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Research Article

LARVICIDAL EFFECT OF ESSENTIAL OILS OF AROMATIC PLANTS OF CONGO-BRAZZAVILLE, AGAINST ANOPHELES GAMBIAE, A VECTOR OF MALARIA

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A B S T R A C T

Malaria is a disease, which poses huge problems for the people. One of the methods of fighting malaria is the elimination of the vector agent which is Anopheles. The use of synthetic insecticides has harmful consequences: pollution of the environment, intoxication of human, inefficiency due to the resistance of these mosquitoes against these products. One of the alternatives is the use of biological insecticides. In this work, we extracted essential oils from 10 plant species of Congo-Brazzaville: Chenopodiumambrosioides, Cyperus articulatus, Cyperusrotundus, Hyptissuaveolens, Guibourtia demeusei, Lippiamultiflora, Aframomumgigateum, Aframomumstipilatum, Zewgiberofficinale. These oils were evaluated for their larvicide activity on the Anopheles gambiae, a vector agent of malaria in Congo. The results obtained show the interestinglarvicide activity with the essential oils of Chenopodiumambrosioides, Guibourtiademeusei and Lippiamultiflora with 100% mortality, followed by Aframomumstipulatum (97.33%), Cyperusarticulatus (95, 00%), Zewgiber officinale (95.00%), Hyptissuaveolens (93.32%) of mortality at 0.2 g / L. Other essential oils were also active. Essential oils of Chenopodiumambrosioides (LC50 = 0.088 g / L), followed by Hyptissuaveolens (LC50 at 0.090 g / L), Guibourtiademeusei (LC50 = 0.098 g / L), Zewgiber of ficinale (LC50 = 0.11 g/L), were more active.

INTRODUCTION

Malaria remains one of the most troubling endemics in sub-Saharan Africa in general and in Congo in particular. It is caused by a Hemococcidae of the Plasmodium genus, transmitted to humans by a female Anopheles mosquito. Statistical data estimate that 445,000 deaths because of malaria in 2016, of which 81% are in Africa, (WHO, 2017). Children under 5 and pregnant women constitute the most vulnerable segment of the population (WHO, 2017). Despite many efforts by the scientific community to reduce the prevalence of malaria at its lowest level, the epidemic has been intensifying for several decades. This would be linked to economic problems (high costs of modern antimalarials, mosquito nets and insecticides) and especially to resistances of the plasmodium against antimalarials and the vector against using insecticides. Most vectors were resistant to Dieldrin, DDT, Permethrin, Deltamethrin and Lambda-cyhalothrin WHO (1992).

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Because of this resistance phenomenon, which has become a major obstacle in the prevention and the treatment of malaria, the use of natural substances with insecticidal properties, extracted from plants is very encouraged. In the African tradition, the using of plants as insecticide is very frequent. Plants are proving to be a potential source of new insecticides. Pyrethers, nicotine and rotenone from plants have been used as insect control agents. The aim of this study is assessed the larvicide effect of essential oils from 10 plant species of Congo-Brazzaville

MATERIAL AND METHOD

Animal Material

Floating larvae on the surface of stagnant water were collected by sieving with a plastic strainer and reintroduced into a plastic container with the stagnant water, then transported to the Faculté des Sciences et Techniques where they were washed. They were fed with cookies without cream 24 hours before exposure to essential oils.

Collection and Identification of Plants

The plant material was collected at Brazzaville for samples of *Cyperusrotundus* (Cyperaceae), *Cyperusesculentus* (Cyperaceae), *Hyptissuaveolens* (Limiaceae), *Aframomumgigateum* zingiberaceae), *Aframomumstipilatum* (Zingiberaceae)), *Zegimberofficinale* and atOwando in the department of Central-Cuvette in Congo for the samples of *Cyperusarticulatus* (Cyperaceae), *Chenopodiumambrosioides* (Chenopodiceae), *Guibourtiademeusei* (Fabaceae-Caesalpinioideae), *Lippiamultiflora* (Verbenaceae). Samplesweredried in the Laboratoire de l'unité de chimie du végétal et de la vie, Faculté des Sciences et Techniques, Université Marien Ngouabi, at room temperaturesafefrom sunlight. Botanical identification was done by professor Mountsamboté from National Herbarium.

Essential oils Extraction

Plants samples were dried and powdered using a mechanical grinder. A total of 1.5 kg of leaves of each dried plant specimen, for the samples of *Hyptissuaveolens, Aframomumgigateum, Aframomumstipilatum, Lippiamultiflora, Chénopodiumambrosioides* and 2 kg of rhizomes for samples of *Zwgiberofficinale, Cyperusrotundus, Cyperusarticulatus, Cyperusesculentus* and exudates of *Guibourtiademeusei* were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. The essential oil collected by decantation at the end of the distillation was dried over anhydrous sodium sulphate and then introduced into tightly sealed dark glass vials. Essential oils were kept in a refrigerator at a temperature of 4 ° C.

Realization of Larvicide Activity

A stock solution of each essential oil sample was prepared, by dissolving the essential oil in 2 mL of ethanol and then in water to have a concentration solution of 0.4 g / L, and the successive dilutions of Half gave solutions of concentration of 0.2 g / L, 0.1 g / L and 0.05 g / L and 80 mL of each solution were put in 3 plastic pots to have three tests of each concentration. In each pot, we put 25 *Anopheles gambiae* larvae in stage 4, we added 25g of uncooked cookies in each pot.

In the control pot, we put only 2 mL of ethanol and 25 g of uncooked cookies, all in 80 mL of water. The counting of dead larvae were done after 24 hours of exposure to essential oils. The immobile larvae were considered dead and the mortality percentage were calculated using the following formula:

$$\% \text{ of mortality} = \frac{\text{Number of ded larvae}}{\text{Number of exposure larvae}} \times 100$$

% of mortality = (Number of dead larvae) / (Number of exposed larvae) × 100

Figure 1 Picture of Mosquito larvae in culture in presence of increasing concentrations of essential oil

RESULTS AND DISCUSSION

Extraction of Essential oils

The essential oils, jumps those of Guibourtiademeusei, Aframomumgigateum, Aframomumstipilatum, Zegimber officinale colorless, are all of yellow-light color were obtained by hydrodistillation of the organs have yields yields as shown in Table I

Table I Extraction yields of essential oils

species	oilcolor	Organsused	Yield%
Chenopodiumambrosioides	Yellow	Leaf/Stem	0,73
Cyperusarticulatus	Yellow	Rhizomes	0,53
Cyperusrotundus	Yellow	Rhizomes	0,33
Cyperusesculentus	Yellow	Rhizomes	0,12
Hyptissuaveolens	Yellow	leaves	0,05
Guibourtiedemeusei	Colorless	exudates	0,21
Lippiamultiflora	Yellow	leaves	1,71
Aframomum gigateum	Colorless	leaves	1,20
Aframomum stipilatum	Colorless	leaves	1,20
Zegimber officinale	Colorless	Rhizomes	1,75

From all samples, it follows that the sample of *zegimberofficinale* is the sample having more essential oils with a yield of 1.75%, followed by the sample of *Lippiamultiflora* (1.71%), *Aframomumgiganteum* (1.20%) However, it was noted a difference in yield compared to the work of Mesléard *et al*. and Lavialle-Defaix C. *et al* which describe a yield of 0.9% instead of 0.73% for our extraction for *Chenopodiumambrosioides*. There are several reasons for this type of shift: the harvest period, the nature of the soil, the climate and even all manipulations before, during and after extraction.

Larvicidalactivity

Table II shows the percent mortality of Anopheles larvae after 24 hours of exposure to different concentrations of essential oils.

Table II Mortality of larvae exposed to increasing concentrations of oils and LC50

Concentration Samples	0,4 g/L	0,2 g/L	0,1 g/L	0,05 g/L	LC_{50}(g/L)
Chenopodiumambrosioides	100±00%	100±00%	60,0±5,3%	25,32±2,66%	0,088
Hyptissuaveolens	100±00%	93,32±8±%	58,67±1,77%	22,68±0,44%	0,090
Guibourtiademeusei	100±00%	100±00%	50,67±3,56%	22,68±1,77%	0,098
Lippiamultiflora	100±00%	100±00%	24,0±2,66%	9,32±0,44%	0,13
Cyperusarticulatus	100±00%	95±2,23%	37,33±1,77%	6,56±00%	0,13
Cyperusrotundus	100±00%	65,33±3%	21,33±5,3%	6,67±0,44%	0,17
Cyperusesculentus	100±00%	56,0±3%	17,30±3%	0±00%	0,12
Aframomum gigateum	88±2,66%	45±1,77%	5,33±5,3%	0±00%	>0,2
Aframomum stipulatum	100±00%	97,33±%	29,33±00%	8,0±00%	0,13
Zingiber officinale	100±00%	95,0±1,17%	45±2,66%	9,32±0,44%	0,11

The following figures show the shape of the larval mortality curves in function of concentration.

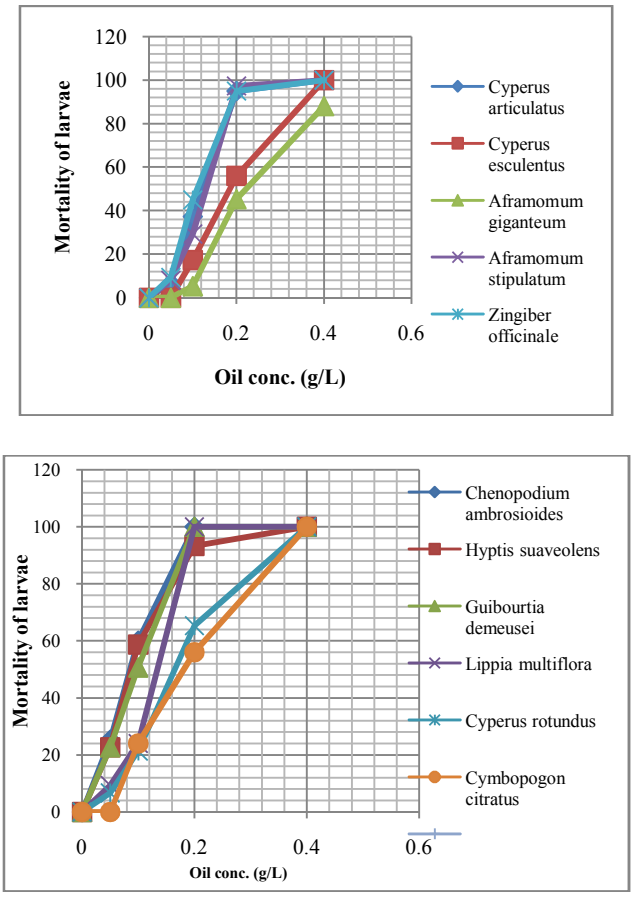

Figure1 Illustration of the larvicidal effect of essential oils after 24 hours of exposure

All these oils had a 100% of mortality percentage at the concentration of 0.4 g / L, except the essential oils of *Aframomumgiganteum*. At a concentration of 0.2 g / L, the essential oils of *Chenopodiumambrosioides, Guibourtiademeusei* and *Lippiamultiflora* showed a 100% of mortality, followed by *Aframomumstipulatum* (97.33%), *Cyperusarticulatus* (95.00%) , *Zingiberofficinale* (95.00%), *Hyptissuaveolens* (93.32%) of mortality at 0.2 g / L; other essential oils have been as active.

As for the lethal dose, these are the essential oils of *Chenopodiumambrosioides* with an LC50 = 0.088 g / L, followed by Hyptissuaveolens with an LC50= 0.090 g / L, *Guibourtiademeusei* with an LC50= 0.098 g / L, *Zegimberofficinale* =0 , 11 g / L, were more remarkable.

CONCLUSION AND PERSPECTIVES

We can say of this, that all these plants presented a mortality of 100% at the concentration of 0,4 g / L. These research results encourage the use of essential oils of Congolese plants as insecticides in vector control against malaria, and may contribute to the study of Congolese medicinal plants in these aspects relating to the recognition of traditional African medicine and pharmacopoeia.

In perspective, we intend to perform analysis by gas chromatography coupled with mass spectrometry, these essential oils, to allow us to understand the structures of molecules responsible activities.

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