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Comparison of larvicidal activities of ethanolic extract of *Cymbopogon citratus* (Poaceae) and *Ocimum basilicum* L. (Lamiaceae) leaves in malaria vector control in Dogbo district in south-western Benin, West Africa

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Abstract

The alteration of mosquito life cycle using larvicides is key in the control of diseases transmitted by mosquitoes. The residual and environmental effect of some of these synthetic larvicides has given an opportunity to search for a potent larvicide as an alternate control measure against mosquitoes. The current study aimed to compare the larvicidal activities of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in malaria vector control in Dogbo district in south-western Benin, West Africa. Larvae of *Anopheles gambiae* sensu lato mosquitoes were collected from breeding sites using the dipping method from September to November 2023 during the small rainy season in Dogbo district of Couffo department. A batch of twenty-five (25) larvae of four instars were exposed to ethanolic extracts of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves with different concentrations of 1 mg/liter, 2mg/liter, 3 mg/liter, 4 mg/liter and 5 mg/liter in some glass jars or plastic test cups of same dimensions covered with small cutting untreated net and in some control, jars containing no trace of these ethanolic extracts. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure. The results showed that the ethanolic extract of *Cymbopogon citratus* leaves had the highest larvicidal activity. It was the most effective against larvae of *Anopheles gambiae* sensu lato. *Cymbopogon citratus* was found to be effective against the larvae of *Anopheles gambiae* sensu lato in laboratory conditions. More effort must be done in order to explore the potentiality of these plant parts available for botanical insecticide preparing.

Keywords: Botanical Insecticide; Ethanolic Extract; *Anopheles Gambiae*; Malaria Control; Benin

1. Introduction

Malaria remains an important global health problem, with 92 % of all deaths occurring in Africa [1]. Since 2015, the annual malaria burden has not fallen any further in Africa [2].

The WHO's global vector control strategy recommends the scaling up of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) to control malaria, towards achieving the Millennium Development Goals for malaria [3-4].

Malaria vectors have developed resistance to the insecticides employed in vector control programmes and indeed to almost all the classes of available insecticides [5]. Insecticide use has been associated with widespread physiological resistance and behavioural changes of malaria vectors which may contribute in maintaining residual malaria transmission [6-7]. So, the rapid spread of insecticide resistance in malaria vectors and the rebound in malaria cases

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observed recently in some endemic areas underscore the urgent need to evaluate and deploy new effective control interventions. The emergence and spread of insecticide resistance may have impacts on the continued effectiveness of insecticide-based interventions [8]. Currently researchers have been exploring several alternative avenues of controlling malaria, and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature stages (i.e., mosquito eggs, larvae and pupae). This strategy is becoming increasingly important in many countries especially in sub-Saharan Africa and involves different species of mosquitoes including those that transmit malaria. The strategy depends on the use of various larvicidal techniques and environmental management practices aimed at reducing larval density and therefore minimizing or reducing vector abundance [9].

Extracts from plants may be alternative sources of mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Plant extracts in general have been recognized as an important natural resource of insecticides [10]. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level [11-12].

Very few researches were published on the larvicidal activities of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in malaria vector control in Dogbo district in south-western Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to compare the larvicidal activities of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in malaria vector control in Dogbo district in south-western Benin, West Africa.

2. Material and methods

2.1. Study area

Figure 1 Map of Republic of Benin showing Dogbo district Surveyed

The study area is located in Republic of 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 (Figure 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 km^2 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 compare the larvicidal activities of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in malaria vector control in Dogbo district in south-western 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.

2.2. Mosquito sampling

Anopheles gambiae sensu lato mosquitoes were collected from September to November 2023 during the small rainy season in Dogbo district of Couffo department. Larvae were collected from breeding sites using the dipping method [13] and kept in labeled bottles (Figure 2). The samples were then carried out to the insectary of Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district (Figure 3).

Figure 2 Mosquito larvae collection in a breeding site

Figure 3 Larvae in labeled plastics in insectary

2.3. Collection of the plant leaves

The leaves of both plants were collected in their predilection areas in Dogbo district of Couffo department (Figure 4 and Figure 5).

Figure 4 Plant of *Ocimum basilicum* L

Figure 5 Plant of *Cymbopogon citratus*

2.4. Plant leaves extraction

To prepare botanical insecticide of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves, we collected fresh green leaves of both plants and we washed them with tap water. The leaves were dried outside of the laboratory at ambient temperature in a class room for a period of 3 days. Then, the dried leaves were crushed or grounded into powder with an electronic mix anda weight of 100 grammes of the leaves powder of each plant was extracted with 250 milliliters of ethanol for a period of 48 hours at temperature of 25°C.Each extract was then filtered with the aid of Whatman No. 1 filter paper. Then, the mixture was dried and stored in some labeled bottles for bioassays.

2.5. Bioassays

A batch of twenty-five (25) larvae of four instars reared in the insectary of the Department of Sciences and Agricultural Techniques was added to each of five glass jars or test cups of same dimensions. These glass jars or test cups contained the dilutions of 1.0mg/liter, 2.0mg/liter, 3.0mg/liter, 4.0 mg/liter and 5.0mg/liter respectively of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves previously obtained and stored. These tests cup were covered with small cutting untreated net. At each range of dilutions, there is a corresponding control. The control jars contained no trace of ethanolic extracts of these plant leaves.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. The test containers were held at 25-28°C.

Larval mortality was recorded after 24hours, 48hours and 72hours exposure. 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 incapables of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

2.6. 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%.

3. Results

3.1. Evaluation of larvicidal effect of ethanolic extract of *Cymbopogon citratus* leaves against larvae of *Anopheles gambiae* s.l.

The analysis of figure 6 showed that after the exposure of *Anopheles gambiae* sensu lato larvae of four instars (L4) to ethanolic extract of *Cymbopogon citratus* leaves, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. The analysis of the same figure showed that very few dead larvae were registered after 24 hours exposure to all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l (P>0,05). The recording of 48 hours exposure showed that dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l et 5mg/l (P <0,05). The number of dead larvae recorded after 48hours exposure was higher than that registered after 24 hours exposure. The recording of 72 hours exposure showed that more dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l (P <0,05). Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. Finally, the highest mortality rate was recorded with the concentration of 5 mg/l (80 dead larvae on a total of 125 tested larvae).

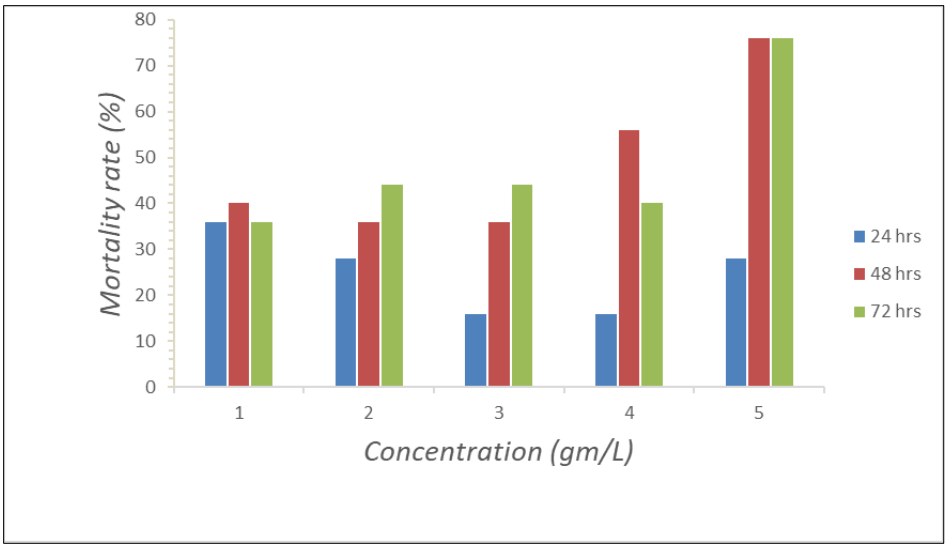

Figure 6 Larvicidal activity of *Cymbopogon citratus* leaves against larvae of *Anopheles gambiae* s.l.

3.2. Evaluation of larvicidal effect of ethanolic extract of *Ocimum basilicum* L. leaves against larvae of *Anopheles gambiae* s.l.

The analysis of figure 7 showed that after the exposure of *Anopheles gambiae* sensu lato larvae of four instars (L4) to ethanolic extract of *Ocimum basilicum* L. leaves, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. The analysis of the same figure showed that very few dead larvae were registered after 24 hours exposure to all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l (P>0,05). The recording of 48 hours exposure showed that dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l (P <0,05). The number of dead larvae recorded after 48hours exposure was higher than that registered after 24 hours exposure. The recording of 72 hours exposure showed that more dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l (P <0,05). Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. Finally, the highest mortality rate was also recorded with the concentration of 5 mg/l (40 dead larvae on a total of 125 tested larvae).

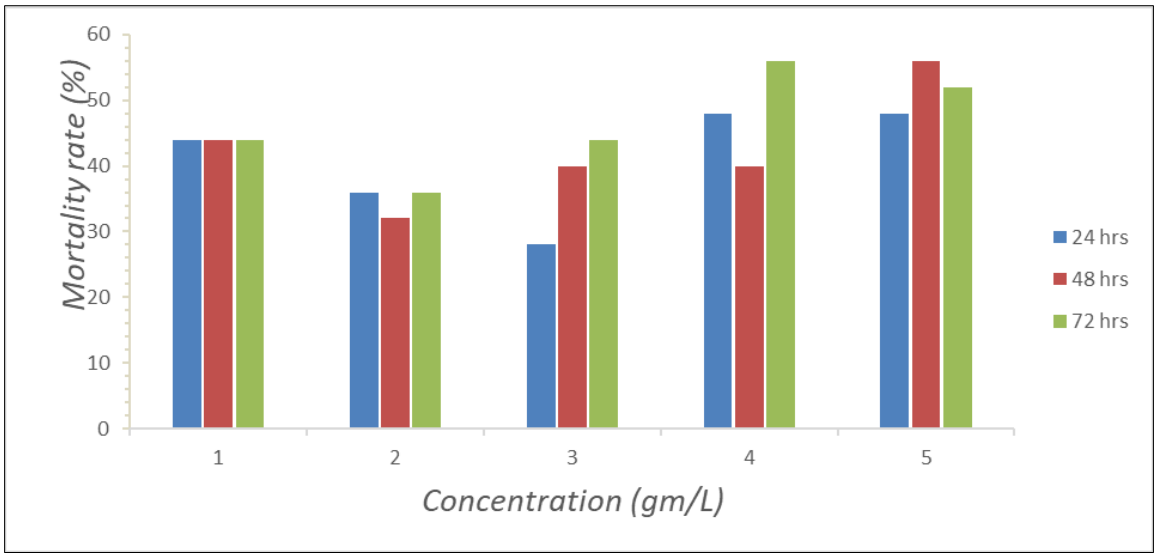

Figure 7 Larvicidal activity of *Ocimum basilicum* L. leaves against larvae of *Anopheles gambiae* s.l.

The table 1 showed the advantages and disadvantages of the use of ethanolic extract of both *Cymbopogon citratus* and *Ocimum basilicum* L. leaves.

Table 1 Advantages and disadvantages of the use of ethanolic extract of both *Cymbopogon citratus* and *Ocimum basilicum* L. leaves

Advantages	**Disadvantages**
Cymbopogon citratus and *Ocimum basilicum* L. are cultivated in many regions in Benin country Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. Leaves is miscible with water after mixture Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. Leaves is a cheap and easy method for larval control in their breeding sites Mosquitoes may not develop resistance to ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. Leaves is not toxic to most non-target organisms including mammals and fish.	Limited effectiveness of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leavesin the presence of vegetation and floating debris (is the main disadvantage)

The analysis of the table 1 showed that the use of ethanolic extract of both *Cymbopogon citratus* and *Ocimum basilicum* L. leaves presented many advantages and very few disadvantages.

4. Discussion

The results obtained in the current study showed that after the exposure of *Anopheles gambiae* s.l. larvae of four instars (L4) to ethanolic extract of *Cymbopogon citratus* leaves, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. Very few dead larvae were registered after 24 hours exposure with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. The recording of 48

hours exposure showed that dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. The number of dead larvae recorded after 48hours exposure was higher than that registered after 24 hours exposure. The recording of 72 hours exposure showed that more dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. These results showed that ethanolic extract of *Cymbopogon citratus* was effective against larvae of *Anopheles gambiae* sensu lato collected in Dogbo district. Our results corroborated with those obtained by Love *et al* [14] in Nigeria. In fact, these authors showed that the plant extracts may be used as alternative insecticides against *Anopheles gambiae* mosquitoes, with a further study on their phytochemicals, characterization and synergistic activities and their adaptability to field assay highly recommended. The mortality recorded by these authors with *Cymbopogon citratus* against larvae of *Anopheles gambiae* sensu lato mosquitoes was 93% comparatively to 80% recorded in the current study, showed that the extract of *Cymbopogon citratus* was effective against larvae of Anopheles gambiae sensu lato mosquitoes.

The results obtained in the current study also showed that after the exposure of *Anopheles gambiae* sensu lato larvae of four instars (L4) to ethanolic extract of *Ocimum basilicum* L. leaves, no dead and moribund larvae were registered in the control plastic cups after 24 hours, 48 hours and 72hours recording, they were all alive. Very few dead larvae were registered after 24 hours exposure to all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. The recording of 48 hours exposure showed that dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. The number of dead larvae recorded after 48hours exposure was higher than that registered after 24 hours exposure. The recording of 72 hours exposure showed that more dead larvae were registered in the all-test plastic cups with all tested concentrations of 1mg/l, 2mg/l, 3 mg/l, 4mg/l and 5mg/l. Otherwise, the number of dead larvae recorded after 72 hours exposure was higher than that registered after 48 hours exposure. These results showed that the efficacy of ethanolic extract of *Ocimum basilicum* L. leaves was less than that of *Cymbopogon citratus* against larvae of *Anopheles gambaie* sensu lato. Our results corroborated with those obtained by Grid and Hamaidi [15] in Algeria. In fact, these authors had shown in their study the larvicidal activity of *Ocimum basilicum* against larvae of *Culex pipiens* after 48 hours and 72 hours exposure with different concentrations of 1g/l, 2g/l, and 3g/l. After 48 hours exposure to the extract of *Ocimum basilicum*, the mortality was 11% with 3 g/l against 4 % with 1g/l whereas after 72 hours exposure, the mortality was 19% with 3 g/l against 13% with 1g/l.

The use of ethanolic extract of both *Cymbopogon citratus* and *Ocimum basilicum* L. leaves presented many advantages and very few disadvantages. In fact, *Cymbopogon citratus* and *Ocimum basilicum* L. are cultivated in many regions in Benin country. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is miscible with water after mixture. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is a cheap and easy method for larval control in their breeding sites. Mosquitoes may not develop resistance to ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is not toxic to most non-target organisms including mammals and fish. However, limited effectiveness of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in the presence of vegetation and floating debris is the main disadvantage in its use.

5. Conclusion

The current study clearly shows that among both ethanolic extracts of plants leaves used in the current study, *Cymbopogon citratus* extract has the highest larvicidal activities. It is found to be effective against the larvae of *Anopheles gambiae* sensu lato in laboratory conditions. More effort must be done in order to explore the potentiality of these plant parts available for botanical insecticide preparing. Researches must also be carried out in field conditions by treatment of mosquito larvae breeding sites with these ethanolic extracts in a context where it is useful to search for alternative solutions to damages cause by chemical insecticides to environment and human health. 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.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest regarding the publication of this paper.

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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