Effect of coconut oil on *Anopheles gambiae sensu lato* (Diptera: Culicidae) larvae tolerance in malaria vector control in Dogbo district in south-western Benin, West Africa

Nazaire Aïzoun 1, *, Arlette Adjatin 2 and Géorcelin Alowanou 3

1 Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa, Cotonou, Benin.

2 Laboratory of Biochemistry, Food and Medicinal Formulations (LaBFAM), National High School of Applied Biosciences and Biotechnologies (ENSBBA) of Dassa-Zoumè, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey.

3 Laboratory of Ethnopharmacology and Animal Health (LESA), Faculty of Agronomic Sciences, University of Abomey-Calavi (UAC), Cotonou, Bénin.

GSC Advanced Research and Reviews, 2021, 09(02), 001–007

Publication history: Received on 27 August 2021; revised on 23 October 2021; accepted on 25 October 2021

Article DOI: https://doi.org/10.30574/gscarr.2021.9.2.0221

Abstract

The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. This study aims to investigate on the effect of coconut oil on *Anopheles gambiae sensu lato* larvae tolerance in malaria vector control in Dogbo district in south-western Benin, West Africa. Larvae of *Anopheles gambiae s.l.* mosquitoes were collected from breeding sites using the dipping method in May 2020 during the rainy season in Dogbo district. A batch of 25 larvae of fourth instar were exposed to a mixture of coconut oil with distilled water saturated with oxygen containing in each of five glass jars or test cups of same dimensions contained each 48 ml distilled water saturated with oxygen plus 2 ml of coconut oil and one control jar containing no trace of coconut oil. Larval mortality was recorded after 24 hours, 48 hours and 72 hours exposure. The results show that the use of coconut oil causes full-grown Anopheles larvae to die by suffocation. After the application of this mixture, the larvae of four instars cannot breathe. The use of coconut oil is effective method for disturbing the siphonal respiration of mosquito larvae. Coconut oil is effective method for mosquito larvae control.

**Keywords:** Coconut oil; Siphonal respiration; Malaria vector control; Benin

1. Introduction

Malaria remains one of the most important infectious diseases worldwide with an estimated 228 million cases and 405,000 deaths occurring in 2018 [1]. Despite reductions in morbidity and mortality during the last decade, the number of cases globally has now plateaued and is even rising again in some settings. In particular children under the age of five in the African region are most affected, with (severe) morbidity largely attributable to infection with *Plasmodium falciparum*. Efforts to further reduce malaria burden would benefit from an improved understanding of malaria transmission dynamics [2].

*Corresponding author: Nazaire Aïzoun
Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa Cotonou, Benin.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.
Insecticide based vector control interventions have reduced malaria incidence [3]. However, the increasing use of a limited number of insecticides, primarily pyrethroids, places an immense selection pressure on insect populations, which has not left disease vectors unaffected [4, 5]. The resulting insecticide resistance in the major malaria vector *Anopheles gambiae* (s.l.) represents one of the greatest challenges in malaria control. In *Anopheles* mosquitoes, resistance is primarily conferred by mutations at the insecticide’s target site that alter its sensitivity, and by the upregulation of enzymes that detoxify or sequester the insecticide [6].

Pyrethroid and organophosphate resistance in the malaria vector *Anopheles gambiae* has led to the search for alternative insecticides. The control measures for mosquitoes involve chemical control [7-9], biological control, environmental management, genetic control, and physical control [10]. Among the control measures, several methods have been controversial because of ecosystem disturbance and the tolerance development of mosquitoes against the given control methods [10, 11, 12]. However, mosquitoes have not acquired tolerance against physical control methods [10]. Oil, surface film, and polystyrene beads have been introduced to disturb the respiration of mosquito larvae and pupae submerged in water [10, 11, 13, 14, 15, 16].

Very few researches were published on the use of essential oils in *Anopheles gambiae* s.l. larvae tolerance in Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to measure the effect of coconut oil on *Anopheles gambiae* s.l. larvae tolerance in Couffo department in south-western Benin.

### 2. Material and methods

#### 2.1. Study area

![Figure 1 Map of Republic of Benin showing Dogbo District](image)

The study area is located in Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Fig.1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km² and belongs to geographic region of ADJA. The choice of the study site took into account the economic activities of populations, their
usual protection practices against mosquito bites, and peasant practices to control farming pests. We took these factors into account to study the effect of coconut oil on *Anopheles gambiae* s.l. larvae tolerance in Dogbo district in Couffo department. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

2.2. Mosquito sampling

*Anopheles gambiae* s.l. mosquitoes were collected in May 2020 during the rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Fig.2). The samples were then carried out to the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

![Figure 2](image-url)

**Figure 2** An *Anopheles gambiae* s.l. larvae breeding site surveyed in Dogbo district

2.3. Purchase of coconuts

![Figure 3](image-url)

**Figure 3** Coconuts used in our study
The coconuts (Fig.3) used in the current study were bought in Dogbo market. This market is not far (about 500 meters) from the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) of Lokossa. These coconuts are held in a bag and carried out to Laboratory. Then, oil was extracted from them.

2.4. Bioassays

A batch of 25 larvae of fourth instar reared in the insectary of the Laboratory of Applied Entomology and Vector Control (LAEVC) was added in each of five glass jars or test cups of same dimensions contained each 48 ml distilled water saturated with oxygen plus 2 ml of coconut oil and one control jar containing no trace of coconut oil. Otherwise, the control jar or control cup containing only 50 ml distilled water saturated with oxygen and 25 larvae of four instars.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. Each test was run three times on different days. The test containers were held at 25-28°C.

Larval mortality was recorded after 24 hours, 48 hours and 72 hours exposure. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

2.5. Statistical analysis

Analysis using t-test was performed with 95% confidence interval in SPSS version 16.0 (SPSS Inc., Chicago, IL). The p-value acquired by t-test for all cases of this study is less than 5%. Abbott's formula was not used in this study for the correction of mortality rates in test jars because the mortality rates in all controls was always less than 5%[17].

3. Results

The recording of the number of dead larvae was done after 24 hours, 48 hours and 72 hours exposure. The analysis of Table 1 shows that no dead larvae was registered in control jar or control cup during the different bioassays. After 24 hours exposure, there was no alive larvae in test cups, but six (06), nine (09) and five (05) moribund larvae respectively were registered during the bioassay 1, 2 and 3.

Table 1 Recording the number of dead larvae after 24 hours exposure

| Control | Bioassay 1 | Bioassay 2 | Bioassay 3 |
|---------|------------|------------|------------|
| Number tested | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead |
| 25 | 25 | 0 | 0 | 25 | 0 | 6 | 19 | 25 | 0 | 9 | 16 | 25 | 0 | 5 | 20 |

In the same way, the analysis of Table 2 shows that no dead larvae was registered in control jar or control cup during the different bioassays. After 48 hours exposure, there still was no alive larvae in test cups, but Three (03), Zero (00) and One (01) moribund larvae respectively were registered during the bioassay 1, 2 and 3. These results show that some of moribund larvae were died after 24 hours exposure to the mixture of coconut oil with distilled water saturated with oxygen.

Table 2 Recording the number of dead larvae after 48 hours exposure

| Control | Bioassay 1 | Bioassay 2 | Bioassay 3 |
|---------|------------|------------|------------|
| Number tested | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead | Alive | Moribund | Dead |
| 25 | 25 | 0 | 0 | 25 | 0 | 3 | 22 | 25 | 0 | 0 | 25 | 25 | 0 | 1 | 24 |
The same remark was made when we analyze the Table 3. In fact, after 72 hours exposure, there was no alive and no moribund larvae in the test cups of the different bioassays. They were all died due to the effect of the mixture of coconut oil with distilled water saturated with oxygen.

**Table 3** Recording the number of dead larvae after 72 hours exposure

| Control | Bioassay 1 | Bioassay 2 | Bioassay 3 |
|---------|------------|------------|------------|
| Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead | Number tested | Alive | Moribund | Dead |
| 25 | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 25 | 0 | 0 | 25 |

The analysis of Table 4 shows that there are many advantages in the use of coconut oil to control mosquito larvae. But, also there are very few disadvantages.

**Table 4** Advantages and disadvantages of the use of coconut oil

| Advantages | Disadvantages |
|------------|---------------|
| Coconut palm is cultivated in many regions in Benin country | Limited effectiveness of coconut oil in the presence of vegetation and floating debris (is the main disadvantage) |
| Coconut oil is a cheap and easy method of larval control for some breeding sites such as borrow-pits, pools and so on | |
| Mosquitoes may not develop resistance to coconut oil | |
| Coconut oil is not toxic to most non-target organisms including mammals and fish. | |
| Coconut oil cannot soil the earth after its action or effect where it has been applied | |

4. Discussion

The results obtained in the current study shows that the coconut oil causes full-grown Anopheles larvae to die: by suffocation, due to a mechanical barrier being formed between them and the air and also by suffocation, due to the essential oil entering their breathing siphons to an extent sufficient to physically block the passage of air. But also, by poisoning, due to the toxic properties of the volatile portions of this oil penetrating the tracheal tissues.

Our obtained results also show that mosquito larvae fail to do siphonal respiration with the application of coconut oil. Consequently, mosquito larvae mainly depend on the dissolved oxygen in water. Certain species of mosquito larvae breathe underwater by piercing their air tube called a siphon [16].

Given that mosquitoes in the immature stages (eggs, larvae, and pupae) are restricted to small-scale aquatic habitats, avoiding the control measures is difficult for them [18, 19]. Some plant oils act by suffocating larvae or disrupting surface tension, inhibiting the ability of larvae to rest and breathe at the surface of the water causing them to drown and interfering with adult emergence. They are considered effective in control of *Anopheles* larvae, but may be impacted by wind or absorbed by vegetation. These agents will affect any aquatic invertebrate requiring use of the air-water interface for breathing, resting or egg-laying. Re-treatment is needed weekly [20].

The application of coconut oil to water containing *Anopheles gambiae* s.l. larvae has many advantages. In fact, coconut palm is cultivated in many regions in the Benin country and therefore coconuts are available on the markets. The use of coconut oil is a cheap and easy method of larval control for several breeding sites such as brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the gutters. In addition, mosquitoes may not develop resistance to coconut oil. It is not toxic to most non-target organisms including mammals and fish. Coconut oil cannot soil the earth after its action or effect where it has been applied. But, the...
application of coconut oil also presents a few disadvantages and the main is that its effectiveness is limited in the presence of vegetation and floating debris.

Although the mostly artificial habitats have been found to be more productive in terms of pupal production than the “traditional” *Anopheles gambiae* s.s. (Diptera: Culicidae) habitats (such as hoof prints and tire ruts) [21], larval control is widely considered to be too labor intensive in sub-Saharan Africa. However, new tools exist to easily identify such habitats [22] that can facilitate targeted larval control.

5. Conclusion
The use of coconut oil disallows mosquito larvae to acquire tolerance. It directly disturbs their siphonal respiration. In the current study, the use of coconut oil is effective method for mosquito larvae control. After the exposure to the mixture of coconut oil with distilled water saturated with oxygen, the larvae of four instars cannot breathe. However, this study was conducted in laboratory conditions and there is also a need to carry it out in field conditions for better conclusions.

Compliance with ethical standards

Acknowledgments
The authors would like to thank people from locations surveyed who had helped us in mosquito collection. We would also like to thank KOUASSI Prisca for technical assistance in laboratory during the current study.

Disclosure of conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of ethical approval
The study follows proper ethical procedures.

Statement of informed consent
Informed consent was obtained from all individual participants included in the study.

References
[1] WHO. World malaria report. Geneva: World Health Organization. 2019.
[2] malERA. Refresh Consultative Panel on Characterising the Reservoir and Measuring Transmission. malERA: an updated research agenda for characterising the reservoir and measuring transmission in malaria elimination and eradication. *PloS Med.* 2017; 14: e1002452.
[3] Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, *et al.* The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature.* 2015; 526: 207–211.
[4] Ranson H, N’Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol.* 2011; 27:91–8.
[5] Edi CV, Koudou BG, Jones CM, Weetman D, Ranson H. Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, southern Côte d’Ivoire. *Emerg Infect Dis.* 2012; 18:1508–11.
[6] Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol.* 2015; 60: 537–559.
[7] Aïzoun N, Ossé R, Azondekon R, Alia R, Oussou O, Gnanguenou V, Aïkpon R, Padonou GG, Akogbéto M. Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. *Parasit Vectors.* 2013a; 6:147.
[8] Aïzoun N, Aïkpon R, Padonou GG, Oussou O, Oké-Agbo F, Gnanguenou V, Ossé R, Akogbéto M. Mixed-function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in *Anopheles gambiae* s.l. in the south-north transect Benin, West Africa. *Parasit Vectors.* 2013b; 6:223.
[9] Aïzoun N, Aïkpon R, Gnanguenon V, Oussou O, Agossa F, Padonou GG. Akogbéto M. Status of organophosphate and carbamate resistance in Anopheles gambiae sensu lato from the south and north Benin, West Africa. Parasit Vectors. 2013; 6:274.

[10] Shidrawi GR. Laboratory tests on mosquito tolerance to insecticides and the development of resistance by Aedes aegypti. Bulletin of the World Health Organization. 1957; 17: 377–411.

[11] Schorkopf DLP et al. Combining attractants and larvicides in biodegradable matrices for sustainable mosquito vector control. PLoS Neglected Tropical Diseases. 2016;10: e0005043.

[12] Poupardin R, et al. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito Aedes aegypti: impact on larval tolerance to chemical insecticides. Insect Biochemistry and Molecular biology. 2008; 38: 540–551.

[13] Corbet SA, et al. Surface films as mosquito larvicides: partitioning the mode of action. Entomologia Experimentalis et Applicata. 2000; 94: 295–307.

[14] Nayar JK, Ali A. A review of monomolecular surface films as larvicides and pupicides of mosquitoes. Journal of Vector Ecology. 2003; 28: 190–199.

[15] B, et al. Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. Fitoterapia. 2007; 78: 205–210.

[16] Turki H, Soltani A. Semi-field and field studies on the efficacy of monomolecular surface film (Agnique®) against immature mosquitoes in the malarious areas of Iran. Asian Pac. J. Trop. Dis. 2017; 7(8): 472–476.

[17] Abbott WS. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Cont. Assoc. 1987; 3(2): 302-303.

[18] Killeen GF, Fillinger U & Knols BG. Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal. 2002; 1:8.

[19] Amer A, Mehlhorn H. Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). Parasitology Research. 2006; 99: 466–472.

[20] WHO. Vector Control: methods for use by individuals and communities. Geneva: World Health Organization; 1997.

[21] Mutuku FM, Bayoh MN, Gimnig JE, Vulule JM, Kamau L, Walker ED, Kabiru E and Hawley WA. Pupal habitat productivity of Anopheles gambiae complex mosquitoes in a rural village in western Kenya. Am. J. Trop. Med. Hyg. 2006; 74: 54-61.

[22] Mushinzimana E, Munga S, Minakawa N, Li L, Feng C, Bian L, Kitron U, Schmidt C, Beck L, Zhou G, Githeko AK, Yan G. Landscape determinants and remote sensing of anopheline mosquito larval habitats in the western Kenya highlands. Malar J. 2006; 5: 13.