gene drives, RNA interference (RNAi) technology enables selective silencing of essential mosquito genes involved in survival or reproduction, offering a species-specific bioinsecticide strategy with minimal off-target effects (Yadav et al., 2023).

RNAi-based methods have demonstrated effectiveness in laboratory settings by targeting genes critical at various developmental stages of mosquitoes. Delivery mechanisms include microinjection, feeding, or topical application of double-stranded RNA molecules designed to trigger gene silencing pathways. While promising, challenges such as stability of RNA molecules in field conditions and efficient delivery to wild mosquito populations must be addressed to realize practical applications. Together with gene drives, RNAi represents a cutting-edge toolkit for integrated vector management aiming to overcome limitations of traditional control methods (Wang et al., 2021; Yadav et al., 2023).

5.3 Advanced formulations and delivery systems

Nanotechnology offers innovative solutions for enhancing the efficacy and sustainability of mosquito control agents through advanced formulations and delivery systems. Nanopesticides formulated with plant-derived compounds or synthetic insecticides improve solubility, stability, and controlled release profiles, thereby increasing target specificity while reducing environmental contamination. These nanoformulations can penetrate mosquito cuticles more effectively or provide prolonged residual activity on treated surfaces compared to conventional products (Benelli et al., 2018; Kumar et al., 2020).

Moreover, nanocarriers enable novel delivery strategies such as slow-release larvicides in breeding habitats or attract-and-kill devices that exploit mosquito behavior for targeted control. Despite their potential benefits,

concerns about non-target effects and ecotoxicity require thorough evaluation before large-scale implementation. Integrating nanotechnology with biological controls and environmentally friendly compounds could form a multifaceted approach that addresses current challenges like resistance development and operational constraints in malaria vector management (Benelli, 2015; Benelli et al., 2018).

6 Development and Implementation of Integrated Vector Management (IVM)

6.1 Theoretical framework and core principles of IVM

Integrated Vector Management (IVM) is a rational decision-making process designed to optimize the use of resources for vector control by combining multiple strategies tailored to local contexts. Its core principles emphasize evidence-based decision-making, integration of various control methods, intersectoral collaboration, advocacy, social mobilization, legislation, and capacity building. This approach recognizes that effective vector control is not solely the responsibility of the health sector but requires coordinated efforts across multiple sectors and stakeholders to address the complex determinants of vector-borne diseases (Beier et al., 2008; Onoh et al., 2020). IVM aims to enhance efficacy, cost-effectiveness, ecological soundness, and sustainability by promoting interdisciplinary integration and adapting interventions to changing environmental and epidemiological conditions (Onoh et al., 2020; Tourapi and Tsioutis, 2022).

The theoretical framework of IVM also incorporates adaptability to emerging challenges such as climate change, urbanization, and evolving vector behaviors. It advocates for locally adapted strategies that consider environmental impacts from human activities and demographic shifts influencing disease transmission dynamics. The Circular Policy concept highlights the need for continuous planning, implementation, enforcement, and validation cycles within IVM programs to maintain effectiveness amid these dynamic factors. This holistic approach ensures that vector control remains responsive to new scientific knowledge and technological advancements while aligning with planetary health goals (Figure 4) (Tourapi and Tsioutis, 2022; Tiffin et al., 2025).

IVM: Responding to Complex Systems

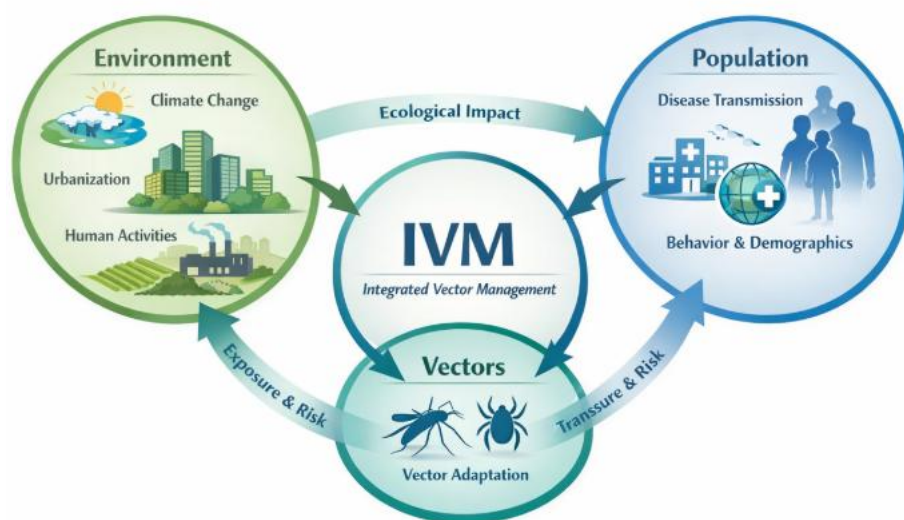


Figure 4 Interactions among environment, vectors, and human populations within the Integrated Vector Management (IVM) framework under dynamic socio-environmental changes (Adopted from Tourapi and Tsioutis, 2022)

6.2 Multi-strategy integration models

Multi-strategy integration within IVM involves combining chemical, biological, environmental, and genetic control methods in a complementary manner to maximize impact on vector populations. For example, integrating insecticide-treated nets with larval source management and biological agents like Wolbachia-infected mosquitoes can address different mosquito life stages and behaviors simultaneously. Such combinations help mitigate

limitations inherent in single-method approaches, including insecticide resistance and behavioral adaptations by vectors (Abbasi, 2025). The integration model also supports the inclusion of novel genomic tools like gene drives alongside traditional interventions to achieve sustainable reductions in disease transmission.

Successful multi-strategy models require robust surveillance systems to guide targeted interventions based on local entomological and epidemiological data. Frameworks like Integrated Aedes Management (IAM) exemplify this approach by incorporating integrated vector surveillance, community mobilization, intersectoral collaboration, capacity building, research, advocacy, and supportive policies. These pillars ensure that diverse tools are deployed effectively according to risk scenarios while fostering adaptability and sustainability in vector control programs (Beier et al., 2008; Roiz et al., 2018).

6.3 Community participation and public health policy support

Community participation is fundamental to the success of IVM as it fosters local ownership, enhances compliance with control measures, and facilitates sustainable behavior change. Engaging communities through education campaigns, social mobilization events such as “mosquito days,” and involvement in environmental management activities empowers residents to contribute actively to vector reduction efforts. Partnerships with local organizations and government departments further strengthen these initiatives by integrating income-generating activities like fish farming or tree planting that align with vector control goals (Ng’ang’a et al., 2021; Ni et al., 2025). Such multisectoral collaboration enhances resource mobilization and capacity building at the grassroots level.

Public health policy support is equally critical for institutionalizing IVM frameworks within national malaria control programs. Strong leadership from governments ensures sustained funding, regulatory backing for innovative tools, and coordination across sectors involved in vector management. Policies aligned with global strategies like the WHO Global Vector Control Response provide guidance for scaling up integrated approaches while addressing emerging challenges such as insecticide resistance or climate change impacts (Roiz et al., 2018; Tourapi and Tsioutis, 2022). Together with community engagement, supportive policies create an enabling environment for effective implementation of IVM strategies that reduce malaria transmission risk sustainably (Beier et al., 2008; Ni et al., 2025).

7 Safety, Environmental Impact, and Sustainability Assessment

7.1 Toxicity to non-target organisms and ecological risks

The use of pesticides in mosquito control poses significant risks to non-target organisms across terrestrial and aquatic ecosystems. Studies have documented adverse effects on growth, reproduction, behavior, and physiological functions in a wide range of species including invertebrates, vertebrates, plants, and microorganisms. These negative impacts contribute to biodiversity loss and ecosystem disruption, with insecticidal compounds such as neonicotinoids notably affecting amphibians and other sensitive taxa. The severity of these effects varies by region but remains consistent across different environments even under realistic exposure scenarios, raising concerns about the sustainability of current pesticide practices (Silva et al., 2023; Wan et al., 2025). Furthermore, natural bioherbicides like matricaria lactones show variable toxicity profiles; while they degrade rapidly in the environment, they can still pose acute risks to aquatic organisms through runoff or leaching, indicating that even plant-based alternatives require careful ecotoxicological evaluation (Suarez et al., 2025).

Ecological risk assessments increasingly emphasize the need to consider indirect effects on food webs and ecosystem services alongside direct toxicity. Emerging methodologies advocate integrating molecular to ecosystem-level endpoints to better capture the complex consequences of pesticide exposure on wildlife populations. However, regulatory frameworks often lag behind scientific advances in assessing these broader ecological impacts comprehensively. This gap underscores the importance of developing more holistic risk assessment approaches that incorporate both standard toxicity data and novel biomarkers to safeguard biodiversity while maintaining vector control efficacy (Rattner et al., 2023; Wan et al., 2025).

7.2 Environmental degradation, residue, and ecological impact

Pesticide residues persist widely in soils, water bodies, sediments, crops, air, and indoor dust environments due to intensive agricultural and vector control applications. Monitoring studies reveal that a majority of environmental samples contain multiple pesticide residues at varying concentrations, including both approved and non-approved compounds. These residues contribute to contamination of food chains and drinking water sources with potential human health implications. The presence of complex mixtures complicates risk assessments since interactions among chemicals may amplify toxic effects beyond those predicted for individual substances (Damalas and Eleftherohorinos, 2011; Silva et al., 2023). Although newer pesticides tend to be more biodegradable than legacy compounds, their degradation products can still affect soil microbiota and aquatic ecosystems adversely if not properly managed (Carvalho, 2017; Suarez et al., 2025).

Efforts to reduce environmental contamination focus on improving pesticide formulations and application techniques alongside promoting alternative pest management strategies. Sustainable agriculture practices such as organic farming or integrated pest management reduce reliance on chemical inputs while enhancing ecosystem resilience. Additionally, regulatory policies increasingly call for comprehensive environmental risk evaluations that include persistence data and ecotoxicity profiles during product approval processes. These measures aim to minimize long-term ecological damage while ensuring effective vector control interventions remain available (Carvalho, 2017; Giovagnoni et al., 2025).

7.3 Cost-effectiveness and long-term sustainability

Assessing the cost-effectiveness of mosquito control strategies requires balancing immediate public health benefits against potential environmental costs and sustainability considerations. While chemical insecticides often provide rapid reductions in vector populations at relatively low initial costs, their long-term use can lead to resistance development, non-target toxicity, and environmental degradation that undermine overall program success (Damalas and Eleftherohorinos, 2011; Hauschild et al., 2022). Incorporating sustainability metrics into decision-making frameworks helps identify interventions that optimize health outcomes without compromising ecosystem integrity or future resource availability.

Sustainable vector management increasingly integrates economic analyses with environmental risk assessments to support safe-and-sustainable-by-design approaches. This combined evaluation facilitates robust policy decisions by highlighting trade-offs between efficacy, safety, cost, and ecological impact throughout a product's lifecycle. Moreover, expanding benefit-risk assessments to include environmental data enables healthcare providers and policymakers to select interventions that align with planetary health goals while maintaining disease control effectiveness (Hauschild et al., 2022; Giovagnoni et al., 2025). Ultimately, fostering multi-sectoral collaboration and investing in innovative technologies will be critical for achieving durable malaria transmission reduction within sustainable development frameworks.

8 Discussion and Future Perspectives

Integrated vector management (IVM) strategies that combine multiple control methods have demonstrated significant potential in reducing malaria transmission, particularly in high-burden regions such as sub-Saharan Africa. Systematic reviews indicate that combining insecticide-treated nets (ITNs), indoor residual spraying (IRS), larval source management, and environmental modifications results in greater reductions in malaria incidence and mosquito density compared to single interventions. These integrated approaches address different mosquito life stages and behaviors, enhancing overall effectiveness and mitigating the limitations of individual methods. Moreover, the use of advanced surveillance tools, including molecular diagnostics and geographic information systems (GIS), supports targeted deployment of interventions, improving resource allocation and program outcomes. Despite these successes, the heterogeneity of local ecological and social contexts means that integrated strategies must be tailored to specific settings for optimal impact. Evidence suggests that community engagement and multisectoral collaboration are critical components that enhance uptake and sustainability of interventions. Additionally, integrating novel technologies such as genetically modified mosquitoes or Wolbachia-infected

vectors with traditional methods shows promise but requires further evaluation under field conditions. Overall, integrated strategies represent a comprehensive framework capable of adapting to evolving transmission dynamics while maximizing public health benefits.

Resistance to insecticides among mosquito populations remains a major obstacle undermining the long-term efficacy of vector control programs. The widespread emergence of resistance mechanisms reduces the effectiveness of chemical-based interventions like ITNs and IRS, necessitating the development of new insecticides and resistance management strategies. Climate change further complicates malaria control by altering vector distribution, breeding patterns, and transmission seasons through rising temperatures and changing rainfall patterns. These environmental shifts expand malaria risk zones into previously unaffected areas, challenging existing control frameworks. Predictive models incorporating climate data are increasingly used to anticipate outbreaks but require integration into operational planning. Implementation barriers also hinder progress in many endemic countries. Weak health infrastructure, inadequate funding, fragmented policy coordination, and limited intersectoral collaboration reduce program efficiency. Studies from Kenya and Zambia highlight gaps between climate-resilient malaria policies and their execution due to misaligned stakeholder roles and insufficient monitoring systems. Social factors such as low community awareness, cultural barriers, and limited access to healthcare further impede intervention uptake. Addressing these multifaceted challenges demands strengthened governance, capacity building, and inclusive policy frameworks that integrate climate adaptation with malaria control.

Future research should prioritize filling critical knowledge gaps related to vector biology in diverse ecological settings, especially urban environments where transmission dynamics differ markedly from rural areas. Investigations into non-vector transmission pathways alongside vector control will provide a more comprehensive understanding of malaria epidemiology. The development of novel tools such as vaccines tailored to region-specific parasite strains and gene-drive technologies for vector population suppression warrants accelerated evaluation through rigorous field trials. Policy recommendations emphasize the need for multi-sectoral collaboration integrating public health, environmental management, climate science, and community stakeholders to design adaptive malaria control programs resilient to emerging threats. Strengthening surveillance systems with real-time data integration will enhance early warning capabilities for outbreaks driven by climatic variability. Additionally, sustainable financing mechanisms coupled with capacity building at local levels are essential for scaling up integrated approaches effectively. Emphasizing equity by addressing social determinants of health will improve intervention reach among vulnerable populations. Collectively, these efforts can advance toward durable malaria reduction aligned with global health goals amid changing environmental landscapes.

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