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COMPARISON OF LARVICIDAL ACTIVITIES OF ETHANOLIC EXTRACT OF SIX PLANTS LEAVES IN MALARIA VECTOR CONTROL IN COUFFO DEPARTMENT IN SOUTH-WESTERN 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**: The current study aimed to compare the larvicidal activities of ethanolic extract of six plants leaves in malaria vector control in Couffo department in south-western Republic of Benin, West Africa. **Methodology**: 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 and from the March to July 2024 during the great rainy season in the six districts of Couffo department. A batch of twenty five (25) larvae of fourth instar were exposed to ethanolic extracts of *Sida acuta* Burm F. (Malvaceae), *Eucalyptus globulus* Labill. (Myrtaceae), *Anacardium* occidentale, *Allium sativum L.* (Amaryllidaceae), *Parkia biglobosa* (Mimosaceae) and *Petroselinum crispum (Mill.) Nyman ex A.W. Hill* (Apiaceae) 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. **Results:** The results showed that among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Allium sativum* L. leaves was the most effective on *Anopheles gambiae s.l.* larvae following by ethanolic extract of *Sida acuta* Burm F. leaves, following by ethanolic extract of *Parkia biglobosa* leaves, following by ethanolic extract of *Petroselinum crispum (Mill.)* Nyman *ex A.W. Hill* leaves, following by ethanolic extract of *Eucalyptus globulus* Labill leaves and following by ethanolic extract of *Anacardium* occidentale leaves. **Conclusion:** Among the six ethanolic extracts of plants leaves used in the current study, *Allium sativum L.* extract have the highest larvicidal activity. It was found to be effective against the larvae of *Anopheles gambiae* sensu lato in laboratory conditions. The indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control.

KEYWORDS: botanical insecticide, ethanolic extract, *Anopheles gambiae*, malaria control, Benin.

INTRODUCTION

The World Health Organization (WHO) estimated to 229 million the number of cases of malaria and to 409,000 the number of death having occurred worldwide in 2019.[1] The same year, 94% of all malaria deaths occurred in sub-Saharan African[1] countries, where malaria control consumes a major part of the national health budgets.[2-3]

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme

has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale.[4-5] Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature.[6] It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friedly alternatives such as biological control of vectors has become the central focus of the control programmme in place of the chemical insecticides.

Conventionally, synthetic insecticides organochlorines, carbamates, organophosphates, and pyrethroids temephos, fenthion, malathion, and dichlorodiphenyltrichloroethane (DDT) were expensive, leaving a residual effect, adapting resistance, non-biodegradable, toxicity to non-target organisms.[7] These problems urged the researchers for an expeditious search for new alternatives. Botanical-based insecticides were currently one of the most promising approaches, much research currently being devoted to plant extracts for the development of sustainable botanical insecticides.[8] Plant extracts contain a mixture of several chemical active ingredients and thus may be able to effectively kill the mosquito through a different mechanism.[9] For the past two decades, numerous researches have been conducted on the biological activity of plant extracts against larvae of mosquitoes and insects, in that few plant extracts were commercialized. This shows that plant extracts were environmentally safe, non- toxicity against humans and other organisms.[10]

Very few researches were published on the use of botanical insecticide against *Anopheles gambiae s.l.* larvae in 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 six plants leaves in malaria vector control in Couffo department in south-western Republic of Benin, West Africa.

MATERIAL AND METHODS

Study area

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 the six districts of this department (Fig.1). 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 six plants leaves in malaria vector control 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.

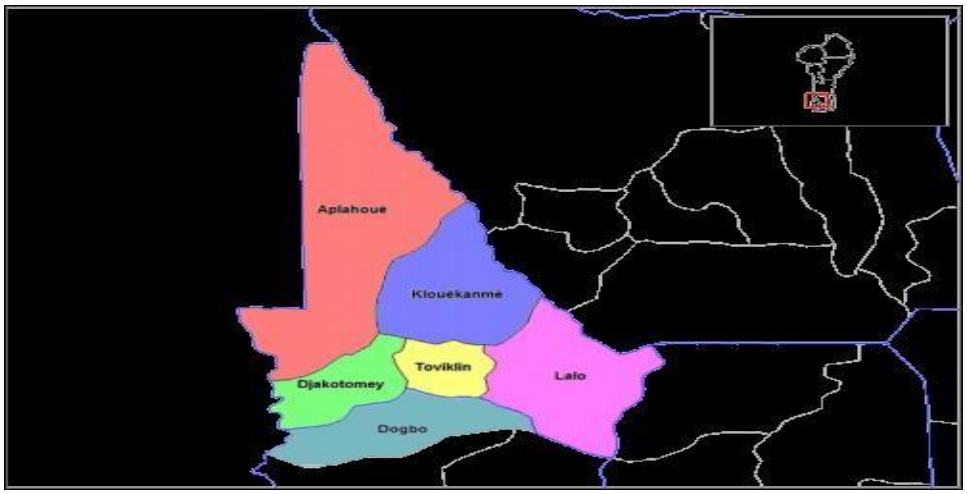

Fig. 1: Map of Republic of Benin showing the six districts surveyed in Couffo department.

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected from September to November 2023 during the small rainy season and from March to July 2024 during the great rainy season in the six districts of Couffo department. Larvae were collected from breeding sites using the dipping method[11] and kept in labeled bottles (Fig.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 (Fig.3).

Fig.2: Mosquito larvae collection in a breeding site.

Fig.3: Larvae in labeled plastics in insectary.

Collection of the plant leaves

The leaves of the six plants were collected in their predilection areas in Couffo department (Fig.4).

		
***Sida acuta* Burn F.**	***Eucalyptus globulus* Labill.**	***Anacardium* occidentale**
		
***Alium sativum* L.**	***Parkia biglobosa***	***Petroselinum crispum (Mill.)* Nyman**
Fig. 4: Trees which leaves were used.		

Plant leaves extraction

To prepare botanical insecticide of *Sida acuta* Burm F., *Eucalyptus globulus* Labill., *Anacardium* occidentale, *Allium sativum L.*, *Parkia biglobosa* and *Petroselinum crispum (Mill.*) Nyman leaves, we collected fresh green leaves of the six 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 and a 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.

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 containing 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 *Sida acuta* Burm F, *Eucalyptus globulus* Labill., *Anacardium* occidentale, *Allium sativum L., Parkia biglobosa* and *Petroselinum crispum (Mill.*) Nyman 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 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%.

RESULTS

Evaluation of larvicidal effect of ethanolic extract of *Sida acuta* Burm F. leaves on larvae of *Anopheles gambiae* s.l. from Dogbo district

The analysis of figure 5 showed that after the exposure of *Anopheles gambiae* s.l. larvae of four instars (L4) to ethanolic extract of *Sida acuta* Burm F. 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 with 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 recorded with the concentration of 5 mg/l (80 dead larvae on a total of 125 tested larvae).

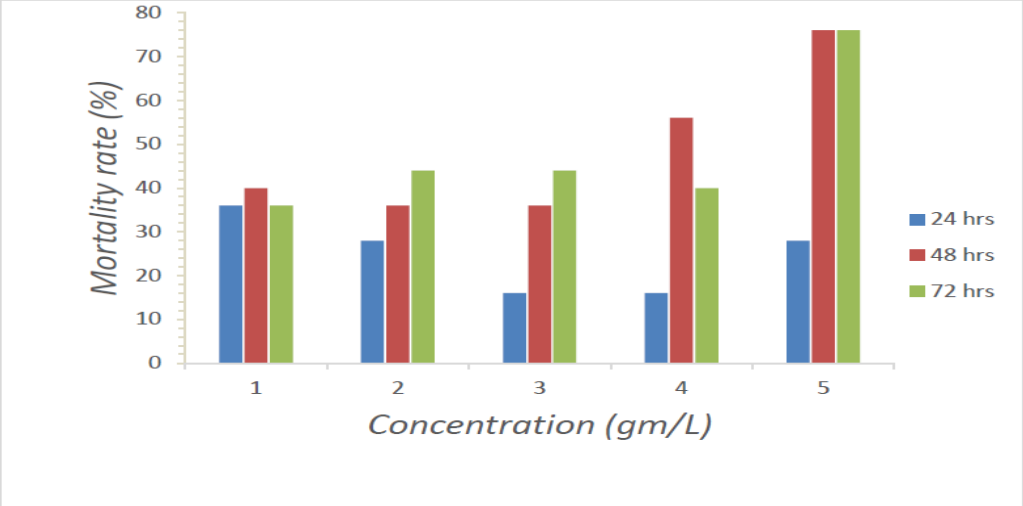

Fig.5: Larvicidal activity of *Sida acuta* Burm F. leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Eucalyptus globulus* Labill leaves on larvae of *Anopheles gambiae* s.l. from Aplahoué district

The analysis of figure 6 showed that after the exposure of *Anopheles gambiae s.l.* larvae of four instars (L4) to ethanolic extract of *Eucalyptus globulus* Labill 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 with 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 recorded with the concentration of 5 mg/l (45 dead larvae on a total of 125 tested larvae).

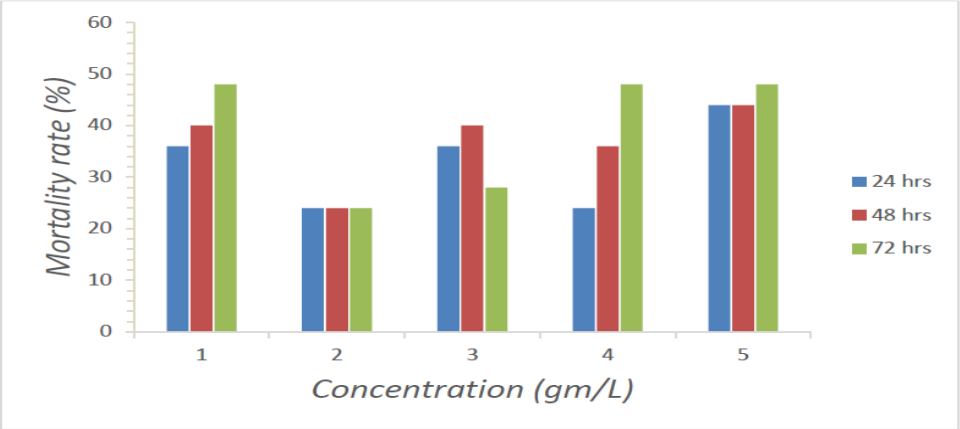

Fig.6: Larvicidal activity of *Eucalyptus globulus* Labill leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Anacardium* occidentale leaves on larvae of *Anopheles gambiae* s.l. from Djakotomey district

The analysis of figure 7 showed that after the exposure of *Anopheles gambiae s.l.* larvae of four instars (L4) to ethanolic extract of *Anacardium* occidentale 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 with 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 recorded with the concentration of 5 mg/l (40 dead larvae on a total of 125 tested larvae).

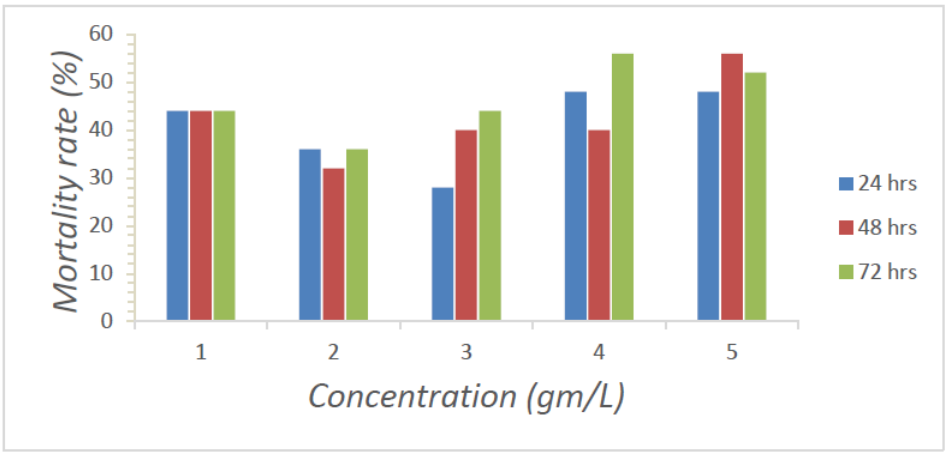

Fig.7: Larvicidal activity of *Anacardium* occidentale leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Allium sativum* L. leaves on larvae of *Anopheles gambiae* s.l. from Klouekanmè district

The analysis of figure 8 showed that after the exposure of *Anopheles gambiae s.l.* larvae of four instars (L4) to ethanolic extract of *Allium sativum* 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 a lot of 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 (P < 0,05). The recording of 48 hours exposure also showed that a lot of 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). In addition, all the tested

larvae with concentrations of 3 mg/l, 4mg/l and 5mg/l were dead. 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 concentrations of 3 mg/l, 4mg/l and 5mg/l (125 dead larvae on a total of 125 tested larvae).

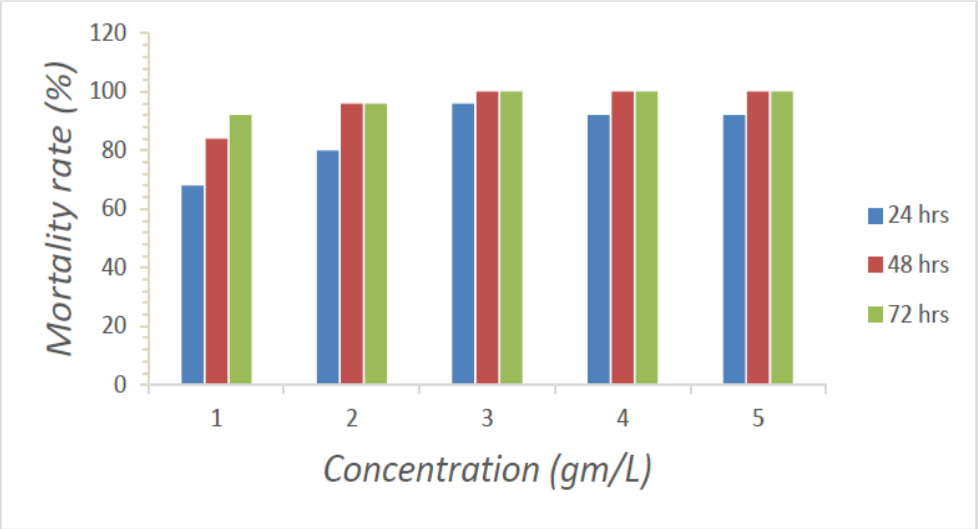

Fig.8: Larvicidal activity of *Allium sativum* L. leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Parkia biglobosa* leaves on larvae of *Anopheles gambiae* s.l. from Lalo district

The analysis of figure 9 showed that after the exposure of *Anopheles gambiae s.l.* larvae of four instars (L4) to ethanolic extract of *Parkia biglobosa* 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 with 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 recorded with the concentration of 4 mg/l (70 dead larvae on a total of 125 tested larvae).

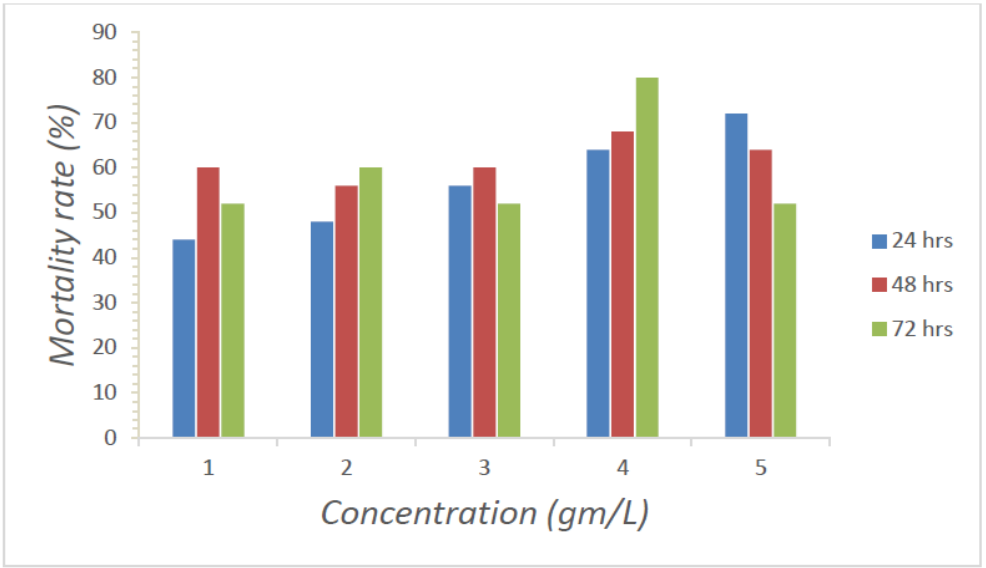

Fig.9: Larvicidal activity of *Parkia biglobosa* leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Petroselinum crispum (Mill.)* Nyman leaves on larvae of *Anopheles gambiae* s.l. from Toviklin district

The analysis of figure 10 showed that after the exposure of *Anopheles gambiae s.l.* larvae of four instars (L4) to ethanolic extract of *Petroselinum crispum (Mill.)* Nyman 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 with 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 recorded with the concentration of 3 mg/l (50 dead larvae on a total of 125 tested larvae).

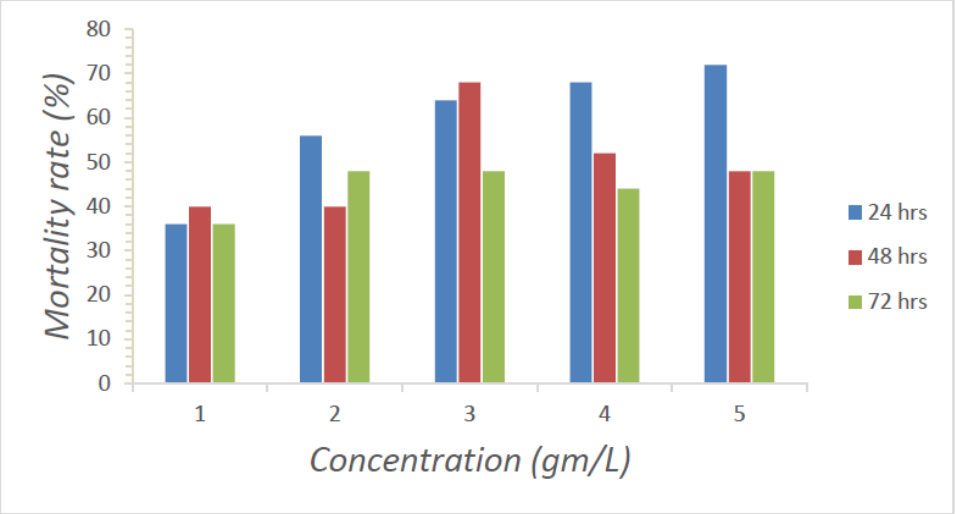

Fig.10: Larvicidal activity of *Petroselinum crispum (Mill.)* Nyman leaves on larvae of *Anopheles gambiae* s.l.

Comparison of larvicidal activities of ethanolic extract of six plants leaves in malaria vector control in Couffo department

The analysis of figure 11 showed that among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Allium sativum* L. leaves was the most effective against *Anopheles gambiae s.l.* larvae following by ethanolic extract of *Sida acuta* Burm F. leaves, following by ethanolic extract of *Parkia biglobosa* leaves, following by ethanolic extract of *Petroselinum crispum (Mill.)* Nyman leaves, following by ethanolic extract of *Eucalyptus globulus* Labill leaves and following by ethanolic extract of *Anacardium* occidentale leaves.

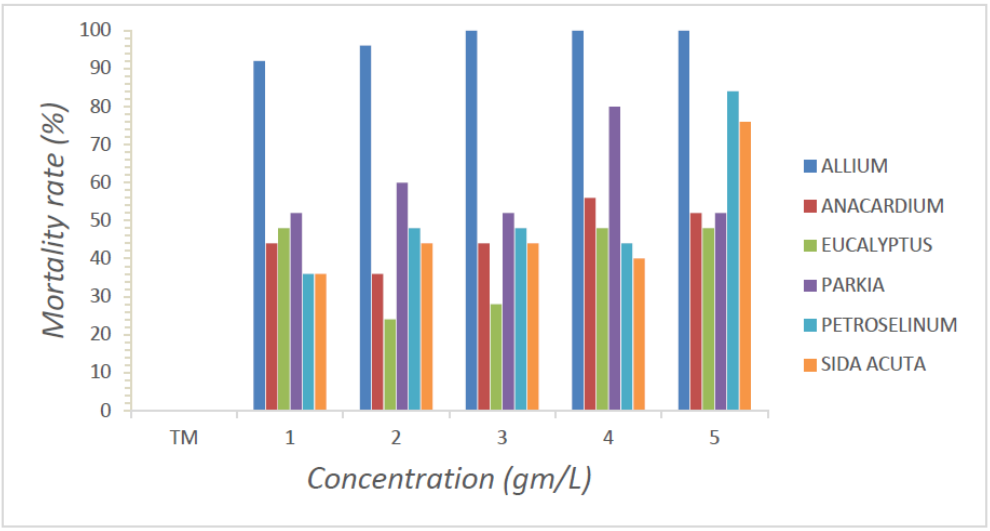

Fig.11: Larvicidal activity of the six plant leaves on larvae of *Anopheles gambiae* s.l.

DISCUSSION

Among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Allium sativum* L. leaves was the most effective against *Anopheles gambiae* sensu lato larvae of four instars (L4) collected in Couffo department. Despite that very few studies were published on the efficacy of ethanolic extract of *Allium sativum* L. leaves on larvae of *Anopheles gambiae* s.l., our results corroborated with those obtained by Kasim *et al.*[12] who demonstrated the potency of garlic (*Allium sativum*) in managing the larvae and thus contributes as an affordable way to control anopheles and culex larvae of mosquito in Nigeria. In their study, they had shown a remarkable effect of aqueous extract of garlic on the fourth instar of Anopheles and Culex mosquito larvae collected at Kofar Dundaye in Sokoto metropolis. They had also demonstrated that the mortality of Culex and Anopheles depends on time of exposure and concentration of the extract.

The efficacy of ethanolic extract of *Allium sativum* L. leaves was followed by that of *Sida acuta* Burm F. on larvae of *Anopheles gambiae s.l.* Despite that very few studies were published on the efficacy of ethanolic extract of *Sida acuta* Burm F. leaves on larvae of *Anopheles gambiae* s.l., our results corroborated with those obtained by Govindarajan[13] in india. This author had shown the efficacy of extract of *Sida acuta* Burm F. on larvae of third instar (L3) of three main mosquitoes, vectors of human disease which are: *Culex quinquefasciatus, Aedes aegypti* et *Anopheles stephensi.*

The efficacy of ethanolic extract of *Sida acuta* Burm F. leaves was followed by that of *Parkia biglobosa* on larvae of *Anopheles gambiae s.l.* As very few studies were still published on the efficacy of ethanolic extract of *Parkia biglobosa* on larvae of *Anopheles gambiae s.l.*, our results were not compared to eventual previous study.

The efficacy of ethanolic extract of *Parkia biglobosa* leaves was followed by that of *Petroselinum crispum (Mill.)* Nyman on larvae of *Anopheles gambiae s.l.* In the similar way, as very few studies were still published on the efficacy of ethanolic extract of *Petroselinum crispum (Mill.)* Nyman on larvae of *Anopheles gambiae s.l.*, our results were not compared to eventual previous study.

The efficacy of ethanolic extract of *Petroselinum crispum (Mill.)* Nyman leaves was followed by that of *Eucalyptus globulus* Labill on larvae of *Anopheles gambiae s.l.* Despite that very few studies were published on the efficacy of ethanolic extract of *Eucalyptus globulus* Labill leaves on larvae of *Anopheles gambiae* s.l., our results corroborated with those obtained by Grid and Hamaidi[14] in Algeria. These authors had shown that the aqueous extract of *Eucalyptus globulus* Labill was effective on *Culex pipiens* larvae collected in this country. Our results also corroborated with those obtained by Okbatinsae *et al*[15] who studied in vitro the larvicidal effects of some plant extracts against *Anopheles gambiae* larvae (Diptera: Culicidae) including *Eucalyptus globulus* Labill.

The efficacy of ethanolic extract of *Eucalyptus globulus* Labill leaves was followed by that of *Anacardium* occidentale on larvae of *Anopheles gambiae s.l.* Despite that very few studies were published on the efficacy of ethanolic extract of *Anacardium* occidentale leaves on larvae of *Anopheles gambiae* s.l., our results corroborated with those obtained by Nnamani *et al*[16] in Nigeria.

These authors had shown that the aqueous extract of *Anacardium* occidentale was effective on larvae of *Anopheles gambiae* collected in this country.

CONCLUSION

Among the six ethanolic extracts of plants leaves used in the current study, *Allium sativum* L. extract has the highest larvicidal activities. It 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. Researches must also be carried out in field conditions by treatment of mosquito larvae breeding sites with these ethanolic extracts of local plants leaves in a context where it is useful to search for alternative solutions to damages cause by chemical insecticides to environment and human health.

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CONFLICT OF INTEREST

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

REFERENCES

1. WHO. World Malaria Report 2020. Geneva. World Heath Organization, 2020.
2. WHO. World Malaria Report 2019. Geneva. World Heath Organizatio, 2019.
3. WHO. World Malaria Report 2018. Geneva. World Heath Organization, 2018.
4. Brown AW. Insecticide resistance in mosquitoes: a pragmatic review. J Am Mosq Control Assoc, 1986; 2: 123–40.
5. Russell TL, Kay BH, Skilleter GA. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. Aquat Biol, 2009; 6: 77–90.
6. Bhatt RP and Khanal SN. Environmental impact assessment system in Nepal - an overview of policy, legal instruments and process. Kathmandu Univ J Sci Enginnering Tech, 2009; 5: 160–70.

7. Anyaele OO and Amusan AAS. Toxicity of hexanoic extracts of *Dennettia tripetala* (G. Baxer) on larvae of *Aedes aegypti* (L.). Afr J Biomed Res, 2003; 6: 49–53.
8. Liang Y, Li Xiuwei, ZuminGu Peiwen Qin, Ji Mingshan. Toxicity of amorphigenin from the seeds of *Amorpha fruticosa* against the larvae of *Culex pipiens pallens* (Diptera: Culicidae). Molecules, 2015; 20: 3238–3254.
9. Pavela R. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. Parasitol Res, 2015; 114: 3835–3853.
10. Govindarajan M, Rajeswary M, Bhattacharyya A, Benelli G. Eugenol, apinene and ß- caryophyllene from *Plectranthus barbatus* essential oil as ecofriendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. Parasitol Res, 2016; 115(2): 807-815.
11. O'Malley C. Seven ways to a succesful dipping carrer. Wing beats, 1995; 6: 23-24.
12. Kasim M, Ann O, Yahaya MM. Larvicidal activity of garlic (*Allium sativum*) on anopheles and culex mosquito larvae. Pharmacology Online, 2019; 2: 130-138.
13. Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. Asian Pacific Journal of Tropical Medicine, 2010; 691- 695.
14. Grid et Hamaidi. Etude comparative de l'effet des extraits aqueux et des huiles essentielles de certaines plantes contre les larves de *Culex pipiens* (Diptera, Culicidae). Mémoire présenté en vue de l'obtention du Diplôme de Master. Faculté des Sciences de la Nature et de la Vie, Université des Frères Mentouri Constantine. République Algérienne Démocratique et Populaire. Soutenu le, 28 /06/2018, 70 pages.
15. Okbatinsae G and Haile A. In vitro studies of larvicidal effects of some plant extracts against *Anopheles gambiae* larvae (Diptera: Culicidae). Journal of medicinal plants research, 2017; 11(4): 66-72.
16. Nnamani CV, Osayi EE, Atama CI and Nwachukwu C. Larvicidal effects of leaf, bark and nutshell of *Anacardium* occidentale on the larvae of *Anopheles gambiae* in Ebonyi State, Nigeria. Animal Research International, 2011; 8(1): 1353-1358.