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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.

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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 72hours 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.

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

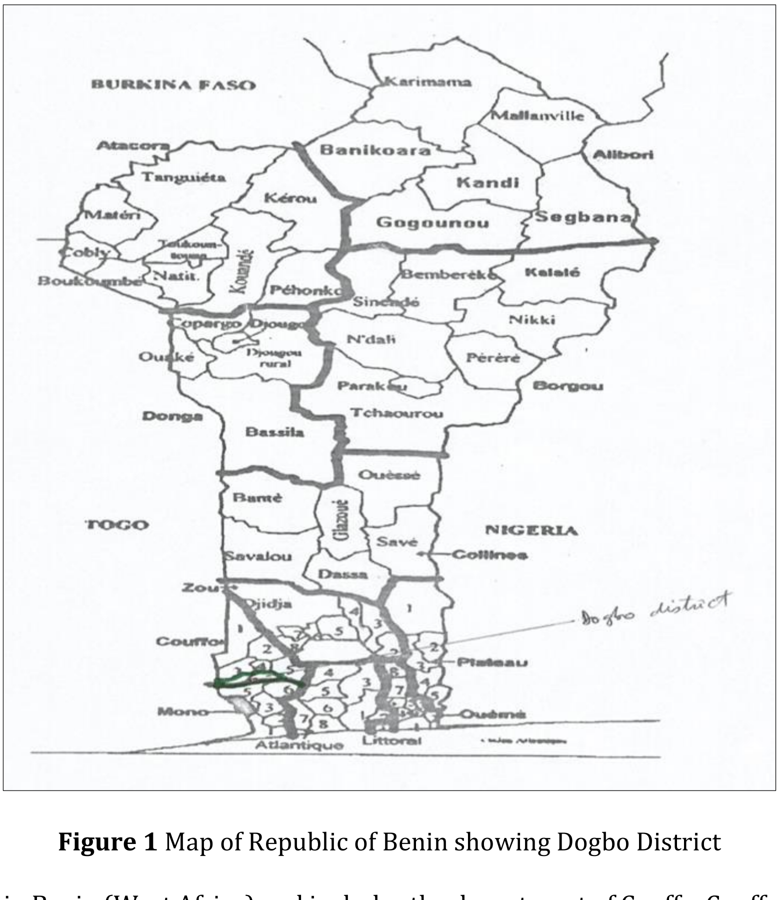

Figure 1 Map of Republic of Benin showing Dogbo District

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

2.3. Purchase of coconuts

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 each48 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 72hours 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 24hours, 48hours and 72hours exposure. The analysis of Table 1 shows that no dead larvae was registered in control jar or control cup during the different bioassays. After 24hours 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 24hours exposure

Control				**Bioassay 1**				**Bioassay 2**				**Bioassay 3**			
Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	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 48hours 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 24hours exposure to the mixture of coconut oil with distilled water saturated with oxygen.

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

Control				**Bioassay 1**				**Bioassay 2**				**Bioassay 3**			
Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	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 72hours 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 72hours exposure

Control				**Bioassay 1**				**Bioassay 2**				**Bioassay 3**			
Number tested	Alive	Moribund	Dead	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	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 gambaie 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* Giles 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

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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.

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