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Effect of carica papaya linn. (Caricaceae) latex gel in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district in couffo department in south-western republic of Benin, West Africa

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Abstract

Background: The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions.

Objective: This study aims to investigate on the effect of *Carica papaya* latex gel on *Anopheles gambiae sensu lato* larvae tolerance in malaria vector control in Dogbo district in south-western Benin, West Africa.

Methodology: Larvae of *Anopheles gambiae s.l* mosquitoes were collected from breeding sites using the dipping method in June 2020 during the rainy season in Dogbo district. A batch of 10 larvae of fourth instar were exposed to a mixture of *Carica papaya* latex gel 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 *Carica papaya* latex gel and one control jar containing no trace of *Carica papaya* latex gel. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure.

Results: The results show that the use of *Carica papaya* latex gel 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 *Carica papaya* latex gel is effective method for disturbing the siphonal respiration of mosquito larvae.

Conclusion: *Carica papaya* latex gel is effective method for mosquito larvae control.

Keywords: *Carica papaya* latex gel, siphonal respiration, malaria, vector control, republic of Benin

Introduction

Long Lasting Insecticidal mosquito Nets (LLINs) and indoor residual spraying (IRS) are major malaria vector control strategies [1]. Both strategies have had substantial impacts on the malaria burden over the past 15 years. Indeed, LLIN and IRS accounted for an estimated 68 and 11% of the malaria averted cases, respectively, between 2000 and 2015 [2].

Vector control is a corner stone in the fight against vector-borne diseases particularly malaria [3]. Insecticides are considered to be a powerful weapon in order considerably to improve the major public health indices. However, over the past six decades, insecticides are playing the crucial role to contain the vector-borne diseases and have saved hundreds of millions of lives every year. In the last decade, we have attained a remarkable success to combat with many arthropod-borne diseases particularly malaria. All credits go to the combined effect of IRS, long-lasting insecticidal nets (LLINs), and effective case management. However, now malaria vector control is facing a serious challenge in terms of insecticide resistance, particularly against pyrethroids [4]. Pyrethroid resistance is a potential threat to the global public health concern. It is largely due to the heavy reliance, recurrent and inappropriate pyrethroid usage. It calls for the searching of new insecticides with novel mode of action. As a result, it is the hour to launch an extensive search to explore eco-friendly biological materials for the control of insect pests, and we are all just around the corner to reinstate the ubiquitous chemical of concern by plant-based products in the insect control [5].

Also, over the centuries, even before the advent of modern synthetic pesticides our ancestors completely depended upon the usage of plant-derived products as a pesticidal agent against various insects. At the moment, a renewed interest has been observed to exploit the insecticidal control potentialities of various plant-based products [6]. The rationale behind the reawakened desire for searching the new plant-based insecticides with novel mode of actions is necessary for new Integrated Pest Management (IPM) strategy.

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 *Carica papaya* latex gel in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district in Couffo department in south-western Republic of Benin.

Materials and Methods

Study area

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 measure the effect of *Carica papaya* latex gel in the management of larvicide resistance in larvae of *Anopheles gambiae sensu lato* from Dogbo district in Couffo department in south-western Republic of Benin. 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.

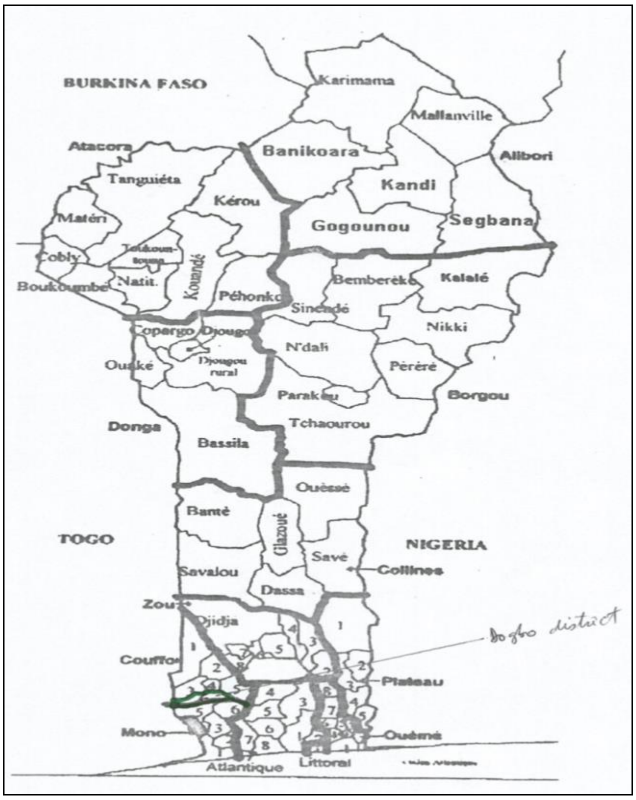

Fig 1: Map of Republic of Benin showing Dogbo District

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected in June 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.

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

Latex collection

Latex gel was collected in sterile bottles from unripe mature fruits on paw paw (Fig.3) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district. For that, unripe mature fruits were pierced with needles and latex gel was taken in the sterile bottles. Then, these bottles were carried out to Laboratory of Applied Entomology and Vector Control (LAEVC) for bioassays.

Fig 3: *Carica papaya* tree

Bioassays

A batch of 10 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 *Carica papaya* latex gel and one control jar containing no trace of *Carica papaya* latex gel. Otherwise, the control jar or control cup containing only 50 ml distilled water saturated with oxygen and 10 larve 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.

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% [7].

Results

The recording of the number of dead larvae was done after 24hours, 48 hours 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 three (03), two (02) and four (04) 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	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead
10	10	0	0	10	0	3	7	10	0	2	8	10	0	4	6

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 one (01), Zero (00) and two (02) 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 *Carica papaya* latex gel 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	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead	Number tested	Alive	Moribund	Dead
10	10	0	0	10	0	1	9	10	0	0	10	10	0	2	8

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 *Carica papaya* latex gel 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	Number tested	Alive	Moribund	Dead
10	10	0	0	10	0	0	10	10	0	0	10	10	0	0	10

The analysis of Table 4 shows that there are many advantages in the use of *Carica papaya* latex gel to control mosquito larvae. But, also there are very few disadvantages.

Table 4: Advantages and disadvantages of the use of *Carica papaya* latex gel

Advantages	Disadvantages
Carica papaya tree (paw paw) is cultivated in many regions in Benin country *Carica papaya* latex gel 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 *Carica papaya* latex gel *Carica papaya* latex gel is not toxic to most non-target organisms including mammals and fish.	Limited effectiveness of *Carica papaya* latex gel in the presence of vegetation and floating debris (is the main disadvantage)
Carica papaya latex gel cannot soil the earth after its action or effect where it has been applied	

Discussion

The results obtained in the current study shows that the *Carica papaya* latex gel 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 *Carica papaya* latex gel. 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 [8].

In the current study, the use of *Carica papaya* latex gel had inhibited the development of resistance with the existing chemical insecticides, it further reduced the heavy reliance of chemical pesticides as well as their adverse impact on human health and environment. In general, green pesticides/risk reduced pesticides are target-specific and can be non-toxic. Latex was a fluid secreted by laticifer cells in plants. The biological role of latex secretion in plants remains unknown, but several types of research proved that it has insecticidal property [9]. Latex contains highly active chemicals such as aromatics, terpenoids, alkaloids, phenols, glycosides, carbohydrates and proteins [10].

The application of *Carica papaya* latex gel to water containing *Anopheles gambaie s.l.* larvae has many advantages. In fact, *Carica papaya* tree is cultivated in many regions in Benin and therefore available in the country. The use of *Carica papaya* latex gel 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 *Carica papaya* latex gel. It is not toxic to most non-target organisms including mammals and fish. *Carica papaya* latex gel cannot soil the earth after its action or effect where it has been applied. But, the application of *Carica papaya* latex gel also presents a few disadvantages and the main is that its effectiveness is limited in the presence of vegetation and floating debris.

Regrading larvicidal activity of Carica papaya, crude extracts of the plant have also been tested such as crude methanol extract of the leaf of *C. papaya* and showed highest activity against the first and fourth instar of *Aedes aegypti* with LC50 values of 51.76ppm and 82.18ppm and pupae was 440.65ppm [11]. Okolie [12] tested the crude aqueous extract of the leaves against anopheles and culex species and reported 100% morality at doses of 0.06mg/ml and 0.10mg/ml respectively. In Indonesia, 70% crude ethanol extract of leaves and seeds were tested against the larvae of *Anopheles farauti*, *koliensis*, *subpictus* and *punctulatus* with LC50 and LC90 values for leaves and seeds being 422.311 ppm, 1399.577 ppm and 21.983 ppm and 137.862 ppm respectively [13]. A presentation at the 3rd International congress on Global Warming in Bharathiar University, Colmbatore, India on the larvicidal and pupicidal activity of crude aqueous extract of *Carica papaya* leaf and seed extract against *Aedes aegypti* found that the extracts inhibited larval growth [14]. Infra red analysis of the seeds of *Carica papaya* against the larvae of *Culex quinquefasciatus* and *Anopheles stephensi* showed the presence of aliphatic amide that may be responsible for the larvicidal activity [15]. Acetone extract of *Carica papaya* showed highest mortality when compared to methanol, ethanol, ethylacetate and chloroform extract against *Culex quinquefasciatus*. Mortality observed was 61.6% at 24 hours at concentration of 500 ppm. LC50 and LC90 values were 80.56 and 380.67 ppm and 60.89 and 150.75 ppm at 24 and 48 hours respectively [16]. A lot of emphasis has been placed on the seed of the plant but even at that very little is known of the active principles responsible for this activity. On the other hand nothing is known of the active principles that may exist in the leaf, stem or root of the plant that may be responsible for larvicidal activity (if it exists) or mechanisms of action by these principles. Though several extracts and compounds from different plant families have been evaluated to show new and promising larvicides, very few plant products have been developed for controlling mosquitoes [17].

Conclusion

The use of *Carica papaya* latex gel is effective on larvae of fourth instar of *Anopheles gambiae sensu lato* in the current study. *Carica papaya* latex gel can help for new stratagem in the control of mosquito larvae, in malaria vector management program. However, this study was conducted in laboratory conditions and there is also a need to carry it out in field conditions for better conclusions. Also, the bio-active molecules responsible of larvae mortality or lethality and their mode of actions remain indistinct and imprecise, and this calls for further pharmacological and clinical research on them.

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Conflicts of interest

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

References

1. WHO. World malaria report. Geneva: World Health Organisation, 2019.
2. 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,2015:526:207-11.
3. Karunamoorthi K. Vector Control: A Cornerstone in the Malaria Elimination Campaign. Clin. Microbiol. Infect,2011:17:1608-1616.
4. Karunamoorthi K, Sabesan S. Relative efficacy of repellents treated wristbands against three major mosquitoes (Insecta; Diptera: Culicidae) vectors of disease, under laboratory conditions. Int. Health,2009:1:173-177.
5. Karunamoorthi K, Ramanujam S, Rathinasamy R. Evaluation of leaf extracts of Vitex negundo L. (Family: Verbenaceae) against larvae of Culex tritaeniorhynchus (Insecta; Diptera; Culicidae) and repellent activity on adult vector mosquitoes. Parasitol. Res,2008:103:545-550.
6. Karunamoorthi K, Ilango K, Endale A. Ethnobotanical survey of knowledge and usage custom of traditional insect/mosquito repellent plants among the Ethiopian Oromo ethnic group. J. Ethnopharmacol,2009:125:224-229.
7. Abbott WS. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Cont. Assoc,1987:3(2):302-303.
8. 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.
9. Rajkuberan C, Sudha K, Sathish Kumar G, Sivaramakrishnan S. Antibacterial and cytotoxicity of silver nanoparticles synthesized using latex of

Calotropis gigantea. Spectrochimica Acta A,2015:136(5):924-930.
10. Konno K. Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. Phytochemistry,2011:72(13):1510-1530.
11. Kovendan K, Murugan K, Naresh KA, Vincent S, Hwang JS. Bioefficacy of larvicidal and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). Parasitology Research,2012:110(2):669-78.
12. Okolie NJC. Larvicidal effects of pawpaw (*Carica papaya*) aqueous extract on mosquito vectors. International Journal of Natural and Applied Sciences,2006:2(4):417-420.
13. Sesanti H, Arsunan AA, Hasanuddin I. Potential test of papaya leaf and seed extract *Carica papaya* as larvicide against Anopheles mosquito larval mortality. Sp Jayapura Papau Indonesia. International Journal of Science and Research Publications,2014:4(6):1-8.
14. Noortheen A, Muthuselvi M, Meenambigai K. Evaluation of the larvicidal and pupicidal activity of the aqueous extract of *Carica papaya* leaf and seed against the dengue vector, *Aedes aegypti*. International Congress (3rd) on Global Warming on Biodiversity of Insects Management and Conservation Strategies (GW-BIMC,13, Nov 26-28), 2013.
15. Rawani A, Ghosh A, Laskar S, Chandra G. Aliphatic amide from the seeds of *Carica papaya* as mosquito larvicide, pupicide, adulticide, repellant and smoke toxicant. Journal of Mosquito Research,2012:2(2):8-18.
16. Ravichandran R, Thangaraj D, Alwarsamy M. Antimosquito activity of leaf extract of Neem (*Melia azedarach*) and Papaya (*Carica papaya*) detected against the larvae *Culex quinquefasciatus*. International Journal of Innovative Research in Science, Engineering and Technology,2014:3(4):11928-11935.
17. Magadula JJ, Innocent E, Otieno JN. Mosquito larvicidal and cytotoxic activities of 3 *Annona* species and isolation of active principles. Journal of Medicinal Plants Research,2009:3(9):674-680.