UNIVERSITY OF YAOUNDE I
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UNIVERSITE DE YAOUNDE I

FACULTY OF SCIENCE
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FACULTE DES SCIENCES

DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES

Insecticidal effects of *Cupressus lusitanica* (Cupressaceae), *Ocimum basilicum* (Lumiaceae) and *Petroselinum crispum* (Apiaceae) leaves on the developmental stages of *Anopheles coluzzii* Coetzee & Wilkerson 2013 (Diptera: Culicidae) malaria vector

Thesis submitted in partial fulfillment of the requirements for the award of

Doctorate/Ph.D. degree in Biology of Animal Organisms

Option: Zoology

Specialty: Medical Entomology

By

TAMUNJOH Stella Shinwin Ateyim

Registration number: 12Q1333

M. Phil. Entomology

Co-supervisors:

FOKO DADJI Gisele Aurelie
Associate Professor

and

TAMESSE Joseph Lebel
Professor

Year 2023

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****
UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
****
B.P. 812 Yaoundé
Tél: (237) 242 23 95 84
Fax: (237) 242 23 44 96

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UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
****
P.O. BOX 812 Yaounde
Phone: (237) 242 23 95 84
Fax: (237) 242 23 44 96

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES
DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY

ATTESTATION DE CORRECTION

****

Nous soussignés, membres du jury de soutenance de la **Thèse de Doctorat/Ph.D** en **Biologie des Organismes Animaux**, Option : **Zoologie**, de Madame **TAMUNJOH Stella Shinwin Ateyim**, matricule **12Q1333,** soutenance autorisée par la correspondance N° 240804/UYI/VR-EPDTIC/DAAC/DAACA/DRD/SR/CB-ER du Recteur de l'Université de Yaoundé I en date du 10 avril 2024 sur le sujet intitulé : « **Insecticidal effects of Cupressus lusitanica (Cupressaceae), Ocimum basilicum (Lamiaceae) and Petroselinum crispum (Apiaceae) leaves on the developmental stages of *Anopheles coluzzii* Coetzee and Wilkerson, 2013 (Diptera : Culicidea) malaria vector»,** attestons que les corrections exigées au candidat lors de cette évaluation, qui a eu lieu le **mercredi 10 avril 2024** dans la **salle Multimédia** de la **Faculté des Sciences**, ont réellement été effectuées et que le présent document peut être déposé sous sa forme actuelle.

En foi de quoi, la présente attestation lui est délivrée pour servir et valoir ce que de droit.

Fait à Yaoundé, le 17/05/2024

Les Examinateurs

Le Chef de Département

Pr. Sévilor KEKEUNOU
Faculté des Sciences
Université de Yaoundé I

Le Président du Jury

Fomena Abraham
Professeur

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LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

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(Par Département et par Grade)
DATE D'ACTUALISATION 31 MAI 2023

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15	KIKI Maxime Armand	Chargé de Cours	En poste
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29	GUIDZAVAI KOUCHERE Albert	Assistant	En poste
30	MANN MANYOMBE Martin Luther	Assistant	En poste
31	MEFENZA NOUNTU Thiery	Assistant	En poste
32	NYOUMBI DLEUNA Christelle	Assistant	En poste
33	TENKEU JEUFACK Yannick Léa	Assistant	En poste

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11	LAMYE Glory MOH	Chargé de Cours	En poste
12	MEYIN A EBONG Solange	Chargé de Cours	En poste
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14	NKOUDOU ZE Nardis	Chargé de Cours	En poste
15	TAMATCHO KWEYANG Blandine Pulchérie	Chargé de Cours	En poste
16	TCHIKOUA Roger	Chargé de Cours	*Chef de Service de la Scolarité*
17	TOBOLBAÏ Richard	Chargé de Cours	En poste
18	NKOUE TONG Abraham	Assistant	En poste
19	SAKE NGANE Carole Stéphanie	Assistant	En poste
20	EZO'O MENGO Fabrice Télésfor	Assistant	En poste
21	EHETH Jean Samuel	Assistant	En poste
22	MAYI Marie Paule Audrey	Assistant	En poste
23	NGOUENAM Romial Joël	Assistant	En poste
24	NJAPNDOUNKE Bilkissou	Assistant	En poste

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Répartition chiffrée des Enseignants de la Faculté des Sciences de l'Université de Yaoundé I

NOMBRE D'ENSEIGNANTS					
DÉPARTEMENT	**Professeurs**	**Maîtres de Conférences**	**Chargés de Cours**	**Assistants**	**Total**
BCH	8 (01)	15 (11)	13 (03)	7 (05)	**43 (20)**
BPA	14 (01)	16 (09)	18 (04)	4 (02)	**52 (16)**
BPV	6 (01)	12 (02)	13 (07)	3 (00)	**34 (10)**
CI	7 (01)	15 (04)	5 (01)	1 (00)	**28 (06)**
CO	6 (01)	18 (04)	11 (04)	2 (00)	**37 (09)**
IN	2 (00)	2 (00)	14 (01)	4 (00)	**22 (01)**
MAT	1 (00)	8 (00)	17 (01)	7 (02)	**33 (03)**
MIB	2 (01)	7 (03)	8 (04)	7 (02)	**24 (10)**
PHY	15 (01)	15 (04)	11 (01)	2 (00)	**43 (06)**
ST	8 (00)	17 (03)	15 (04)	3 (01)	**43 (08)**
Total	**69 (07)**	**125 (40)**	**125 (30)**	**40 (12)**	**359 (89)**

Soit un total de **359 (89)** dont :

- Professeurs **69 (07)**
- Maîtres de Conférences **125 (40)**
- Chargés de Cours **125 (30)**
- Assistants **40 (12)**

( ) = Nombre de Femmes **89**

DEDICATION

This work is dedicated to my family, especially to my beloved husband, Tchoffo Benjamain, of blessed memory, who was very patient with my absence for several years and to my father Tamunjoh James Ateyim (of blessed memory) and mother Theresia Ngum who believed in the education of a female child when many were still in doubt and lastly to my kids and grand kids.

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SUMMARY

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LIST OF ABBREVIATIONS

An **-** *Anopheles*

BSV- Blood-stage vaccines

CSP- Circumsporozoite protein

CDC- Center for disease control and Prevention

DDVP- Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate

DDT- Dichloro-Diphenyl-Trichloroethane

DEET- N, N-diethyl-3-methylbenzamide

DEF- S-triuty phosphotrithioate

DEM- Diethyl maleate

DMSO - Dimethyl sulfoxide

EUAS – European Union for Animal Safety

HTTC- Higher Teachers Training College

IRS - Indoor residual spraying

ITNs - Insecticide treated nets

KD - Knockdown

LC_{50} - Lethal concentration causing 50% mortality

LD_{50} - Lethal dose causing 50% mortality

LLINs - Long-lasting insecticidal nets

MCE - Methanolic crude extract

NMCP – National Malaria Control Programme

OCEAC- Organization of Coordination for the Fight against Endemic Diseases in Central Africa

OECD - Organization for Economic Corporation and Development

PBO - Pieroyl butoacide

PEV- Pre-erythrocytic vaccines

Pfs - Post fertilization stage

SSM-TBV- Sexual-stage Mosquito-transmission-blocking vaccines

WHO - World Health Organization

ABSTRACT

The interest for plant-based insecticidal formulations is fast growing as alternative solutions to current synthetic insecticides which are associated with harmful effects on the environment and human beings. We have tested this hypothesis by assessing the potential deterrent effect of *Petroselinum crispum* (parsley), *Ocimum basilicum* (basil), and *Cupressus lusitanica* (cypress) formulations on different stages of the malaria vector, *Anopheles coluzzii*. Powder, methanolic extract and essential oil of these plants were phytochemically characterized using colorimetric test method and Gas Chromatography coupled with Mass Spectrometry (GC-MS). Invitro standard bioassays were performed with laboratory mosquito strain of *Anopheles coluzzii*, to evaluate larvicidal deterrence i.e., egg-hatching inhibition and larval mortality 24 hours post exposure, at 0.1g/mL, 0.3g/mL and 0.5 g/mL of plant powder, at 0.1, 0.3 and 0.5 mg/mL for methanol extract and at 0.01, 0.02 and 0.03 mg/mL for essential oil. The knockdown effects of plant extracts on adult mosquitoes were also recorded using the CDC bottle method. The acute toxicity of these formulations was tested on Wistar rats, dragonfly larvae and gambusia fish, and the potential repellent effect of a cream produced from the essential oil of *Petroselinum crispum* was also monitored in the field as preliminary assessment. The qualitative phytochemical analysis of plant powders and methanolic extracts revealed the presence of tannins, terpenoids and phenolic compounds in *Ocimum basilicum* and *Cupressus lusitanica* whereas saponin alkaloids and phenolic were the main compounds in *Petroselinum crispum*. Concerning the chemical composition of essential oils, myristicin was the main compound of *Petroselinum crispum* (67.1%), Linalool showed a proportion of 65.7% in *Ocimum basilicum* and (-)-4-Terpineol reached 12.7% in *Cupressus lusitanica*. *Cupressus lusitanica* powder-based formulation showed the most significant (H = 14.32; p =0.003) deterrent effect on the immature stages of development of *An. coluzzii* compared with *Petroselinum crispum* and *Ocimum basilicum*. The egg hatching inhibition induced by *Cupressus lusitanica* powder increased with concentrations from 43 to 83% (versus 23 to 60% by *P. crispum* and 14 to 30 by *O. basilicum*). The ranges of larval mortality followed similar tendency with 17-57%, 12-15% and 3-22% mortality rate for *Cupressus lusitanica*, *Petroselinum crispum* and *Ocimum basilicum*, respectively. The knockdown rates at lowest concentration were 65%, 35% and 44% for parsley, basil and cypress respectively in methanolic extract and 80%, 55%, 65% for the essential oil after 60 min exposure. Mortality rates after 24 hours were 46%, 33% and 38% for methanolic extract and 60, 40 and 45% for essential oil at the lowest concentration. The LC50 of parsley was 0.11 and 0.008 mg/mL/bottle for the methanolic extract and the essential oil respectively. *Petroselinum crispum* cream had a percentage reduction of 71.8%, 56.3% and

64% at Ngoa Kelle, Olezoa and Biyem Assi respectively. Plant products caused significant dose-dependent activity on immature stages and adults of *An. coluzzii*. Herbal products showed no signs of acute oral toxicity or dermal toxicity in Wistar rats at the maximum concentration of (0.1mg/mL). These products also showed low mortality on dragonfly larvae and gambusia fish in the laboratory. Thus, these plants could be a source of safe insecticidal substances, rich in active principles important for the preparation of traditional or refined commercial formulation of repellents.

Keywords: Mosquitoes, Mosquito activity, Repellent, Powder, Methanolic extracts, Essential oils.

RESUME

L'intérêt pour les formulations insecticides à base de plantes est en pleine croissance comme solution alternative aux insecticides de synthèse actuels qui sont associés à des effets nocifs sur l'environnement et les êtres humains. Nous avons testé cette hypothèse en évaluant l'effet dissuasif potentiel des formulations de *Petroselinum crispum* (persil), *Ocimum basilicum* (basilic) et *Cupressus lusitanica* (cyprès) sur différents stades du vecteur du paludisme, *Anopheles coluzzii*. La poudre, l'extrait méthanolique et l'huile essentielle de ces plantes ont été caractérisés phytochimiquement à l'aide de la méthode de test colorimétrique et de la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Des essais biologiques standard in vitro ont été effectués avec une souche de moustiques de laboratoire *d'Anopheles coluzzii*, pour évaluer la dissuasion larvicide, c'est-à-dire l'inhibition de l'éclosion des œufs et la mortalité larvaire 24 heures après l'exposition, à 0,1 g/mL, 0,3 g/mL et 0,5 g/mL de poudre de plante, à 0,1, 0,3 et 0,5 mg/mL pour l'extrait de au méthanol et à 0,01, 0,02 et 0,03 mg/mL pour l'huile essentielle. Les effets de choc des extraits de plantes sur les moustiques adultes ont également été évalués à l'aide de la méthode de la bouteille CDC. La toxicité aiguë de ces formulations a été testée sur des rats Wistar, des larves de libellules et des poissons gambusia, et l'effet répulsif potentiel d'une crème produite à partir de l'huile essentielle de *Petroselinum crispum* a également été suivi sur le terrain en tant qu'évaluation préliminaire. L'analyse phytochimique qualitative des poudres végétales et des extraits méthanoliques a révélé la présence de tanins, de terpénoïdes et de composés phénoliques chez *Ocimum basilicum* et *Cupressus lusitanica* alors que les alcaloïdes saponiques et phénoliques étaient les principaux composés chez *Petroselinum crispum*. Concernant la composition chimique des huiles essentielles, la myristicine était le principal composé de *Petroselinum crispum* (67,1%), le linalol présentait une proportion de 65,7% dans *Ocimum basilicum* et le (-)-4-terpinéol atteignait 12,7% dans *Cupressus lusitanica*. La formulation à base de poudre de *Cupressus lusitanica* a montré l'effet dissuasif le plus significatif ($H = 14,32$; $p = 0,003$) sur les stades immatures de développement *d'An. coluzzii* par rapport à *Petroselinum crispum* et *Ocimum basilicum*. L'inhibition de l'éclosion des œufs induite par la poudre de *Cupressus lusitanica* augmente avec des concentrations de 43 à 83 % (contre 23 à 60 % par *P. crispum* et 14 à 30 par *O. basilicum*). Les fourchettes de mortalité larvaire ont suivi une tendance similaire avec un taux de mortalité de 17-57%, 12-15% et 3-22% pour *Cupressus lusitanica*, *Petroselinum crispum* et *Ocimum basilicum*, respectivement. Les taux de knockdown à la concentration la plus faible étaient de 65 %, 35 % et 44 % pour le persil, le basilic et le cyprès respectivement dans l'extrait méthanolique et de 80 %, 55 %, 65 % pour l'huile essentielle après 60 min

d'exposition. Les taux de mortalité après 24 heures étaient de 46 %, 33 % et 38 % pour l'extrait méthanolique et de 60, 40 et 45 % pour l'huile essentielle à la plus faible concentration. La CL50 du persil était de 0,11 et 0,008 mg/mL/bouteille pour l'extrait méthanolique et l'huile essentielle respectivement. La crème à base de *Petroselinum crispum* a eu un pourcentage de réduction de 71,8%, 56,3% et 64% à Ngoa EKelle, Olezoa et Biyem Assi respectivement. Les produits végétaux ont provoqué une activité dose-dépendante significative sur les stades immatures et les adultes *d'An. coluzzii*. Les produits à base de plantes n'ont montré aucun signe de toxicité orale aiguë ou de toxicité cutanée chez les rats Wistar à la concentration maximale de (0,1 mg/mL). Ces produits ont également montré une faible mortalité sur les larves de libellules et les poissons gambusia en laboratoire. Ainsi, ces plantes pourraient être une source de substances insecticides sûres, riches en principes actifs importants pour la préparation de formulations commerciales traditionnelles ou raffinées de répulsifs.

Mots-clés : Moustiques, Activité moustique, Répulsion, Poudre, Extraits méthanoliques, Huiles essentielles.

INTRODUCTION

Malaria remains a major public health problem despite the various means implemented to fight this pandemic. Globally, 249 million people were infected with the disease with 608,000 deaths recorded in 2023, 94% of global cases and 95% of the total deaths are from Africa (WHO, 2024). The rapid growth of Africa's population of around 2-6% per year in recent years has affected the epidemiology of vector-borne diseases (WHO, 2018). Africa remains the most affected continent with the highest number of the disease cases and deaths (WHO, 2024). Thus, malaria is increasingly becoming an important public health problem. In Cameroon, more than 3 million cases of malaria are recorded annually and more than 2,481 deaths in hospital facilities (NMCP, 2023). The level of malaria transmission is quite high estimated at more than 33 infectious bites per man per year due almost exclusively to *An. gambiae sl* (Van-Der-Kolk *et al*., 2003, Tchuinkam *et al.,* 2010; Doumbe-Belisse *et al.,* 2018; Djamouko-Djonkam *et al.,* 2020). Various factors can justify this state of affairs: in particular, the anarchic and uncontrolled occupation of land, the exploitation of marshy lowlands for the construction of houses and agricultural practices, the absence of cleaning of gutters and ditches or the dumping of household garbage in the riverbeds which block the circulation of water and create pools of water favorable to the development of mosquitoes (Antonio-Nkondjio *et al.*, 2017). All these practices increase the multiplication of vectors and consequently human-vector contact; thus, posing increasing challenges for mosquito control (Knudsen and Slooff, 1992). To control this disease, the World Health Organization (WHO) in 2012 recommended the implementation of an integrated fight for the control of malaria, in order to preserve the effectiveness of the first-line measures currently used, which include the long-lasting insecticide treated net (LLIN) and indoor residual spraying (IRS) (WHO, 2012, 2013; Gueye, 2012). Larval control can be used in addition to the existing measures because it targets the pre-mature stages (Bousema *et al*., 2017). It has the advantage that it reduces the density of mosquitoes that bite inside and outside homes, manages the resistance of anopheles to insecticides and is also effective for control in areas where the mosquitoes exhibit heterogeneous behaviour.

Malaria control programme in Cameroon is based on: i) the case management with affordable diagnostic tools and curative treatment with artemisin based combinations, and ii) the prevention strategy with the universal coverage of long-lasting insecticide treated net (LLIN) and indoor sprays. These strategies have contributed to the decrease of disease burden across the country. However, additional control measures are necessary for boosting vector management toward the global objective of malaria elimination by 2030. To address this challenge, new or alternative control measures need to be developed against parasites and

vectors. The most realistic and short-term reachable malaria control approach is vector control for which various insecticidal candidates are under investigation due to their potential anti-mosquito effects.

The emergence of pyrethroid/DDT which causes resistance in local populations of *An. gambiae sl*, not biodegradable and toxic to the environment has caused researchers to look for alternatives (Antonio-Nkondjio *et al.*, 2012; Étang *et al*., al., 2016; Awono-Ambene *et al.*, 2018). Plants may be a source of alternative agent to replace synthetic insecticides for mosquito control because botanicals are environmentally safer, more biodegradable and more targets specific (Dua *et al*., 2010). More than 1,200 plant species are having potential insecticidal value (Roark 1947). Sukumar *et al*., (1991) discussed 344 plant species that exhibited mosquitocidal activity. The most promising botanical groups are of the families: Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiaceae, Aristolochiaceae and Malvaceae which have provided numerous beneficial principles ranging from pharmaceuticals to insecticides (Kamalakannan *et al*., 2010). Some plants species, such as *Annona squamosa* L, *Gloriosa superba* L., *Millingtonia hortensis, Abuta grandifolia, Minthostachys setose, Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolen* have been reported to effectively control mosquito populations (Okigbo *et al.*, 2010). Previous studies carried out in Cameroon especially in the University of Yaounde1using plant extracts such as *Capsicum annuum, Piper nigum, Zingiber officinale, Clausena anisata, Aframomum sulcatum, Cannabis sativa*… on mosquito control have been effective (Foko *et al* 2007, 2011, 2016, 2018; Abe *et al* 2019). Natural products like pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene, camphor and turpentine are among some important phytochemical insecticides widely used in developed countries (Wood 2003).

Parsley is a species of herbaceous plant of the family Apiaceae. It is commonly used in cooking for its divided leaves, and in Central Europe for its taproot. It is also a medicinal plant with a scientific name *Petroselinum crispum*. Parsley is a biennial plant 25-80 cm high, very aromatic when crumpled, with a characteristic smell. The stems are ridged and the leaves are glabrous. *P. crispum* essential oil has been used for control *Aedes egypti* (Jitrawadee *et al.*, 2019).

Basil has about 150 species of herbaceous or bushy, annual or perennial, generally aromatic plants, the best known of which is the common basil. Basil can also help with relaxation and mental calming. It is also used to help strengthen the body's resistance and stimulate natural defenses. Oils from some *Ocimum* spp. have been shown to repel insects and

have larvicidal activity against houseflies, blue bottle flies, and mosquitoes (Abo-Elseoud MA *et al.,* 2005). Repellency against the adult females of *Culex pipiens* was observed by using essential oils extracted from the dried foliage of *O. basilicum* (Sienkiewicz M *et al.*, 2013).

According to Katende *et al.*, 1995), *Cupressus lusitanica* is an evergreen fast-growing tree. It has a straight trunk, generally conical but irregular in shape, branches hang down with branchlets in all directions and grow up to 35m high. The bark is red-brown with vertical grooves which turn grey with age. It is the Portuguese cypress or Mexican cypress, a native to Central America, grown as an ornamental tree in parks and large gardens. The specific name, lusitanica, was given to it by British botanist Philip Miller who described it in 1768. The essential oil from the leaves of this plant is usually used for store products (Philip *et al.*, 2016)

Nonetheless, no study has been reported on the insecticidal efficacy of the powder, methanolic extracts or essential oils of *C. lusitanica, O. basilicum* and *P. crispum* against *Anopheles coluzzii*. The choice of these three plant species considered in the present study was based mainly on their insecticidal effects against the eggs, larvae, pupae and adults of mosquitoes. Other reasons include information about the plants from the inhabitants, their abundant availability in the North West Region of Cameroon and their accessibility by local populations.

The reseach question of this work was to find out whether *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* extracts could be used in the control of *Anopheles coluzzii* at different developmental stages

The impact of plant extracts on the development of *Anopheles* coluzzii was reviewed through these hypothesis: :(1) Parsley (*Petroselinum crispum*), basil (*Ocimum basilicum),* and cypress (*Cupressus lusitanica*) leaves contain many phytochemical components; (2) Extracts from parsley (*Petroselinum crispum*), basil (*Ocimum basilicum),* and cypress (*Cupressus lusitanica*) have effects on the developmental stages of *Anopheles coluzzii*; (3) Plant extracts have no toxic effect on non-target organisms; (4) *Petroselinum crispum* cream has a significant repellent effect on adult mosquitoes

Main objective:

The main objective of this work was to investigate the insecticidal effects of three local plants; parsley (*Petroselinum crispum*), basil (*Ocimum basilicum),* and cypress (*Cupressus lusitanica*) against the developmetal stages of *An. coluzzii*.

The specific objectives were to:

1. Evaluate the phytochemical constituents of *Petroselinum crispum, Ocimum basilicum* and *Cupressus lusitanica* extracts;
2. Asses the effects of *Petroselinum crispum, Ocimum basilicum* and *Cupressus lusitanica* extracts on the different developmental stages of *Anopheles coluzzii;*
3. Determine the toxic effect of *Petroselinum crispum, Ocimum basilicum* and *Cupressus lusitanica* extracts on non-target organisms (Wister rats, dragonfly and fish);
4. Assess the repellent effect of parsley cream against adults mosquitoes in the field;

This thesis is organized around three main parts: the first part begins by an introduction which presents the knowledge gaps, the problem and the hypothesis to be tested, then ends with a broad literature review that brings together the information important to situate the problem of the subject of study. The second part describes the material and the methods used. Finally, the third part presents the obtained results and their discussion and ends with a conclusion, recommendations and perspectives.

CHAPTER 1: LITERATURE REVIEW

I.1 The overview of malaria

I.1.1: Historical trends

Malaria is an infectious disease caused by a single-celled parasitic protozoan of the genus *Plasmodium,* which spends its life cycle both in humans and certain species of mosquitoes. Under natural condition, malaria is transmitted by female mosquitoes of the genus *Anopheles* (Macdonald *et al*., 1957, Institute Pasteur 2019). The parasites live mainly intracellular multiplying many times, first in the liver and then in infected red blood cells. This leads to the destruction of red blood cells and consumption of haemoglobin, with consequent bouts of fever, followed by cyclically recurring severe sweating (Gullan and Cranston, 1999). Malaria is a mosquito-borne infectious disease of humans and other animals caused by a parasite of the genus *Plasmodium*. The *Plasmodium* parasites are transmitted through a bite from an infested female mosquito of the genus *Anopheles* which introduces the parasite via its saliva into the circulatory system and ultimately to the liver where they mature and reproduce. The disease causes symptoms that typically include fever and headache which in severe cases can progress to coma and death.

Malaria has been documented as far as latitude 64ºN (Archangel, USSR), and latitude 32º S (Cordoba, Argentina), occurring as a focal disease in these areas (Bruce-Chwatt, 1985). According to the latest World malaria report, there were 241 million cases of malaria in 2020 compared to 229 million cases in 2019. The estimated number of malaria deaths stood at 602 000 in 2020 – an increase of 69 000 deaths over the previous year. In this report, 95% cases and 96% deaths occurred in the African Region (WHO, 2021). Malaria is widespread in the tropical and sub-tropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia and America. According to World malaria report released on 30 November 2020, there were 229 million cases of malaria in 2019 compared to 228 million cases in 2018. The estimated number of malaria deaths stood at 602 000 in 2021, compared with 411 000 deaths in 2018. The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of all malaria cases and deaths (WHO, 2020).

Over the last 5 years, the use of preventive measures (LLIN, IRS and chemoprophylaxis) of this disease have increased significantly. For example, about 53% of the population at risk slept under a treated net compared to 30% in 2010. These interventions have contributed to significantly reduce the global disease incidence and mortality rates by 29% and 31%,

respectively, in African Region between 2010 and 2015; other regions have also achieved impressive reductions in their malaria burden (WHO, 2016). Since 2010, the malaria mortality rate has declined by 58% in the Western Pacific Region, by 46% in the South-East Asia Region, by 37% in the Region of the Americas and by 6% in the Eastern Mediterranean Region. In 2015, the European Region was malaria-free: all 53 countries in the region reported at least 1 year of zero locally-acquired cases of malaria (WHO, 2016).

In Cameroon, Malaria still has a devastating impact on public health and welfare with the whole country exposed to the risk of transmission. It is the most prevalent parasitic disease and the leading cause of morbidity and mortality in all age groups (Antonio-Nkondjio *et al*., 2019). Although significant progress has been made in the recent past, the disease remains prevalent with a high number of suspected cases in health care facilities varying between 3.3–3.7 million per year. The climates in Cameroon with a long rainy season or two rainy seasons in some areas provide ideal conditions for the vector throughout the year (Asare, E. O., and Amekudzi, L. K. (2017). This disease imposes a serious obstacle to social and economic development in the country.

I.1.2. Symptoms

Malaria is an acute febrile illness. In a non-immune individual, symptoms usually appear 10–15 days after the infective mosquito bite. The first symptoms – fever, headache, and chills – may be mild and difficult to recognize as malaria. If the disease is not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death. Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria (WHO, 2016). In adults, multi-organ failure is frequent. In malaria endemic areas, people may develop partial immunity, allowing asymptomatic infections to occur. A fever 8 to 30 days after infection may or may not be accompanied by headache, muscle pain, weakness, vomiting, diarrhea, and cough. Typical cycles alternating fever, tremors with cold and intense sweating, can occur: it is known as "malaria attack". The periodicity of these cycles depends on the species of parasite involved, and coincides with the multiplication of the parasites and the bursting of red blood cells, which also leads to anemia. Malaria caused by *P. falciparum* can be fatal if left untreated. In some cases, infected red blood cells can block blood vessels supplying the brain leading to cerebral malaria, which is often fatal. In areas where malaria is highly endemic, part of the population is an asymptomatic carrier. After many years of chronic infection with the parasite, some individuals tolerate its presence and develop natural immunity ("acquired immunity").

I.1.3. Epidemiology

Malaria affects around 100 countries around the world, particularly the less favoured tropical

Regions of Africa, Asia and Latin America. Africa is by far the most affected continent with 90% of malaria cases recorded in its tropical areas (WHO, 2016). Epidemics can occur during movements of populations with little exposure to malaria to highly endemic areas. Europe is experiencing so-called imported cases of malaria. In France, in 2011, 3,560 imported cases were reported (Institute Pasteur, 2019).

I.1.4. Transmission

Malaria is transmitted to humans by the bite of a female mosquito, of the genus *Anopheles*, itself infected after having bitten someone suffering from malaria. The female, by taking the blood meal necessary for her laying, injects the parasite into his host. Male mosquitoes do not bite. The transmission of Plasmodium from one man to another is therefore via the mosquito, the main cause being *Anopheles gambiae* on the African continent and *An. coluzzii* (Antonio-Nkondjio *et al.*, 2012). There is only one case of direct human-to-human contamination, when an infected pregnant woman infects her child through the placenta (WHO, 2018).

I.1.5. Prevention and treatment

There are several anti-malaria molecules that can be used in prophylaxis (prevention during travel in endemic areas) or in therapy. The best known are chloroquine or quinine (WHO, 2000). Others, like mefloquine, are used in areas where parasites that are resistant to chloroquine live. It is dangerous to travel to an area of intense malaria transmission without taking regular preventive treatment, especially for children and pregnant women who are at increased risk of severe disease. Preventive treatment should be prescribed by a doctor. It takes into account the areas visited (risk, existence or not of resistance), the duration of the trip and also the person including; age, pathological history, intolerance to antimalarial drugs, a possible drug interaction and pregnancy (WHO, 2001). Anti-malaria drugs do not guarantee absolute protection against infection, so it is important to protect yourself from mosquito bites (mosquito nets, anti-mosquito products). No preventive measure alone provides total protection and, even if the right treatment has been taken correctly, it is possible to have an attack of malaria, sometimes of late onset (WHO, 2016). The first symptoms are often not very alarming, but malaria can be fatal if treatment is delayed. Also, in the event of even slight fever, nausea,

headaches, aches or fatigue during the stay or in the months following the return from an endemic area, a doctor must be consulted urgently (WHO, 2000). Taking a blood sample is necessary to confirm the diagnosis. Any fever in someone returning from the tropics should be considered a priority as malaria until proven otherwise.

I.1.6. Control

I.1.6.1. Chemotherapy and pharmacoresistance

In anti-malaria fight, treatment of patients is primordial. There are two main aspects in the treatment of malaria as far as chemotherapy is concerned; the reduction of parasites in patients (Drakeley *et al*., 2006) and the treatment of complications of infection (CDC, 2010). These treatments as well as prophylaxis have for the past years based on the use of chloroquine and quinine. However, due to the abusive consumption of chloroquine, *Plasmodium falciparum* has developed resistance to this drug. This led high increase of malaria in the end of the year 1980 with indices ranging between 25% and 40%. Nevertheless, chloroquine remains one of the anti-malaria drugs that are used in malaria endemic regions of Afirica (Trape, 2001). The resistance of *P. falciparum* is due to gene mutation at the level of the digestive vacuole. This mutation caused resistance of about 40% in Uganda (Kamya *et al*., 2002), 62% in Ghana (Koran *et al*., 2005), and 50% in Cameroon (Antonio-Nkondjio · 2019). New molecules that were recommended included: artemether-lumifantrine (coarterm), artesonate-mefloquine, artesonate-amodiaquine and artesonate-sulfadoxine (WHO, 2001, Foko Dadji *et al.*, 2007). Chemoprophylaxis is an effective tool for malaria control in endemic areas especially for young children and pregnant women. Chloroquine was first used for this purpose in endemic regions but it has now become ineffective (MPH, 2009). Over recent years many antimalarial drugs have been rendered useless by the development of resistance by the malaria parasite. New antimalarial are rapidly suffering the same fate as the traditional therapies but new methods are now being discovered.

I.1.6.2. Use of vaccines against malaria parasite

Malaria vaccines may provide a wide range of benefits: providing personal protection from infection and episodes of clinical malaria to vaccinated individuals; reducing population level transmission in a community, and achieving and sustaining elimination in areas of low transmission. Malaria vaccine candidates have conventionally been classified according to the stage of the life-cycle targeted. Pre-erythrocytic Vaccines (PEV) target sporozoites and hepatic forms in the liver, potentially providing protection from infection. Blood-stage Vaccines (BSV)

target merozoites and infected red blood cells, preventing episodes of symptomatic clinical malaria and helping to clear blood-stage infections. Sexual-stage Mosquito-transmission-blocking vaccines (SSM-TBV) target the sexual stages of the Plasmodium parasite in the human or mosquito preventing onwards transmission but not necessarily providing direct protection to the vaccinated individual. Both PEVs and SSM-TBVs are a major focus of current research efforts. PEVs and SSM-TBVs are likely to have similar effects on a population level, causing reductions in transmission in the community. However, on an individual level, it will be possible to measure the effect of PEVs, but not the effect of SSM-TBVs which do not provide direct protection to vaccinated individuals. A number of candidate *P. falciparum* PEVs are currently under development based either on sub-unit approaches where vaccination induces immune responses to targeted antigens, or whole parasite approaches where exposure to attenuated sporozoites may induce strong, broad-spectrum immune responses (Trieu *et al*., 2011). Today, no vaccine is available to fight malaria. At the Institute Pasteur, several teams are working on developing vaccines against malaria and several candidates are under study (WHO, 2018).

I.2. Biology of vector

Mosquitoes belong to the family Culicidae, and are the most prominent of the numerous species of blood sucking arthropods that feed on human and other warm-blooded animals. They are most found within the tropical and subtropical regions of the world (Reiter, 2001). Mosquitoes are semi-aquatic insects since the egg, larval and pupal stages breed in water and the adult stage is terrestrial (White, 2004). They are considered as the most medically significant vectors, since they have harmed human and disrupted societies over the millennia and continue to have socio-economic and devastating impacts on human beings (Guzman *et al* 2010; Kala and Senthilkumar 2010; Kovendan and Murugan 2011; Otranto *et al*. 2013). The species best known for their impacts on human health are primarily in the genera of *Anopheles*, *Aedes* and *Culex*, with each species having its own particular life story, habitat preference, and dispersal ability (Mazzacano and Black 2013). The female mosquitoes require blood meal to complete their oogenesis and during blood feeding, they may transmit the causative agents of harmful diseases, such as malaria, chikungunya, dengue fever, yellow fever, West Nile fever, lymphatic filariasis, etc. (Das and Ansari 2003). Among several mosquito genera, there is the *Anopheles* genus which comprises vectors of the pathogen causing malaria in humans. In Africa, the *Anopheles gambiae* species is the major vector of human malaria parasites and in Cameroon *Anopheles colozzi*i is the major malaria vector (Atonio-Nkondjio *et al*., 2012).

Like all insects, adult anophelines have slender bodies with 3 sections: head, thorax and abdomen. The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odour as well as odour of breeding sites where females lay eggs. The head also has an elongated, forward-projecting proboscis (piercing-sucking mouth parts) used for feeding, and two sensory palps. The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. There are more than 400 different species of *Anopheles* mosquito; around 30 are malaria vectors of major importance (Lehane 2005**)**. Most of the important vector species bite between dusk and dawn. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment (WHO, 2020). The anopheline female mosquito is the only vector of human malaria. It may also transmit filariasis and some viral diseases (Temu *et al*., 2012).

In Africa, the most important vectors of human malaria are *Anopheles gambiae* s.s, *An. arabiensis* and *An. funestus*. But *An. moucheti*, *An. nili*, and *An. hancocki* are secondary vectors (Antonio-Nkondjio *et al*., 2011). Earlier studies on the bionomics of *Anopheles* in Cameroon carried out some years ago demonstrated that the principal vectors of malaria were: *Anopheles gambiae* s.l, *An. funestus* which are cosmopolitan; *An. arabiensis* Patton in the northern parts of Cameroon where malaria is unstable; *An. nili* Theobald in borders of rivers and streams (Carnevale *et al*., 1992) and *An. moucheti* Evans in forest areas (Njan Nloga *et al*., 1993). Some vectors considered as minor take a locally active part in malaria transmission. *An. melas* Theobald is probably a good vector in the coastal region. *An. paludis* Theobald, *An. pharoensis* Theobald, and *An. hancocki* Edwards have been found infected by *Plasmodium falciparum* Welch in Cameroon (Fontenille *et al.,* 2000). *An. wellcomei* Theobald and *An. marshallii* Theobald might occasionally transmit malaria to humans. In the mount Cameroon region, studies carried out revealed there is a high density of *An. gambiae* complex, *An. funestus* and *An. hancocki*, which vary with altitude (Wanji *et al*., 2003). Recent studies have shown that the main malaria vector in Cameroon is *An. coluzzii* (Antonio-Nkondjio *et al*., 2011).

I.2.1.: Geographical distribution of Anopheles

There are about 528 known species of anopheles in the world (Harbach *et al.,* 2007). Among these, about 20 species have at least a complex each. On a global scale, the nations of the Americas benefit from having the lowest *Plasmodium* morbidity, with stable risk areas typically having low levels of endemicity (Harbach *et al*., 2007). Such reduced levels of malaria transmission coupled with continuing reports of decreasing mortality and morbidity for all

major *Plasmodium* species across the region (e.g., between 2000 and 2007) (WHO, 2011). This has been credited to an increasing use of integrated vector control (WHO, 2011). Integrated vector control/management relies on a number of factors, but foremost as given in the World Health Organization, strategic framework for integrated vector management (Shaalan *et al.,* 2005) is the 'selection of proven vector control methods based on knowledge of local vector biology and ecology, disease transmission and morbidity, essentially knowing which vector species is present and understanding how it behaves.

I.2.1.1. *Anopheles gambiae complex*

Anopheles gambiae Giles is the most efficient malaria vector in the Afro-tropical region thus commonly called the African malaria mosquito. The *An. gambiae* complex of sibling species (White, 1974; Coetzee *et al*., 2013) comprises eight reproductively isolated species that are almost indistinguishable morphologically and largely sympatric (geographically co-existing) species (White, 1974; Ayala and Coluzzii 2005; Hunt *et al*., 1998). This species complex comprises four fresh-water breeding species (*An. arabiensis,* Patton, 1904; *An. coluzzii,* Coetzee *et al.,* 2013; *An. gambiae* Giles, 1902 and *An. quadriannulatus* Theobald, 1903). These species love breeding sites represented by sunny and clean water pools devoid of vegetation particularly man-made breeding sites, like wells dogged to prepare mud bricks, small water pools created by cattle footprints or grooves tracked by vehicle tires (etc…). These mosquitoes are extremely well adapted to human settings, as witnessed by their remarkable anthropophily and endophily, which make them efficient malaria vectors. Also are two salt-water breeders *An. melas* Theobald, 1903 in West Africa and *An. merus* Dönitz, 1902 in East Africa. Both species are mostly zoophilic but also bite humans in the absence of animals (Coetzee *et al*., 2000). A seventh member, the halophilic *An. bwambae* White, 1985 that breeds only in mineral water has been described in Semliki Forest National Park in eastern Uganda, where it co-exists with *An. gambiae* as adult mosquitoes, thus an important local vector (White and Kaufman, 2014; Scott *et al*., 2012). The eighth species recently described is another fresh water breeder *An. amharicus* Hunt *et al*., 1998 that occurs in Ethiopia. *An. amharicus* and *An. quadriannulatus* are primarily zoophilic and are not considered to be involved in the transmission of malaria.

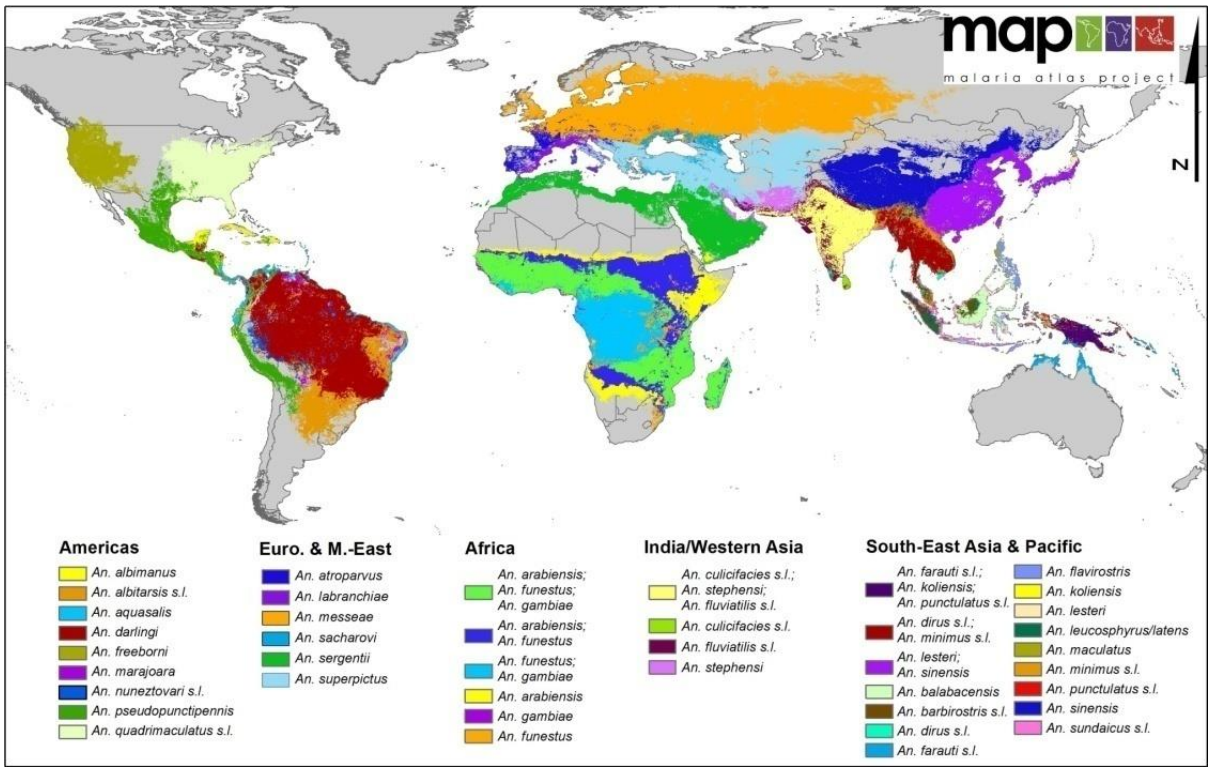

Figure 1: Geographical distribution of *Anopheles* in the World (Sinka M.E 2013)

Anopheles mosquito iswidely distributed in the World especially in Africa (Figure 1). In Cameroon four members of this complex have been identified incuding *An. gambiae, An. coluzzii*, *An. arabiensis* and *An. melas* (Wondji *et al*., 2005; Simard *et al*., 2009). *An. gambiae* and *An. coluzzii* are widely spread throughout the country (Figure 2). However, *An. coluzzii* is predominant in urban areas whereas *An. gambiae* dominates in semi-urban and rural areas (Simard *et al*., 2009; Kamdem *et al*., 2012) but their abundance both, seem to decrease drastically as we move upwards from southern to northern Cameroon where the sibling species *An. arabiensis* becomes the predominant and almost exclusive in the sudan-savanna areas (Wondji *et al*., 2005, Antonio-Nkondjio *et al.*, 2006). *An. melas* has been identified along the coastal areas in Cameroon precisely in Ipono (Wondji *et al*., 2005), Tiko (Bigoga *et al*., 2007), Campo (Simard *et al*., 2009) and in Manoka (Mbida *et al*., 2016). Monitoring of *An. gambiae* s.l susceptibility to insecticides used in public health have been extensively investigated throughout the nation and resistances to pyrethroids, organochlorines and carbamates have been reported for the sibling species *An. gambiae*, *An. coluzzii* and *An. arabiensis* (Etang *et al*., 2007; Wondji *et al*., 2005; Chouaibou *et al*., 2008; Nwane *et al*., 2009; Antonio-Nkondjio *et al.*, 2017; Mandeng *et al*., 2019). Having a heterogeneous environment, 52 *Anopheles* species have been identified in Cameroon, among which, 17 have been found non-randomly distributed within the country and infected with human malaria parasite (Diego *et al*., 2009). Therefore, the

distribution of a large number of species of *Anopheles* corresponding to approximately 85% of 17 the total malaria vectors recorded in Africa (17 out of 20 species) itself explains the diversity, complexities and aggressiveness of malaria transmission in Cameroon. The primary cause of abundance of such a large number of *Anopheles* species in Cameroon is often related to variable local environmental factors, such as precipitation, temperature, habitat availability, etc (Ayala *et al*., 2009).

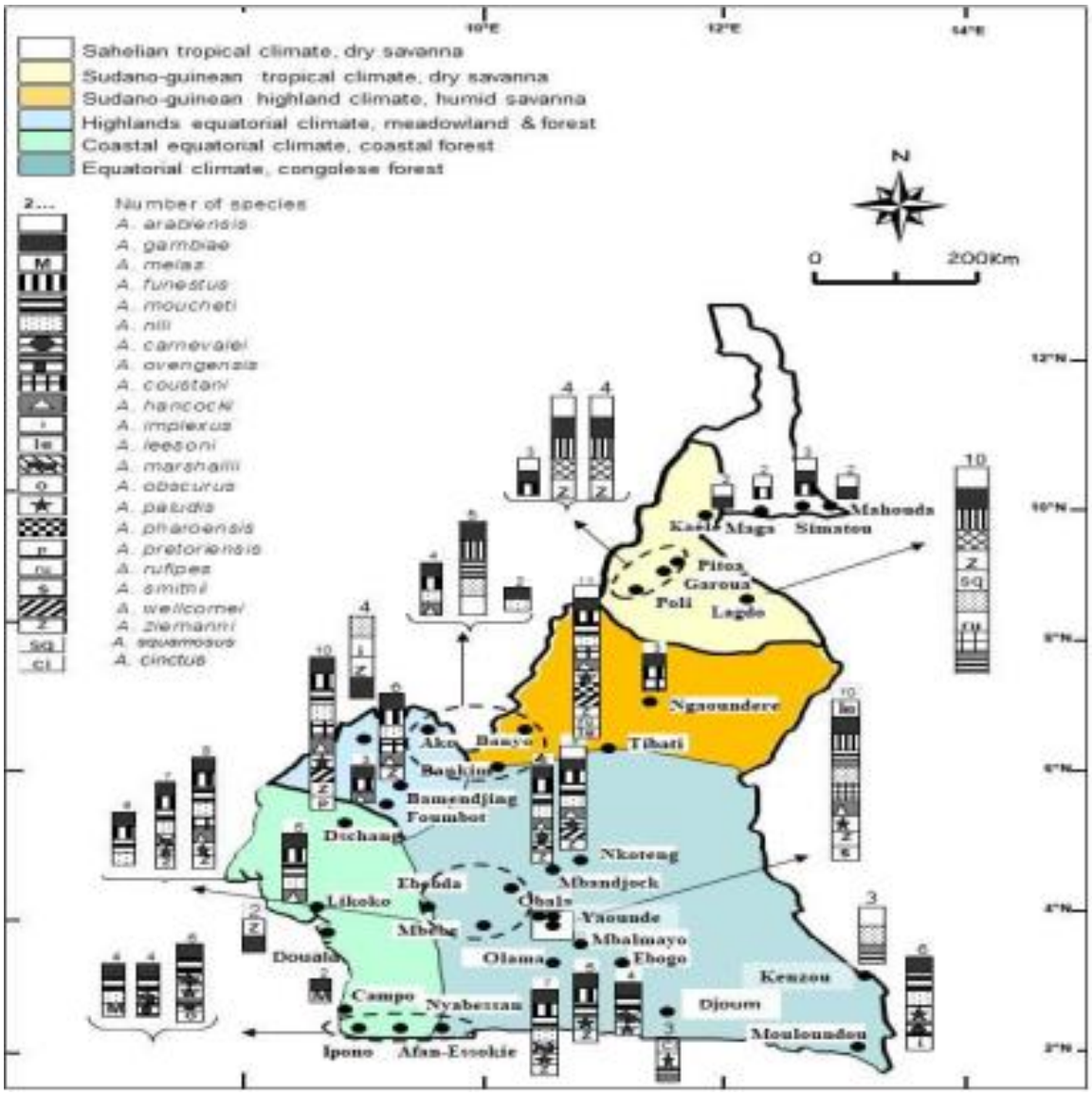

Figure 2: Geographical distribution of *Anopheles* species in Cameroon between 2000 and 2018 (Wondji *et al.*, 2018).

I.2.2. Bioecology of vector

Like all mosquitoes, anophelines go through four stages in their life cycle: egg, larva, pupa, and adult (Figure3). The first three stages are aquatic and last about 5–10 days, depending on the ambient temperature while the fourth stage is aerial i. e the adult (imago). The morphology of the different stages includes:

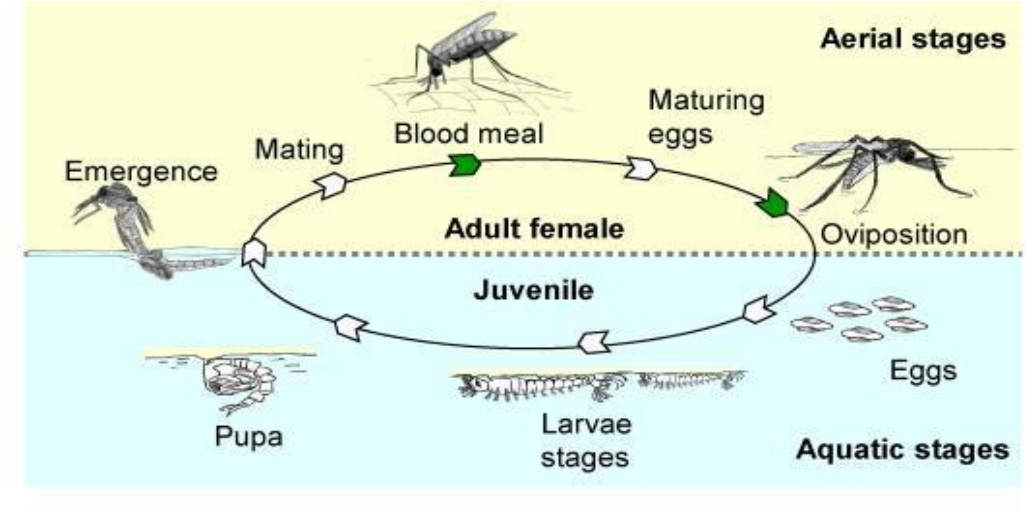

Figure 3: Anopheline life cycle (WHO website, 2010)

Egg: Eggs are laid singly directly on water and are unique in having floats on either side. Eggs are not resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates, the number of eggs produce is dependent on the quantity of the blood meal. A smaller blood meal produces fewer eggs (Nelson, 1986). Eggs are laid on damp surfaces in areas likely to temporal flood, such as tree holes and man-made containers. Not all the eggs are laid at once, but can be spread out over hours or days, depending on the availability of suitable substrates (Clements 1999). Most often, eggs will be placed at varying distances above the water line, and a female will not lay the entire clutch at a single site, but rather spread out the eggs over two or more sites (Foster and Walker 2002).

Mosquito eggs vary significantly among the major groups of species, and in some cases among the individual species. Usually boat-shaped, eggs of *An. gambiae* (Figure 4) are between 0.47 and 0.48 mm long, convex below and concave above, and the surface is covered with a polygonal pattern (Gillies and De Meillon 1968).

Larva: Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax, and a segmented abdomen. They have no legs. In contrast to other

mosquitoes, *Anopheles* larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water. Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently (Becker *et al.* 2010). The larvae spend most of their time feeding on algae, bacteria, and other microorganisms in the surface microlayer. They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Larvae develop through 4 stages, or instars, after which they metamorphose into pupae (Guillaumot 2006). At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth.

The larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. Larvae of *Anopheles* mosquitoes have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, and grassy ditches, the edges of streams and rivers, and small, temporary rain pools (Gillies and De Meillon, 1968).

Pupa: The pupa is comma-shaped when viewed from the side. The head and thorax are merged into a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae do not feed and must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges.

Adult: The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions. Like all insects, adult anophelines have slender bodies with 3 sections: head, thorax and abdomen. The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae (Figure 4). The antennae are important for detecting host odours as well as odours of breeding sites where females lay eggs. The head also has an elongate, forward-projecting proboscis (piercing-sucking mouth parts) used for feeding, and two sensory palps. The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen. *Anopheles* mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black scales on the wings (Gillies and de Meillon, 1968). Adult *Anopheles* can also be identified by their

typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting. Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate. Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies. Females can survive up to a month (or longer in captivity) but most probably do not live longer than 1-2 weeks in nature. Their chances of survival depend on temperature and humidity, but also their ability to successfully obtain a blood meal while avoiding host defenses.

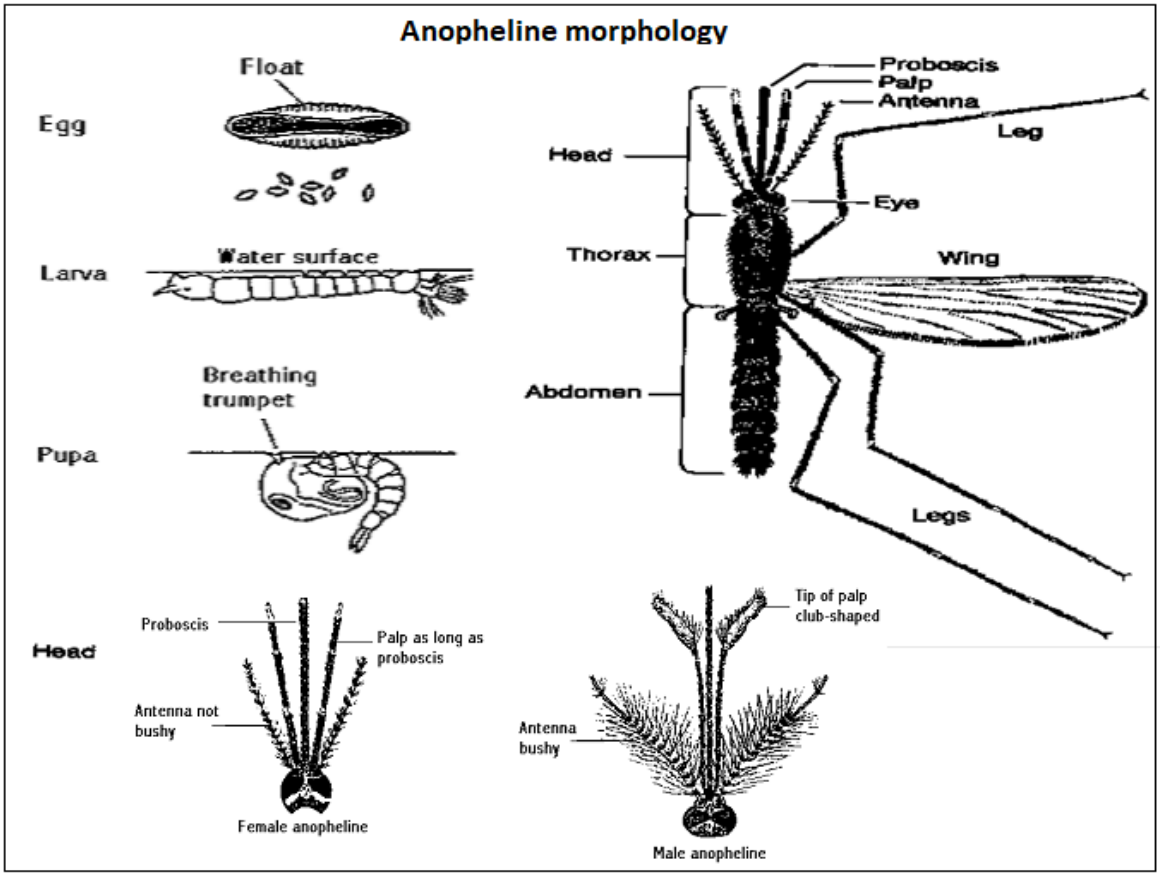

Figure 4: Summary of Anopheline aquatic and adult morphology (Reseach gate)

I.2.2.1. Host-Seeking and feeding behavior of adult *Anopheles*

Anopheles species have well defined behavioral characteristics and this often determines their ability to transmit malaria. Their close associations with human habitat make them likely to use humans as sources for blood meals (Vincent *et al*., 2011). Generally, the first host cues to reach a mosquito are volatile chemicals emanating from the skin, breath and waste products of a potential host (Takken, 1996), carried by air currents. The probability that the mosquito responds to these cues and the intensity of its response depend on the strength of the host-derived stimuli, of the competing external stimuli (e.g., odours from other sources, prohibitive wind speeds, etc), its internal state (e.g., circadian phase, gonotrophic and nutritional status etc), and its genotype (i.e., the genetic component of the responsiveness to given stimuli). Finally, once on the host, probing and biting are affected and initiated by the quality and quantity of stimuli such as heat and phagostimulants (Constantini *et al*., 1999). The feeding behaviour varies greatly (Figure 5), some species feed on human (anthropophilic) while others prefer non-human host (zoophilic). Heavily engorged females with blood usually rest inside houses on clothing hanging on the walls, on the back or underside of pieces of furniture or pictures and ceiling. The females frequently prefer the lower portions of the interior of houses where temperatures are lower and the humidity is higher. This provides a tolerable microclimate for the mosquitoes. The behaviour of an *Anopheles* species may be grouped according to their feeding habits and their relationship to man:

Endophily: the habit of remaining within a man-made shelter throughout the whole or a definite part of the gonotrophic cycle.

Endophagy: the habit of obtaining the blood meal within a man-made structure.

Exophily: the habit of spending greater part of the gonotrophic cycle out of doors.

Exophagy: the habit of seeking the blood meal out of doors.

Normally, the females disperse further than the males. Strong seasonal winds may carry *Anopheles* up to 30 km from their main breeding places to other places (Vincent *et al*., 2011).

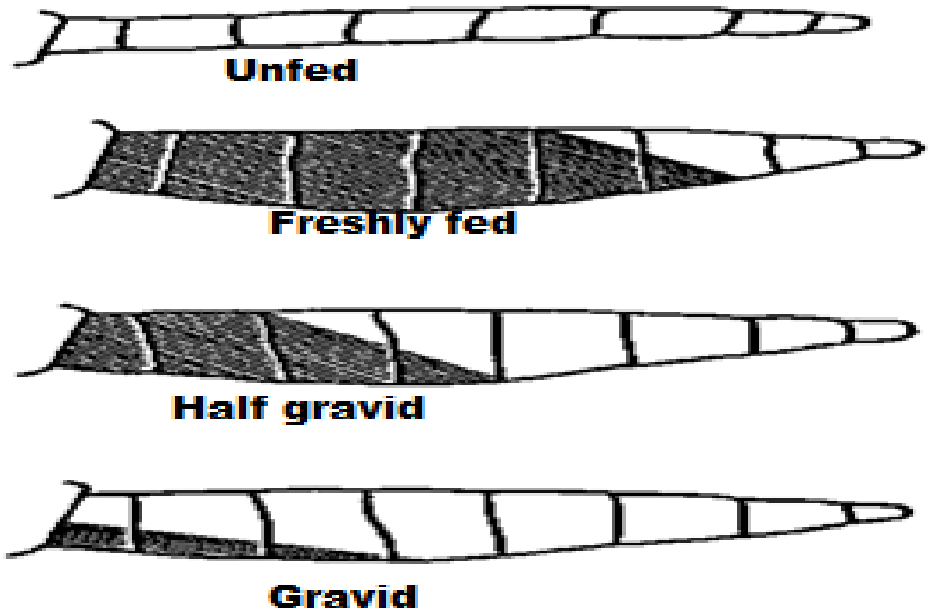

Figure 5: Illustration of a female mosquito Abdomen at different stages of blood meal digestion (WHO, 2010).

I.2.3. Systematic Position of *Anopheles*

The taxonomic position of *Anopheles*, according to Harbach, 2004 and Manguin, 2008 is as follows:

-Kingdom: Animalia

-Presence of movement

-Phylum: Arthropoda (Kühl G and Rust J. 2012)

-Presence of bilateral symmetry

-Presence of articulated appendages

-Body cover with chitin

-Metameric bodies with tagmata

-Sub-Phylum: Mandibulata/Antennata

-Head carries mandibles and a pair of antennae

-Class: Insecta

-Bodies divided into 3 parts; head, thorax and abdomen

-Presence of 2 pairs of wings

-Sub-Class: Pterygota

-Presence of wings at one stage of their post-embryonic development

-Order: Diptera

-Presence of bite and suck buccal pieces

-Presence of a pair of wings, anterior and posterior wings have been transformed into balancers or halters

-Sub-Order: Nematocera (Latreille, 1825):

-Long and multi-articulated antennae

-Pronotum separated from mesonotum by a transverse suture

-Thin palps present 3, 4 or 5 articles

-Family: Culicidae (Latreille, 1825)

-Bodiess covered with filiform scales

-Buccal cavity with an elongated trunk, straight and bent, always pointing to the front

-Sub-Family: Anophelina (Theobald, 1905)

-Eggs with lateral floaters

-Trunk always front and longer than the rest of the head

-Maxillary palps also long like the trunk

-Genus: *Anopheles* (Meigen 1818)

-Presence or absence of lateral shed scales on the costa

-Species: *Anopheles gambiae* (Gilles 1902)

There are about 400 species of Anopheles in the world and about 60 species can transmit malaria parasite (Mazier, 1991). Most of these species are found in the tropical regions of the world and only about 30 species are found in the temperate regions. *An. gambiae* is the principal vector of malaria in Africa (Awolola *et al*., 2005).

I.3. Malaria parasites

Although there are numerous species of Plasmodia worldwide, only six species cause malaria in humans. These are primary *P. falciparum*, *P. vivax*, *P. ovale curtisi*, *P. ovale*

wallikeri and *P. malariae*; the sixth species being *P. knowlesi* which is distributed across south East Asia and P. cynomolgi..

- ***Plasmodium falciparum*** (Welch, 1887) is a one of the species of *Plasmodia* that causes the most dangerous form of malaria in the African Region, accounting for 99.7% of estimated malaria cases in 2018 (WHO, 2019), with the highest rates of complications and mortality. It is the predominant species responsible for malignant tertian malaria. It is one of the commonest forms of malaria and most lethal complication. *Plasmodium falciparum* causes severe malaria via a distinctive property not shared by any other human malaria, that of sequestration (Achidi *et al.*, 2005). Within the 48-hour asexual blood stage cycle, the mature forms change the surface properties of infected red blood cells, causing them to stick to blood vessels (a process called cytoadherence). This leads to obstruction of the microcirculation and results in dysfunction of multiple organs, typically the brain in cerebral malaria.

- ***Plasmodium vivax*** (Grassi and Feletti, 1890) is the benign type of malaria parasitamia is often less heavy than *falciparum* malaria and it is less present in black Africa (Miller, 1975). *P. vivax* can reproduce both asexually and sexually, depending on its life cycle stage. This species is characterized by some of the sporozoites which do not immediately start to grow and divide after entering the hepatocyte, but remain in a dormant (this is the hypnozoite stage) for weeks or months. The duration of latency is variable from one hypnozoite to another and the factors that will eventually trigger growth are not known; this explains how a single infection can be responsible for a series of waves of parasitamia or "relapses". *P. vivax* preferentially penetrates young red blood cells (reticulocytes) and the parasite uses the Duffy blood group antigens to penetrate red blood cells. This antigen does not occur in the majority of humans in West Africa. As a result, *P. vivax* occurs less frequently in West Africa.

- ***Plasmodium ovale*** (Stephen, 1922) is the benign tertian type of malaria with an incubation period of 15 days to about 4 years. It is found exclusively in Africa especially, in the north and west of the equator (Miller, 1975).

- ***Plasmodium malariae*** (Laveran, 1881) is a malaria parasite that causes a disease that has been recognized since the Greek and Roman civilizations over 2,000 years ago, involved in quartan, tertian, and semitertian patterns of fever in patients.

- ***Plasmodium knowlesi*** originally known of zoonotic malaria parasite in India in the early 1930's revealed naturally communicable to human in Asia.

- ***Plasmodium cynomolgi*** (Mayer, 1907) is a simian malaria parasite that has been a central model parasite since it was first described in 1907. Recently it has made the zoonotic jump and started naturally infecting humans. In this paper, the interactions between *Plasmodium cynomolgi* and humans, the environment and the non-human animal intermediates or definitive host will be discussed, with a particular focus on the clinical implications of infection and approaches to management of this novel zoonotic parasite.

I.3.1. *Plasmodium* spp life cycle

In the life cycle of *Plasmodium* (Figure 6), a female *Anopheles* mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins again. Other merozoites develop into immature gametes or gametocytes. When a mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. If a mosquito takes a blood meal containing a gametocyte of each sex, the two sexual stages merge and form a zygote. The zygote develops into a motile stage called the ookinete which penetrates the wall of the mosquito gut and forms a stationary oocyst. The oocyst develops over about 11 days, then begins to release thousands of sporozoites into the mosquito's hemolymph. The sporozoites move through the hemolymph and infect the mosquito salivary glands, where they will again be injected into a mammalian host when the mosquito takes a blood meal. The sporozoites migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal.

Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and thus do not transmit the disease. The females of the *Anopheles* genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted by blood transfusions, although this is rare.

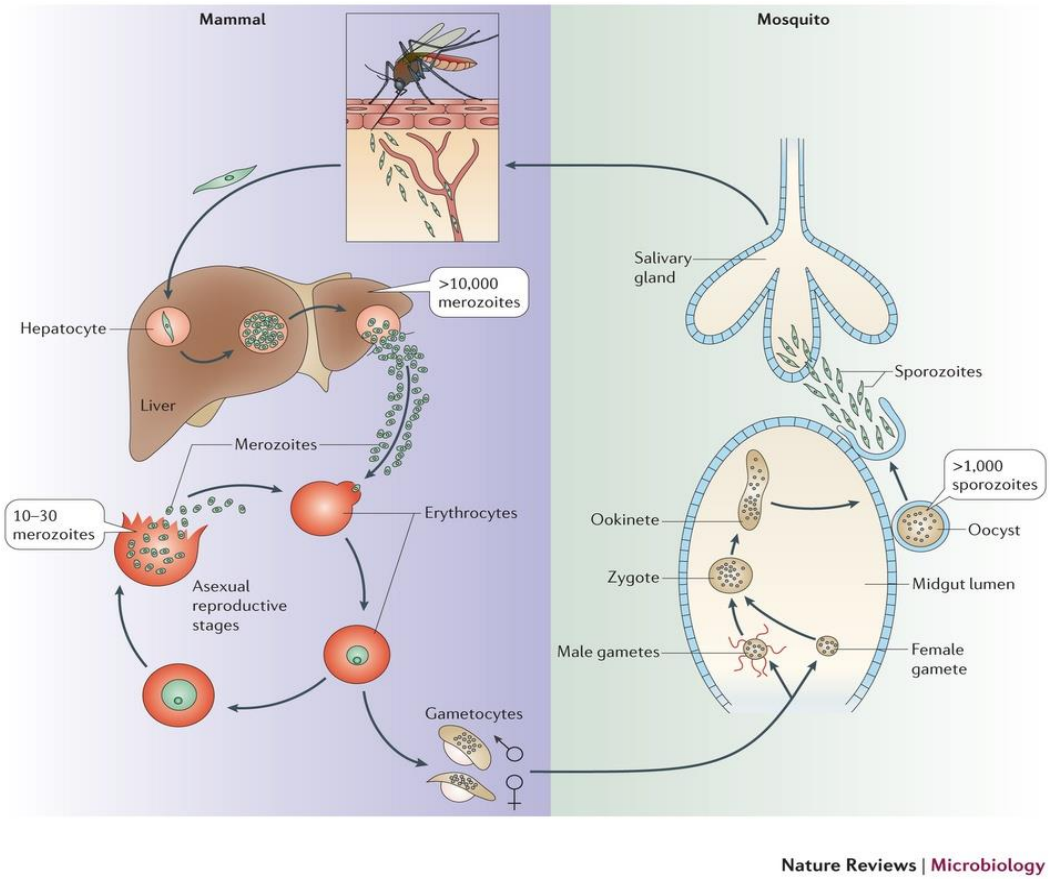

Figure 6: The *Plasmodium* spp. life cycle by Menard *et al*., 2013

I.4. Vector control

Vector control has a proven record of contributing to the reduction of vector-borne disease transmission (WHO, 2004; Townson *et al*., 2005). With the absence of an effective vaccine against malaria so far, most endemic countries have based malaria prevention on reducing vector-human contact through vector control (Kleinschmidt *et al*., 2009; Sharp *et al*. 2007; Protopopoff *et al*., 2007). Prevention and control mechanisms with respect to addressing the vector population largely depends on combining different methods such as chemical control (use of insecticide, repellents, larviciding), biological control (regulate *Anopheles* populations naturally via predation, parasitism, and competition), genetic control (which covers all methods by which a mechanism for pest or vector control is introduced into a wild population through mating), bio-pesticides (plant-derived substances to protect against insect vectors), environmental management by educating the populations on the need for environmental and personal hygiene especially in keeping the immediate surrounding clean with proper drainage system, and above all instigating a change in behaviour of the population at risk (Jamison *et al*.,

2006). The direct output of a vector control activity must be an important reduction of the vectorial capacity. An integrated vector control program would incorporate collection of local information about vector distribution and behavior to identify one or more control techniques that would be effective, affordable, and acceptable to local communities Vector control is the main way to prevent and reduce malaria transmission. If coverage of vector control interventions within a specific area is high enough, then a measure of protection will be conferred across the community (WHO, 2020).

I.4.1. Chemical control

Chemical control of adult female mosquitoes has been the most widely successful vector control method since the 1940s. Before the 2nd world war, the chemicals used for destroying insect pests, were largely inorganic chemicals such as compounds of lead and arsenic which are well known 28 poisons. Some organic chemicals of plant origin, such as nicotine, pyrethrum and rotenone were also used for pest control. The 1940's represent the beginning of the modern era of organic pesticides called "pesticide revolution" when DDT was first used as an insecticide. DDT was first synthesized by Zeidler in 1874, but its insecticidal properties were discovered by Muller in 1939. DDT was commercially manufactured in 1943 and soon became the most extensively used insecticide. Following the discovery and application of DDT, other new groups of synthetic insecticides have been manufactured and used against medically important insects. Chemical methods of malaria vector management can be organized quickly, are effective, and can produce results at relatively low cost if used efficiently. Insecticides are classified according to their chemical composition, origin, their toxicological action and their mode of penetration. Many insecticides act upon the insect's nervous system (e.g., cholinesterase inhibition), while others act as growth regulators or endotoxins. Most act on neurons by causing a sodium/potassium imbalance preventing normal transmission of nerve impulses.

There are several classes of insecticides; however, those of public health significance can be divided into six major classes: organophosphates, carbamates, pyrethroids, insect growth regulators and microbial insecticides.

1.4.1.1. Insecticide based malaria vector control measures

Insecticide-based control measures including indoor residual spraying (IRS) with insecticides and ITNs are the principal way to kill mosquitoes that bite indoors (WHO, 2010). These measures have been proved efficacious in several parts of the world in reducing the

mosquito densities and also reduction in malaria incidence. However, periodic re-impregnation of erratic dose of insecticide diluted the efficacy of ITNs. To overcome these problems, long-lasting insecticidal nets (LLINs) were developed and are being promoted in many malaria endemic areas. While both IRS and ITNs remain the mainstay of malaria vector control (Lengeler, 2004; Protopopoff *et al*., 2007; Kleinschmidt *et al*., 2006), the ownership and utilization of ITNs remains minimal in most endemic countries (Noor *et al*., 2009) and the operational deployment of IRS is more complex than ITNs. Deployment of these interventions together in high malaria risk areas is being advocated. Presently, there is mounting evidence that combining IRS and ITNs affords enhanced protection to exposed populations compared to using one method alone (Kleinschmidt *et al*., 2009). Although these two interventions have been critical in providing community protection the optimal policy for their complementation still remains to be determined.

I.4.1.1.1. Long lasting insecticidal nets (LLITNs)

It is a net treated at factory level with insecticide either incorporated into or coated around fibers resisting to multiple washes and whose biological activity last as long as the net itself (3–4 years for polyester nets, 4–5 years for polyethylene ones) (CDC). The community-wide use of LLINs has been reported to reduce the vector population significantly and when used by a majority of the target population (80%); it provides protection to all people in the community, including those who do not themselves sleep under nets (Lengeler, 1998). As the LLINs shorten the mean mosquito life span, very few mosquitoes can survive long enough for the sporogonic cycle to be completed, thus reducing the transmission (Phillips-Howard *et al*., 2003). As the LLINs also inhibit mosquito feeding, the reproductive potential of highly anthropophilic vectors is also reduced. LLIN is the most technologically advanced form of treated net available today which maintain efficacy without re-treatment for about 3–5 years, and represent an important innovation that could facilitate sustainable scale-up of malaria prevention. LLINs act in three different ways; firstly, through provision of personal protection, by acting as a physical barrier between mosquitoes and the person sleeping under the net, secondly by reducing indoor 31 biting by a combination of increased mosquito mortality, which is caused by the insecticide on the net and the reduction of mosquito house entry caused by the nets excito-repellent properties (Lindsay *et al*., 1991). These properties combined lead to good protection and an even bigger reduction in transmission, producing a community effect where high population coverage is achieved (Maxwell *et al*., 2002, Hawley *et al*., 2003, Killeen and Smith, 2007, Le Menach *et al*., 2007).

I.4.1.1.2. Indoor residual spraying (IRS)

IRS is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide. When carried out correctly, IRS is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, to reduce malaria transmission. The effectiveness of IRS as a malaria control intervention arises from the fact that many important malaria vectors are endophilic. Also, the efficacy and persistence of residual insecticides vary with the type of surfaces sprayed (mud, wood, palm leaves, cement brick…), public acceptance and strong financial support. In these circumstances, IRS can reduce the vector life span, vector population, the number of humans bitten thus malaria transmission. Unfortunately, the availability of low-risk and cost-effective is diminishing due to increasing mosquito resistance and little development of new compounds over the past 20 years (Minisante, 2016). The considerable resources required for IRS combined with the potential for the development of insecticide resistance and the possible environmental hazards, necessitates a strict justification for its use as a vector control measure. Its use is therefore recommended only in high priority areas. One significant difference between the use of IRS and the use of treated mosquito nets is the point at which each intervention works to greatest effect. IRS may provide some small amount of protection to an individual house by repelling and reducing the number of vectors that come into the house. However, the greatest impact of an IRS intervention takes place after feeding, when the anopheline mosquito is more likely to rest on a sprayed surface and pick up a lethal dose of insecticide, thus preventing it from going on to transmit the malaria parasite to others in the vicinity. This means that for IRS to be effective there must be high coverage (usually > 85%) of all structures that are potential resting places in order to obtain the "mass effect" on the vector population. LLINs, however, inhibit feeding before the mosquito can inoculate the person with sporozoites, thus it provides both personal protection and, at high coverage rates, a "mass effect" on the vector population (WHO 2008).

I.4.1.1.3. Mosquito repellents

Prevention of mosquito bites by natural insect repellents has been used for hundreds of years, and commercial repellents have been on the market since the early 20th century (Peterson and Coats 2001). According to Patel *et al*., (2012), mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface. There are also mosquito repellent products available based

on sound production, particularly ultrasound (inaudibly high frequency sounds) (Sah *et al.* 2010). Usually, insect repellents work by masking human scent, or by using a scent which insects naturally avoid (Elissa *et al.* 2004). Carbon dioxide, excretory products and lactic acid present in sweat in warm-blooded animals act as an attractive substance for female mosquitoes. The perception of the odour is through chemo-receptors present in the antennae of mosquitoes. The repellents block the lactic acid receptors thus destroying upwind flight and as a result the mosquito loses its contact with the host (Elissa *et al.*, 2004; Sah *et al*., 2010). The most common mosquito repellent formulations available on the market contain DEET (N, N-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Yap 1986; Coleman *et al*., 1993; Walker *et al*., 1996). Currently, the Environmental Protection Agency's registered insect repellent ingredients approved for application to the skin include DEET, picaridin, MGK-326, MGK-264, IR3535, oil of citronella, and oil of lemon eucalyptus (Tracy *et al*., 2008). DEET has reigned as the most efficacious and broadly used insect repellent for the last 6 decades, with a strong safety record and excellent protection against ticks, mosquitoes, and other arthropods. Newer agents like picaridin and natural products such as oil of lemon eucalyptus are becoming increasingly popular because of their low toxicity, comparable efficacy, and customer approval (Tracy *et al.*, 2008).

I.4.2. Biological Control

The call for malaria control, over the last century, marked a new epoch in the history of this disease. Many control strategies targeting either the *Plasmodium* parasite or the *Anopheles* vector were shown to be effective. Yet, the emergence of drug resistant parasites and insecticide resistant mosquito strains, along with numerous health, environmental, and ecological side effects of many chemical agents, highlighted the need to develop alternative tools that either complement or substitute conventional malaria control approaches. Despite the numerous established findings that explain the process of the parasite propagation within the *Anopheles*, this vector borne disease remains one of the major health threatening problems world-wide. The use of biological means is considered a fundamental part of the recently launched malaria eradication program and has so far shown promising results, although this approach is still in its infancy. This review presents an overview of the most promising biological control tools for malaria eradication, namely fungi, bacteria, larvivorous fish, parasites, viruses and nematodes (Najera, 1999).

I.4.2.1. Use of predators

That depends on whether you are targeting the larval or adult stages, Toxorrhynchite larvae feed well on anopheline larvae but am not sure of any other insect predators. Tadpoles, backswimmers (Hemiptera: Notonectidae), and the *Gambusia afinis* species of fish make the other examples of predators. Dragon fly larvae feed on mosquito larva. Mosquitoes as adults have numerous predators, particulaly spiders. These arachnids spin webs in houses as well in the environment where mosquitoes seek refuge. Lizards too prefer adult mosquitoes (Okigbo *et al*., 2010).

I.4.2.2. Use of plants

Medicinal plants have long history as important components in traditional medicine, and food of humans since ancient Egyptians and Chinese. According to WHO traditional medicine can be defined to be the sum of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether used in health maintenance as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness (WHO, 2018). Since ancient times, plants have been used in medicine and are still used today (Grover *et al* 2002). Plants generally produce many secondary metabolites which constitute important source of many pharmaceutical drugs (Yeshi *et al.*, 2017). It appeared that the essential and volatile oils of the plant were differing according to the plant location and variety (Salem *et al*, 2014). Naturally occurring botanical compounds contain a broad range of chemical active ingredients can intervene in all biological processes of the mosquito, thus interrupt its life cycle and dispersal and reduce harms to humans and animals. Mosquito control is a difficult task and is becoming even more so due to a variety of factors including the development of insecticide resistance and concern over environmental pollution and many of them are immuno suppressants (Srivastava and Sharma, 2000). Because of resistance in the vectors, conventionally insecticides, the chief weapon of the vector, are becoming ineffective (Robert Vincentet al., 1993). Plants are rich source of bioactive organic chemicals and synthesize a number of secondary metabolites to serve as defence chemicals against mosquitoes attack. These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, as well as attractants (Murugan *et al*., 1996). Toxic effects and resistance to synthetic insecticides are barriers in controlling mosquitoes therefore, it is necessary to develop safe alternative contol options, which require minimum care (Mittal and Subbarao, 2003). Plant

based insecticides may be the best option for mosquito control as they have biologically active chemicals that are easily decomposed into products which are not toxic to other species (Mahmood *et al* 2016) and potentially suitable for use in control of mosquito larvae (Yang *et al*., 2004). In fact, many researchers have reported the effectiveness of plant extracts or essential oils against mosquito larvae (Rahuman *et al*., 2008).

I.5. Overview of *Petroselinum crispum* (Mill)

Petroselinum crispum (Figure 7) is a hibiscus plant in the family of Apiaceae with common name parsley. As a native to Mediterranean plant, parsley has been cultivated and used in culinary and medicine for thousands of years (Airy-Shaw, (1938)**.** Fragrant parsley or petrosello is also a spontaneous plant, native to Europe and West Asia (Enam *et al*., 2013). This species is divided into several varieties: *P. crispum* variety crispum which is curly or curly parsley, *P. crispum* variety neapolitanum which is flat parsley or Naples's parsley (these two varieties are cultivated for their foliage), *P. crispum* variety tuberosum which is tuberous parsley and *P. segetum* which is harvest parsley (Petropoulos *et al*., 2004). *Petroselinum crispum* was identified by Daniel Dang (No: 374) and registered by NHC (National Herbarium Cameroon) using code number: 25583/SRF.Cam. (Section de Recherche Forestier du Cameroon).

I.5.1. Classification

It belongs to the Kingdom of **Plantae** (Eukaryotic multicellular and autotrophic organism having cell wall (cellulose) in its structure; Phylum of **Spermatophyta** (reproduces sexually or asexually) Plant that contains vascular tissues, xylem and phloem. Plant which produces yellow flowers grouped by 2 to 3 above base petiole); Class of **Dicotyledonae** (Comprising seed plants that produce an embryo with paired cotyledons and net-veined leaves,divided into six (not always well distinguished); Order of **Apiales** (Herb with thin stems woody vines, and trees; dicotyledonous flowering plants); Family of **Apiaceae (**Non-allergenic resin produce in virtually all plant tissue and the distinctive smooth, yet flaking aromatic leaves); Genus: ***Petroselinum*** (Tiny flowers, a yellowish green, are grouped in umbels of 8-20 radios stamens and a the basal ones serrated or toothed) and the Species: ***Petroselinum crispum*** Mill (About up to 80 cm in height, long, hairless, with thin stems and triangular outline leaves two to three times pinnate, are fragrant) (Spichiger *et al.*, 2002 and Daroui-Mokaddem, 2012).

I.5.2. Botanical description

Parsley is an herb grown for the pungent flavored leaves. Parsley, *Petroselinum crispum*, is a hardy biennial in the carrot family (Umbelliferae/Apiaceae) generally grown for its flavorful, dark green leaves that are a rich source of vitamin C, vitamin A and iron. Parsley can be identified by its long, thin stem and triangular, toothed leaves, which are bright green in colour and come in two main varieties: curly and flat-leafed (Craft and Setzer, 2017). Flat-leafed parsley has a stronger, sweeter flavour than other varieties do, which makes it more preferable for cooking. Parsley is a biennial plant with bright green, feather-like leaves (Figure 7) that is in the same family as dill. Morphology, parsley is a glabrous biennial herb that is grown as an annual in cultivation for its edible leaves. Leaves are triangular in outline, consisting of three-lobed cuneate lobes. Umbels are lax, and flowers have yellow, inflexed at the apex, petals. The Italian variety (flat leaf) has a more fragrant and less bitter taste than the curly variety. There is also another type of parsley known as turnip-rooted (or Hamburg) that is cultivated for its roots, which resemble salsify and burdock. It is pertinent to note that the ingestion of this type of plant is toxic to many domestic animals including horses, cats and dogs due to the action of furocoumarins causing symptoms such as photosentisization, ulcerative and exudative dermatitis and ocular toxicity that may require veterinary consultation. Therefore, consideration should be giving to sources of plant cultivation due to the fact that absorption of heavy metals by the plants has been observed in soil irrigated with untreated waste water making the plant a potential source of heavy metal toxicity (Jonathan D and William N, 2017).

Figure 7: *Petroselinum crispum* leaves

I.5.3. Geographical distribution of parsley

Petroselinum crispum has been cultivated in British gardens since at least 995 years (Harvey, 1981). Although most records are casual, it is very persistent in many of its coastal sites (Figure 8). It is difficult to assess any changes in its distribution. A cultivated species of uncertain origin is now widely naturalised in Europe and other continents.

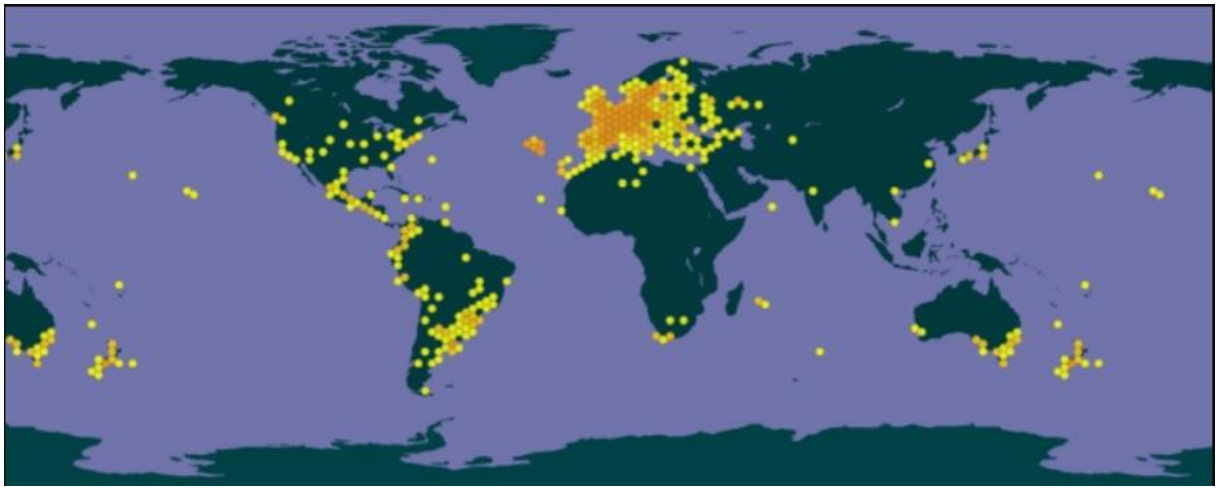

Figure 8: Distribution of *Petroselinum crispum*. Global infotmation 2020

I.5.4. Chemical composition:

Petroselinum crispum is a plant rich in essential oil whose major constituent is Myristicin (could be a chemopreventive agent of cancer) (Zheng *et al*., 1992). *Petroselinum crispum* is a desiccant, seasoning, garnish, condiment, attenuating, appetizer, detergent, diuretic, stimulant, vegetable, decorative, accompaniment to dishes or other fried foods (Petropoulos *et al*., 2004; Fejes *et al.*,1998). Numerous studies carried out on *P. crispum* have demonstrated that it possesses, among other things, a smoke-producing action on Trialeurodes vaporariorum (Hemiptera: Aleyrodidae) in natural conditions on living plants (Mahmoudi *et al*., 2014) and an anti-oxidant activity (Zhang *et al*., 2006). Leaf aqueous and methanol extracts of *P. crispum* have been identified to possess antioxidant activity *in vitro via* the 2, 2,1-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging, ion-chelating and hydroxyl radical assays (Wong *et al*., 2006). Methanol-derived leaf extracts exhibited significantly ($p<0.05$) greater radical-scavenging activity towards both lipid-and water-soluble radicals, which was attributed to the total phenolic content. Ferrous ion-chelating activity was significantly ($p<0.05$) greater in the stem methanol extracts. Seeds of *P. crispum* produce high number of essential oils. Root and leaf also contain essential oils (Bruneton, 1999). Myristicin (phenylpropene) and (Bisabolene <(E)-iso-ɣ>) apiol (phenylpropanoid) are the two main components of *P. crispum* essential oil which are responsible for its antioxidant activity (Zhang *et al*., 2006).

The toxicity of *P. crispum* and its essential oil has not been thoroughly investigated. In ethnomedicine, it has been claimed that *P. crispum* is abortifacient. Photodermatitis due to furocoumarins particularly 55 are responsible for its contact photodermatitis activity in pigs exposed to *P. crispum* (Chaudhary *et al*., 1986).

Furthermore, the plant is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc and these might likely account for its hepato protective effect (Meyer *et al*., 2006).

I.5.5. Uses of parsley

Leaves of *P. crispum* have been employed as food flavour, antitussive and diuretic and also in the treatment of kidney stones, hemorrhoids, gastrointestinal disorder, blurred vision and dermatitis (Aghili *et al*., 2009; Avicenna, 1983). The leaves are also used to manage bleeding, hypertension, hyperlipidemia, hepatic disorders and diabetes in Turkey. Leaves serve are employed as food flavor (Wong and Kitts, 2006) and for treatment for skin diseases (Aljanaby, 2013) in China and Iraq, respectively. In Moroccan traditional healing system, the leaves are used in arterial hypertension, diabetes, cardiac disease, renal disease, lumbago,

eczema and nose bleed (Ziyyat *et al*., 1997; Eddouks *et al*., 2002; Jouad *et al*., 2001.). The leaves are also used for the treatment of amenorrhoea, dysmenorrhea, kidney stones, prostatitis, diabetes, halitosis, anaemia, hypertension, hyperuricaemia, constipation, odontalgy, pain, baldness and induction of abortion in Spain (Benítez *et al*., 2010). In addition to its volatile oils and flavonoids, parsley is an excellent source of two vital nutrients that are also important for the prevention of many diseases: To fight cancer, to relieve join pains, prevent diabetes, break down kidney stones, reduce stress and it contains vitamin C, k and A (notably through its concentration of the pro-vitamin A carotenoid, beta-carotene). In Thessaloniki (northern Greece), leaves of parsley are taken to relieve hypertension, treat cystitis, kidney stones, and prostatism (Hanlidou *et al*., 2004). Cyprus (Greece) uses infusion of the leaves taken as a tonic to treat anemia, as a stimulant, and a diuretic (Karousou *et al*., 2011). Granada province (southern Spain) uses decoction of the leaves orally as an abortifacient, to treat kidney stones, dysmenorrhea, prostatism, diabetes, and hypertension (Benitez *et al*., 2012). In Nebrodi Regional Park (northeasternsicily, Italy), decoction of the aerial parts is taken orally as a diuretic. Poultice of leaves are also used to treat insect bites (Tuttolomondo *et al*., 2014). In Elazığ (eastern Turkey), decoction of the leaves is taken orally to treat kidney stones and mouth sores (Hayta *et al*., 2014). Vesuvio National Park, Campania (southern Italy) uses decoction of the aerial parts orally also as an abortifacient and anticancer substance (Shankar *et al.*, 2017; Menale *et a*l., 2016).

1.6. Overview of *Ocimum basilicum* L. (sweet basil)

Basil is originally a native to India and other tropical regions of Asia, having been cultivated there for more than 5,000 years (Nurzyńska-Wierdak, 2007b). *Ocimum basilicum* L. (sweet basil) belongs to the family Lamiaceae, which includes about 200 species occur in various botanic varieties and forms (Nurzyńska-Wierdak, 2007b). *Ocimum basilicum* is a popular culinary herb and a source of essential oil (Akgul *et al*., 1989). The aromatic character of each type of basil is determined by genotype and depends on the major chemical compounds of essential oils primarily consisting of monoterpenes and phenylpropanoids (Marotti *et al*., 1994). The essential oil has antimicrobial, antifungal, and insect-repelling, anticonvulsant, hypnotic, and antioxidant activities (Politeo *et al*., 2007). *Ocimum basilicum* (Figure 9) is a highly fragrant condiment plant cultivated all over the world. Its antioxidant contribution makes it a remarkable seasoning. The quadrangular stems are generally woody at their base and much branched. The leaves are petiolate, opposite, membranous, with entire or serrate margins,

terminal inflorescence is simple or branched at its base and the whorls are clearly interrupted (Figure 9).

1.6.1 Classification of *Ocimum basilicum* L.

It belongs to the Kingdom of **Plantae** (Eukaryotic multicellular and autotrophic organism having cell wall (cellulose) in its structure); Phylum of **Magnliophyta (**reproduces sexually or asexually, a plant that contains vascular tissues, xylem and phloem, which produces white flowers grouped by 2 to 3 above base petiol); Class of **Magnliopsida** (Comprising seed plants that produce an embryo with paired cotyledons and net-veined leaves; divided into six and not always well distinguished**);** Order of **Lamiales** (Herb with thin stems with a range of culinar; dicotyledonous flowering plants**);** Family of **Lamiaceae** (Non-allergenic resin produce in virtually all plant tissue and the distinctive smooth, yet flaking aromatic leaves**);** Genus: ***Ocimum* (**Tiny flowers, a yellowish green, are grouped in umbels of 8-20 radios stamens with smooth or slightly toothed edges) and Species of ***Ocimum basilicum* (**About 20 to 80 cm in height, tall, hairless, with thin stems and triangular outline leaves two to three times pinnate, are fragrant) (Koffi *et al* 2006).

I.6.2. Botanical description

Ocimum contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Darrah 1980). Very little has been published on basil taxonomy which follows the International Code of Botanical nomenclature (Oleshko *et al*,. 1989). The morphological diversity within basil species has been accentuated by centuries of cultivation with great variation in pigmentation, leaf shape and size, and pubescence. Taxonomy is further complicated by the existence of chemotypes or chemical races within the species that do not differ significantly in morphology. The taxonomy of this genus has undergone frequent upheavals, so the synonymies are numerous; *Ocimum americanum* L., herbaceous plant, 10 to 50 cm; white or light purple flowers and *Ocimum basilicum* L., herbaceous plant from 30 cm to 1m, white to purple flowers (Darrah 1980). Both plants have square stems, fragrant opposite leaves, and whorled flowers on spiked inflorescences (Darrah 1980).

Figure 9: *Ocimum basilicum* leaves

I.6.3. Geographical distribution of basil

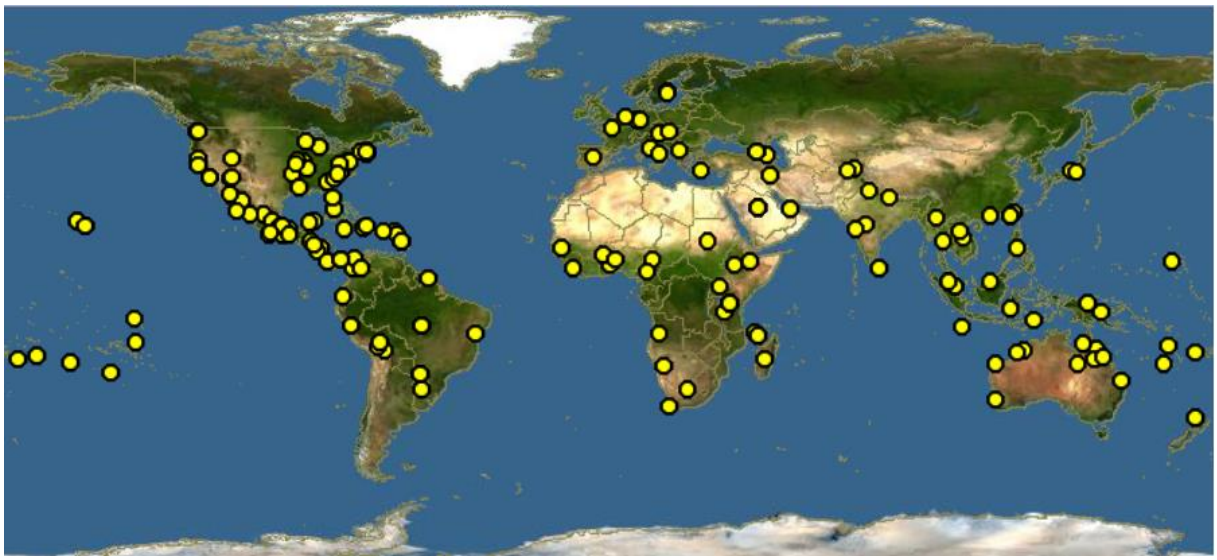

Figure 10: Distribution of *Ocimum basilicum* in the world, American Museum of Natural History. Global Biodiversity Information Facility (484)

Basil was already being cultivated in Egypt 3000 years ago and made its way from the Middle East to Greece, Italy, and the rest of Europe. It reached England in the 16th Century and North America in the early 17th Century (Figure 10). Sweet basil is now cultivated throughout the world. Basil is native to areas in Asia and Africa and grows wild as a perennial on some

pacific islands. Basil was brought from India to Europe through the Middle East in the sixteenth century, and subsequently to America in the seventeenth century (Grieve 1971).

I.6.4. Chemical composition

The various basils have such different scents because the herb has a number of different essential oils components that come together in different proportions for various breeds. The strong clove scent of sweet basil is derived from eugenol, the same chemical as actual cloves. The citrus scent of lemon basil and lime basil reflects their higher portion of citral, which causes this effect in several plants including lemon mint, and of limonene, which gives actual lemon peel its scent. African blue basil has a strong camphor smell because it contains camphor and camphene in higher proportions. Licorice basil contains anethole, the same chemical that makes anise smell like licorice, and in fact is sometimes called "anise basil." Other chemicals that help to produce the distinctive scents of many basils, depending on their proportion in each specific breed, include: citronellol (scented geraniums, roses, and citronella), linalool (a flowery scent also in coriander), myrcene (bay leaf, myrcia) and pinene (which is, as the name implies, the chemical that gives pine oil its smell).

I.6.5. Uses of *Ocimum basilicum* L. (sweet basil)

Traditionally, sweet basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions (Simon *et a*l., 1999). It has multiple uses depending on the desired effect: for internal use; to aid digestion, a dessert spoonful per cup of boiling water. For external use: essence or crumpled leaves on wasp stings. Antioxidant supply: regularly adding basil to food helps provide antioxidants to the body and delay aging, that is to say free radicals, and therefore help prevent the onset of cardiovascular diseases and cancer. The main antioxidant found in basil leaves is rosmarinic acid, which appears to work synergistically with vitamin E (Gülçin *et al*., 2007). This plant is also used to fight against nervous tension, bad breath, and to prevent hair loss. The essential oils of basil extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances, and in traditional rituals and medicines (Guenther 1952, Simon *et a*l. 1999). Two minor components of the essential oil of sweet basil, juvocimene I and II have been reported as potent juvenile hormone analogs (Oliveira *et al*. 2009). The genus *Ocimum*, (Lamiaceae formerly Labiatae), collectively called basil has long been recognized as a diverse and rich source of essential oils. Extracted essential oils have also been shown to contain biologically-active constituents that are insecticidal (Chavan *et al*., 1979).

I.7. Overview of *Cupressus lusitanica* Mill. (1768) (cypress)

Cypress tree is a fast-growing North American native that deserves a prominent place in the landscape according to Katende *et al*., 1995. Many gardeners don't consider planting cypress because they believe it only grows in wet, boggy soil. While it's true that their native environment is constantly wet, once they're established, cypress trees grow well on dry land and can even withstand occasional drought. *Cupressus lusitanica* is also called Mexican cypress. It orinated from Central America. Lusitanica is from the name "Lusitani", which is the formal name of Portugal. It is also known as Mexican white cedar. *Cupressus lusitanica* is an evergreen conifer tree with a conic to ovoid-conic crown, growing to 40 m tall (Figure 11). There are two varieties, treated as distinct species by some botanists. These are: *C. lusitanica var. lusitanica* and C. *lusitanica var. benthami* (Farjon, 2005). *Cupressus lusitanica* (cypress) was identified by Kuate S.P.(No:1) and registered by the National Herbarium in Cameroon (NHC) using code number:62102/HNC.

I.7.1. Classification of *Cupressus lusitanica* Mill.

It belongs to the Kingdom of **Plantae** (Eukaryotic multicellular and autotrophic organism having cell wall (cellulose) in its structure. It reproduces sexually or asexually); Phylum of **Pinophyta (**Plant that contains vascular tissues, xylem and phloem. Plant which produces white flowers grouped by 2 to 3 above base petiole); Class of **Pinopsida** (Comprising cone seed plants that produce an embryo with paired cotyledons and net-veined leaves; divided into six (not always well distinguished)); Order of **Pinales (**Evergreen conifer tree**,** scaly leaves and a large classification, encompassing**,** dicotyledonous flowering plants; Family of **Cupressaceae** (Fast-growing and drought tolerant, Non-allergenic resin produce in virtually all plant tissue and cone leaves); Genus of ***Cupressus* (**The foliage grows in dense sprays, dark green to somewhat yellow-green in colour); and Species of ***Cupressus lusitanica* (**About 40m in height, tall, hairless, the leaves are scale-like, 2–5 mm long and produced on rounded (not flattened) shoots) (Bussmann RW and Sharon D. 2007).

I.7.2. Botanical description:

Cupressus lusitanica is an evergreen conifer tree with a conic to ovoid-conic crown, growing to 40 m tall. The foliage grows in dense sprays, dark green to somewhat yellow-green in colour. The leaves are scale-like, 2–5 mm long, and produced on rounded (not flattened) shoots. Trees are 25-30 m tall. They are crown broadly pyramidal, in older trees broad with pendulous branches. Bark is thick, reddish-brown, with longitudinal fissures. Shoots

quadrangular, pendulous, forming flattened foliage sprays. Foliage is blue-green, four-ranked, ovate, closely pressed, usually with long, pointed apex. Cones are globose, 12 mm across, blue-green in the juvenile stage, turning dark brown when they ripen (Farjon, 1993). The easiest way to distinguish *Cupressus benthamii* from *C. lusitanica* is that the latter species has irregularly arranged sprays of foliage (vs. flattened sprays in *C. benthamii*), and the crown, while often pyramidal in both species, is broader (vs. narrower in *C. benthamii*). Farjon (2005) analysed that all specimens from south of about 22° latitude can be assigned to one or two taxa, which he called *C. lusitanica* var. *lusitanica* and *C. lusitanica* var. *benthamii* (*C. lusitanica* and *C. benthamii)*. An analysis by Li, (2013) also found differences between the two taxa on the basis of several different molecular genetic lines of evidence; however, the differences were very small. There has long been a debate about whether this taxon is indeed "Mexican." It has particularly ranked some Mexican and Central American botanists that Miller described the species from Portuguese material. However, there is strong evidence that the trees in question, which were planted in 1634 at Bussaco near Coimbra in Portugal, were in fact brought there from Mexico or Central America; indeed, there is no other New World source for *Cupressus* at such an early date (Miller, 1769).

Figure 11: *Cupressus lusitanica* Mill. (1969) (cypress)

1.7.3. Geographical distribution

Cupressus lusitanica is naturally distributed in Mexico, Guatemala, Belize, Honduras, El Salvador and Nicaragua (Figure 12). In the 20th century it was introduced as a plantation forest tree into tropical Africa, where it is widely planted at higher elevations. It is also planted in South Africa. The scientific name *lusitanica* (of Portugal) refers to its very early cultivation

there, with plants imported from Mexico to the monastery at Buçaco, near Coimbra in Portugal in about 1634; these trees were already over 130 years old when the species was botanically described by Miller in 1969.

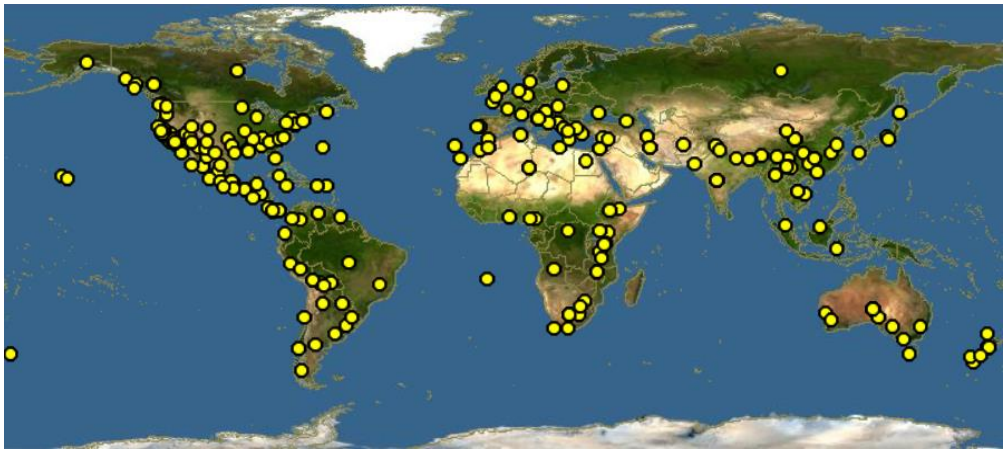

Figure 12: Distribution of *Cupressus lusitanca*. Global Biodiversity Information Facility (98)

I.7.4. Chemical composition:

Chemical constituents and therapeutic properties of Cypress essential oil contain various chemical components, the majority of which are a-pinene, delta carene and limonene. The other constituents include sesquiterpene, properties a-terpinene, sabinene, carvone, 4-terpinol, b-cymene, carveol, cedrol, a-thugene and santene. Contrary to its depiction as a symbol of mourning, Cypress essential oil has a handful of health benefits with its astringent, antimicrobial, anti-bacterial, anti-fungal, firming, anti-seborrheic, anti-dandruff, anti-aging, antioxidant, aromatic, antispasmodic, antiseptic, diuretic, vasoconstricting, mucolytic, fluid retention, decongestant, sedative, hepatic and haemostatic

I.7.5: Uses of *Cupressus lusitanica* Mill.

The wood is used for construction, furniture, poles and posts. It is also suitable for light flooring, ship and boat building, vehicle bodies, agricultural implements, boxes and crates, interior trim, joinery, toys and novelties, turnery, draining boards, veneer and plywood, hardboard and particle board. The wood is used for paper; the bark is used as an astringent. The leaves are used to treat catarrh and headache, leaf sap to treat skin diseases. Essential oil from the leaves is also used in the treatment of rheumatism, whooping cough and as a styptic. The vapour from a leaf decoction is inhaled several times a day for treatment of flu. Some ethnic groups in Mexico use the leaves against cancer.

In Cameroon the leaf juice is used to cure skin diseases and the leaves are used to protect stored grain from insects by the villagers. It is a good fuel wood and health importance to treat ovarian dysfunction, to relieve the symptoms of menopause and also to boost the immune system. Celebrated as one of the 12 sacred oils of Bible, Cypress is also known as one of the oldest trees on earth. It is also used in Ayurvedic healing in the treatment of numerous health disorders for more than 4,000 years. It is used on the skin to prevent bacterial infections and reduce the inflammation and swelling. Also used to speed wound healing and reduce the appearance of scars and blemishes on the skin. Furthermore, cypress can help to soothe the organ systems that may be inflamed by infection or nutrient deficiency, allowing the body to recover more quickly (Gerald *et al*., 2013; Taponjou *et al.*, 2005). This salve can also be applied to hemorrhoids to reduce inflammation and eliminate pain. It can also help to reduce dandruff by preventing the skin from drying out. Essential oil from the leaves, twigs and branches of the tree is used as an adjuvant and perfume in soaps, room sprays, deodorants and other products. *Cupressus lusitanica* is planted as an ornamental tree. Having its uses inscribed in the Holy Bible, Cypress essential oil has been used by mankind for more than 4,000 years. Though associated with death and underworld, this tree is known for regenerating the inner soul. Botanically known as *Cupressus sempervirens.* Cypress trees bear the Eastern Mediterranean region as their native. As the Greek name Sempervirens means 'ever living', cypress trees are found to be ever living with the oldest known as Sarv-e-Abarkooh being found in Iran's Yazd Province that is estimated to be of approximately 4,000 years old (Miller, 1969).

I.8. Overview on plant methanolic extracts:

Extraction in chemistry is a separation process consisting in the separation of a substance from a matrix. Common examples include liquid-liquid extraction, and solid phase extraction. The distribution of a solute between two phases is an equilibrium condition described by partition theory. Solid object (the plant) is placed in contact with a fluid (the solvent). The plant components of interest are then solubilized and contained within the solvent and the solution thus obtained is the desired extract. The process of methanolic extraction involves the plant succus prepared from fresh plant leaves evaporated to dryness and dissolved in 100% methanol overnight. Extracts are then transferred to clean vessels, evaporated to dryness, and redissolved in dimethyl sulfoxide to yield a final concentration. Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time.

I.9. Overview of essential oils:

Essential oils are volatile natural complex secondary metabolites characterized by a strong odour and have a generally lower density than that of water (Bakkali *et al*. 2008). The term "Essential oils" is a generic term which designates the liquid and highly volatile components of plants, marked by a strong and characteristic odour. The first records of essential oils come from ancient India, Persia, and Egypt; and both Greece and Rome conducted extensive trade in odoriferous oils and ointments with the countries of the East. Most probably these products were extracts prepared by placing flowers, roots, and leaves in fatty oils. Terpenes (mainly monoterpenes) make up the bulk (around 90%) of these components. Essential oils are by definition secondary metabolites produced by plants as a defense against phytophagous pests. These extracts contain an average of 20 to 60 compounds which are mostly molecules that are not very complex (monoterpenes, sesquiterpenes, etc. (Meryem, 2009). Essential oil or plant fuel is the concentrated or hydrophobic liquid of aromatic volatile compounds of a plant. It is obtained either by distillation or chemical extraction by solvent (water, alcohol, etc). Essential oils are sub products of photosynthesis elaborated in the secretory cells of plants (Sharifi-Rad *et al* 2017). The synthesis of these sub products is done in three methabolic pathways; Fatty acid pathway which leads to aliphatic compounds (Brunhes, 1999), Mavalonate pathway which leads to the synthesis of terpenes; monoterpenes, sesquiterpenes and triterpenes (Zhang *et al.*, 2011), Shikimic acid pathway made up of aromatic compounds. These are odorant compounds of the phenylpropanoid type (Christopher M. Fraser and Clint Chapple, (2011). The first records of essential oils come from ancient India, Persia, and Egypt; and both Greece and Rome conducted extensive trade in odoriferous oil**s** and ointments with the countries of the East. Most probably these products were extracts prepared by placing flowers, roots, and leaves in fatty oils.

I.9.1. Principal Methods of extracting essential oils

There are several methods to extract essential oils. The main ones are based on vapour entrainment, expression, solubility and volatility. The choice of the most suitable method is made according to the nature of the plant material to be treated, the physicochemical characteristics of the essence to be extracted, the use of the extract and the aroma of the start during the course of extraction (Vashisht *et al.*, 2017).

- **Hydrodistillation**

Hydrodistillation is a traditional method for the extraction of bioactive compounds from plants. The principle of hydrodistillation is the distillation of immiscible binary mixtures. It consists of immersing the plant biomass in a still pot filled with water, which is then brought to the boil. The water vapour and the gasoline released by the plant material form an immiscible mixture. The components of such a mixture behave as if each were alone at the temperature of the mixture that is the partial pressure of the vapour of a component is equal to the vapour pressure of the pure substance. This method is simple in principle and does not require expensive equipment. However, due to the water, acidity, temperature of the medium, reactions of hydrolysis, rearrangement, oxidation, etc. may occur which can very significantly lead to denaturation.

- **Saturated steam distillation**

In this variant, hot steam is forced through the the matrix of raw material. The water vapour is injected through the plant mass arranged on perforated plates. Saturated steam distillation is the most widely used method in industry today for obtaining essential oils from aromatic or medicinal plants. In general, it is carried out at or near atmospheric pressure and at 100 ° C., the boiling point of water. Its advantage is that the alterations of the essential oil collected are minimized;

- **Hydrodiffusion**

It consists of pulsing water vapour through the plant mass, from top to bottom. Thus, the flow of steam passing through the plant biomass is downward, unlike conventional distillation techniques in which the flow of steam is upward. The advantage of this technique is reflected in the qualitative and quantitative improvement of the harvested oil, saving time, steam and energy.

CHAPTER II: STUDY AREA, MATERIAL AND METHODS

II.1. Study Area

The research was conducted in the Central Region of Cameroon-Yaounde urban area which covers 304 km^2 including an urbanized area of 183 km^2. It has an estimated population of 4,100,000 inhabitants or an average density of 13,486 inhabitants per Km. The city of Yaounde is located south of the Central Region and is 250 km east of the coast of the Gulf of Bafia from Encyclopædia Universalis (2018). The soils are mainly ferralitic (Ferralsols**)** and are developed from various parent materials such as micaschists, gneisses and granites (Mesmin *et al*., 2001). The central parts of Cameroon, which the capital is Yaounde, experiences a tropical savanna climate. Cameroon holds three main biomes: dry savanna**,** moist savanna, and tropical rain forest (Mesmin *et al*., 2001).

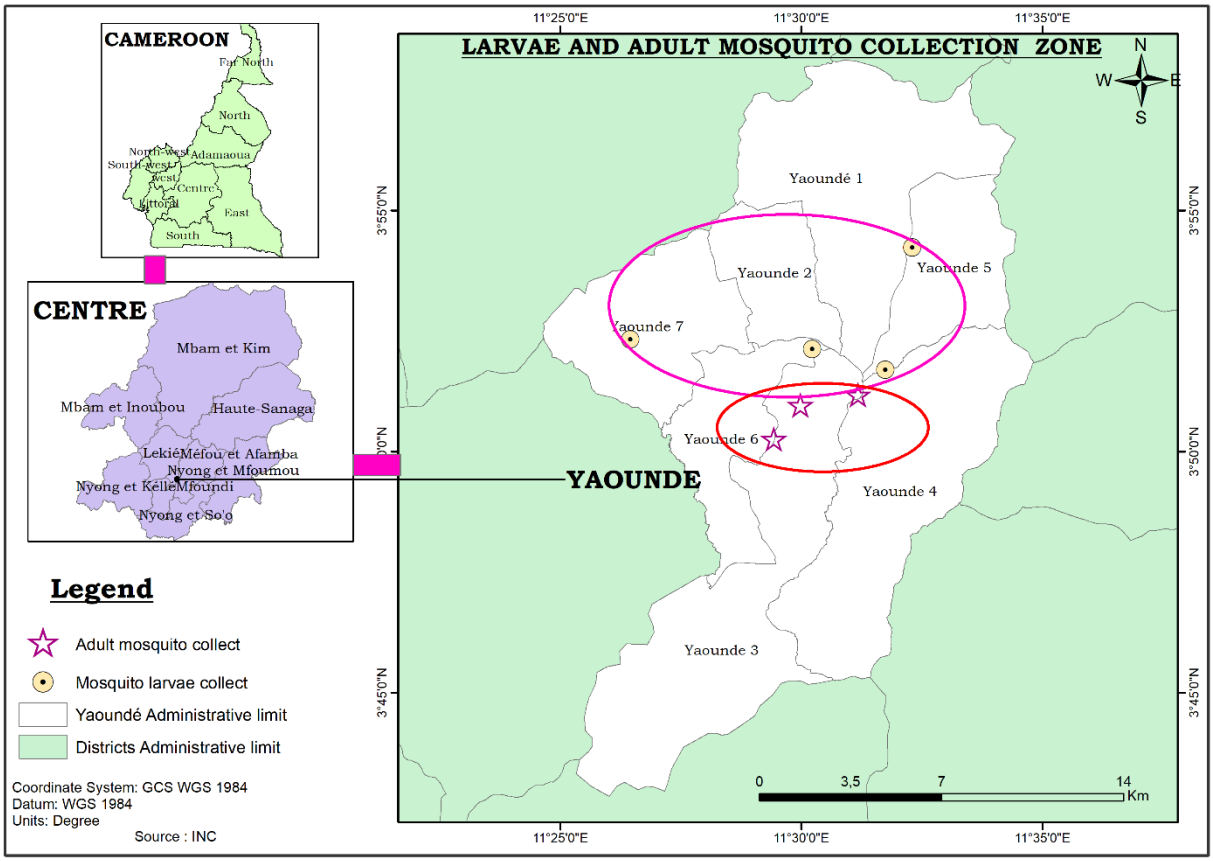

Figure 13: Study site and mosquitoes collection area

II.2. Material and methods

II.2.1. Collection and rearing of *Anopheles coluzzii* larvae

II.2.1.1. Field collection

Anopheles coluzzii larvae were collected from the field in April 2015 in Yaounde (Figure 13) by dipping the ladle into the larvae breeding grounds at Ngoussou, Nkolbisson, Nkoldongo and Messa. Larvae breeding grounds were quiet and sunny temporary waters. The harvest of larvae was made from 10:00 – 12:00 hours when waters were well brightened (Foko *et al,* .2011). The water containing the mosquito larvae was drawn by means of a ladle. Larvae were then transported into the laboratory in bowls (200 ml) and plastic containers (5 L) for breeding to adult stage.

II.2.1.2. Breeding of wild larvae in the laboratory

The wild Anopheles larvae were collected from the field in Yaoundé and taken to the laboratory for rearing. Larvae were identified using the method of Wondji *et al.* (2002) and were distributed in plastic dishes 11cm long, 7.5 cm width and 2.5 cm deep containing spring water. The water level was noted and water was added daily to compensate for evaporation. The larvae were fed with tetramine daily. The nymphs from the different dishes were pipetted and kept in plastic cups in cages. Both male and female imagos were trapped in the cages. Emerging adults were identified using the identification key for species of the genus *Anopheles* by Gillies and Meillon (1968) (experiment carried out from April 2015 to August 2018). The mosquito net of the cages and the breeding materials were constantly washed and disinfected after each breeding cycle. The first-generation females from this strain first receive two consecutive blood meals to stimulate the gonotrophic cycle and obtain maximum eggs. Then a single meal was enough for the other generations. The ideal time for these two blood meals is on the third and fourth day of *Anopheles* adult life (Boudin *et al*., 1991). These adult females were first fed on human blood (the arm), then subsequent generations were fed on the rabbit.

II.2.1.3. Breeding of laboratory strain

Eggs were collected in the morning after they are laid and kept in Petri dishes closed with parafilm for 24 to 36 hours at room temperature for maturation. The eggs were hatched in the center of a hollowed-out paper which floats in the dish half-filled with spring water. We put about 400 eggs in each dish 11 cm long, 7.5 cm width and 2.5 cm deep 40 cm in diameter and 7.5 cm deep containing a liter of spring water and about 0.09g of food (Figure 14). The

larvae were fed with tetramine, a very rich food product originally designed for aquarium fish. The amount of food was 0.3mg/larva/day (Foko *et al* 2007).

Figure 14: Rearing of *Anopheles coluzzii*: Dish with larvae (A), Woody stand with replicates (B), Cages with adult mosquitoes (C).

II.2.1.4. Feeding of adult female mosquitoes in the laboratory using a rabbit

A rabbit of about 6 months old and weighing 5kg was brought from a research institute IRAD (Institut de Recherche Agronomique pour le Developpement) to be sure the organism is healthy. In the laboratory, the ventral part of the animal was shaved with a new blade. The animal was maintained immobile with the ventral part exposed. Mosquitoes were allowed to feed on the animal (Figure 15**)** for 15minutes. This accelerated the maturation of the eggs (Boudin *et al*, 1991). This level was permanently maintained in the laboratory according to the protocol described by Armstrong and Bransby (1961) and Coluzzii (1966).

Figure 15: Adult female mosquitoes feeding on a rabbit

II.2.2. Harvesting and processing of plants

The green leaves of *Petroselinum crispum, Ocimum basilicum* and *Cupressus lusitanica* were harvested locally early in the morning at about 8:10 am in Santa (Figure 16), located on latitude 5.45^{o} N and longitude 10.9^{o} E of the North West Region of Cameroon in March 2015. The leaves were plucked early in the morning and brought to the laboratory in plastic sampling bags. The taxonomic identification was done with the help of experienced botanists at the Department of Botany, St. Johns College, Agra, India. The voucher specimens were numbered and kept in our research laboratory for further reference.

The plants were selected on the basis of of ethanopharmacological information, aromatic smell ethanobotanical literature surveyed and the information gathered from native people living in that part of Cameroon. Specimens collected were brought immediately to Yaounde. The taxonomic identification was done with the help of experienced botanists at the Department of Botany, Higher Teacher Training College, Uiversity of Yaounde 1 and confirmed at the National Herbarium in Yaounde´ (Cameroon) referenced under the numbers 25583/SRF-Cam (*Petroselinum crispum)* 62102/SRF-Cam (*Ocimum basilicum)* and 17300/HNC (*Cupressus lusitanica*). Leaves were then transformed into powder, methanolic extract and essential oil for bioassays.

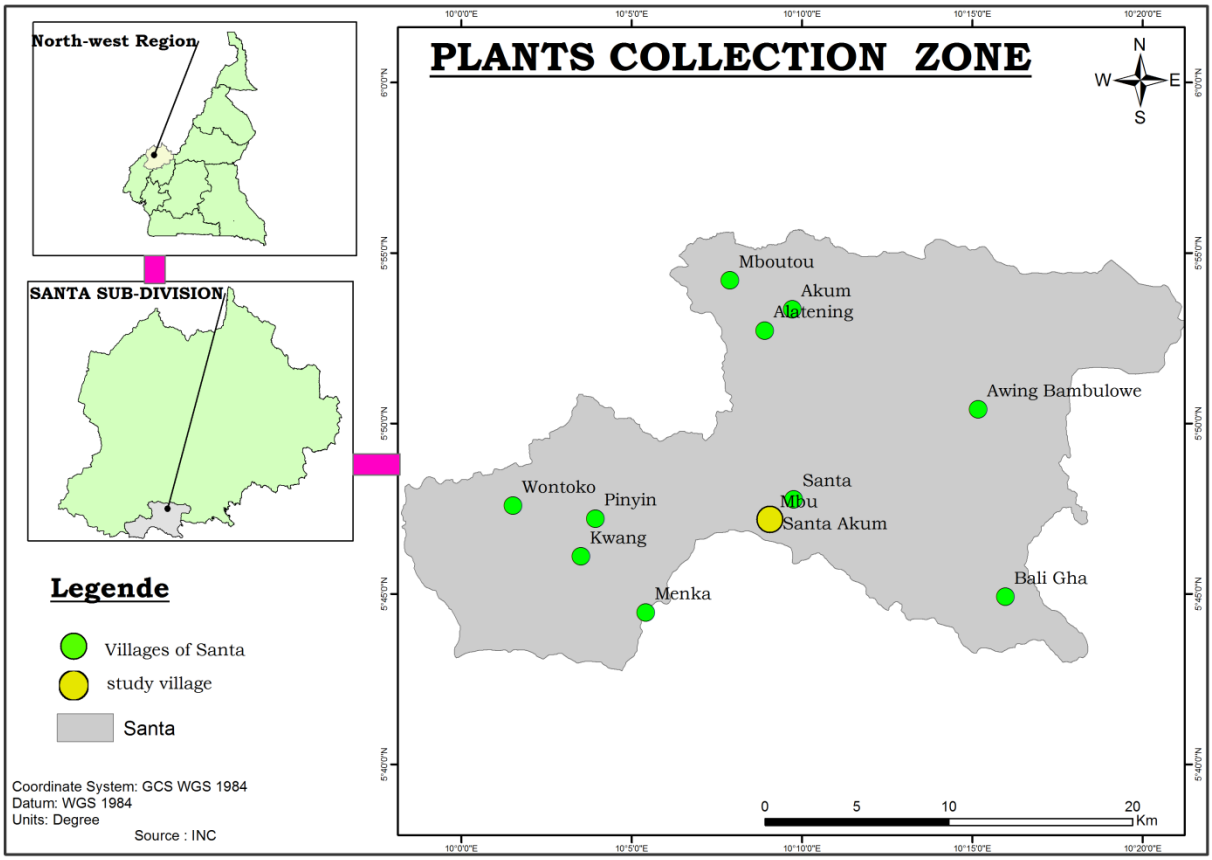

Figure 16: Plants Collection Area

II.2.2.1. Extraction of plant material

II.2.2.1.1. Preparation of leaf powders

Fresh leaves were cleaned and sliced to smaller pieces and dried at shade for 10-15 days in March 2015, then pulverized (reduced to fine partcles) with an electric blender. The grinded leaves were passed through 0.5 mm mesh size sieve and the powders obtained were packed in plastics, labelled and stored in the laboratory until used for extraction and bioassays. Part of the fresh leaves was used for hydrodistilation.

II.2.2.1.2. Methanolic extraction from leaf powders

Extraction of the leaf powders of *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* was carried out in the Chemistry Department laboratory, Higher Teacher Training College, University of Yaounde 1 (Cameroon) during the period from April to May 2015. The initial extraction was processed with the methanol solvent to obtain the residue called methanolic crude extract. The choice of methanol as an initial solvent for extraction was its ability to recover several phytochemical components, especially those which possess

insecticidal effects (Cowan 1999). To obtain the methanolic crude extract, 1000g of powder of each plant were macerated in 3000ml of methanol for 72 h at room temperature and then the maceration was filtrated using filter paper Whatman No.1. The residue of maceration was rinsed and filtrated several times with the fresh methanol until a clear phase was obtained. The filtrate was submmited to Rotary Evaporator apparatus to obtain a residue called crude extract. The yield of extraction was determined following the formula below: The methanolic extracts were then preserved in bottles (Figure 17 D) for bioassays.

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of leaf powder}} \times 100$$

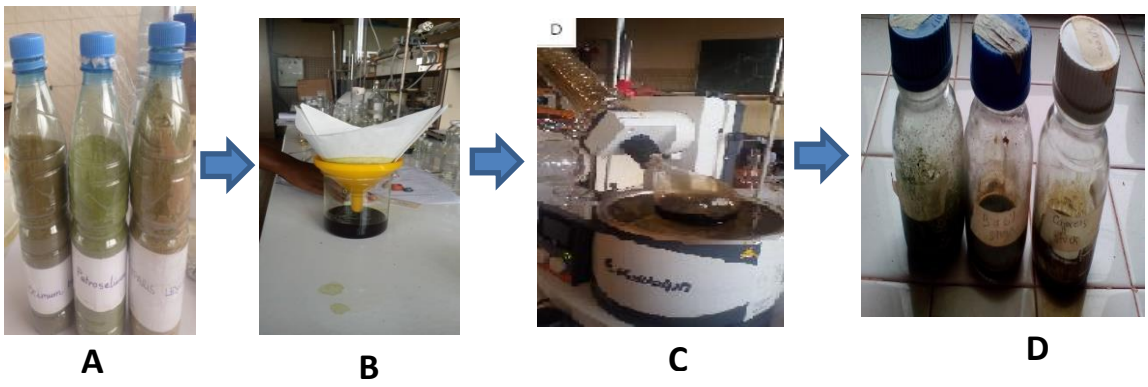

Figure 17: Methanolic extraction process (A = Powder, B = Filtration funnel, C = Rotary apparatus, D = Gummy substance)

II.2.2.1.3. Extraction of essential oils by the process of hydrodistillation

Hydrodistillation entails recovering essential oils from plants using water vapour. Fresh leaves of plant were taken to the Microbiology laboratory, University of Yaounde 1 in March 2015. Leaves were cleaned, weighed and packed in an apparatus, water was added in sufficient amount to immerse the plant and subjected to hydrodistillation for 5 hours using a Clevenger-type apparatus (Figure 18). The vapour mixture of water and oil was condensed by direct cooling with water. Condensed mixture was suspended in the condenser. The oil which is lighter than water was carefully removed from the mixture with a pipette. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C in refrigerator until needed for analysis and bioassay.

Figure 18: Clevenger-type apparatus;

II.2.2.2. Qualitative Phytochemical screening

II.2.2.2.1. Qualitative Phytochemical screening of powders and methanolic extract

The qualitative phytochemical analyses of the components responsible for toxicity on insects were carried out according to the methods of Harborne (1973) and Trease and Evans (1989). Each extract was screened for the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids and terpenoids as described below:

- **Test for Alkaloids**

To 5 g of extract or fraction placed in the test tube, 20mL methanol was added to the tube. The mixture was heated in water bath and allowed to boil for two minutes. It was cooled and filtered. 5mL of the filtrate was tested with two drops Wagner's reagent (solution of iodine and potassium iodide). To another portion of the extract, 2 drops of Hager's reagent (saturated picric acid solution) was added. The presence of yellow precipitate indicates alkaloid.

- **Test for Flavonoids (Sodium hydroxide test)**

The volume of 3 ml of 10% NaOH was added to 3 ml of the extract/fraction. A yellow or golden yellow coloration observed in each extract/fraction tested indicated the presence of flavonoids.

- **Test for Saponins (Foam test)**

The weight of 0.5 g of extract or fraction was shaken with 2 ml of distilled water. If foam produced persists for ten minutes it indicates the presence of saponins.

- **Test for Tannins**

To 1 mL of the extract, a few drops of ferric chloride reagent were added. The appearance of a dark blue or greenish black color showed the presence of tannins.

- **Test for phenolic group**

The quantity of 0.5 g of extract or fraction was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic groups.

- **Test for steroids (Libermann Burchard's test)**

0.1 g of extracts or fractions were treated with 2.5 mL chloroform and filtered. Then, 0.5 mL of the filtrates was treated with few drops of acetic anhydride, boiled and cooled. Then, 1 ml of concentration Sulphuric acid was added to form a lower layer. Formation of brown ring at the junction indicates the presence of steroids.

- **Test for Terpenoids (Salkowski's Test)**

Extracts or fractions were treated with chloroform and filtered. To 0.5 ml of the filtrates, few drops of concentration. Sulphuric acid were added, shakened and allowed to stand for 10 min.

Appearance of golden yellow colour indicates the presence of terpenoids.

II.2.2.2.2. Qualitative Phytochemical screening of essential oils

Essential oils analysis was assessed using Gas Chromatography coupled with Mass Spectrometry (GC-MS) as described by Govindarajan (2011), Granados-Echegoyen *et al*., (2014). Gas Chromatography-Mass Spectrometry (GC/MS) GC-MS Analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer

line temperatures were 220 °C and 250 °C, respectively. The oven program temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH2Cl2, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the m/z range 35-300; electron multiplier 1460 eV; scan rate, 2.96 scan/s. Qualitative analysis: The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of n-alkanes (C9–C22), and their mass spectra with those of the standards (for main components) and those found in the literature and supplemented by the NBS75K database and Wiley 7th NIST 2014 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software). Quantitative analysis: The percentage composition of the essential oils was computed by the normalization method from the GC/FID peak areas, assuming an identical mass response factor for all compounds

II.2.3. Mosquitocidal bioassays (April 2015- October 2019)

II.2.3.1. Determination of test concentration

The mosquito eggs, larvae and pupae were initially exposed to a wide range of test concentration and a control to establish the activity range of plant materials under test. After determining the mortality in this wide range of concentrations, a narrow range of 3 aliquots (0.1; 0.3, 0.5 g/mL; 0.1, 0.3, 0.5 mg/mL; 0.01, 0.03; 0.05 mg/mL for powder, methanolic extract and essential oil respectively). Yielding between 10% and 95% mortality in 24 hours were used to determinate LC_{50} and LC_{90} values. Four replicates and an equal number of controls were set up for each concentration simultaneously using spring water from Simbock neighbourhood. The volume of the stock solution was 1%, obtained by weighing 200mg of technical material and adding 20ml solvent (95% alcohol), shook vigorously to dissolve the material and was kept in screw-cap vial with aluminium foil over the mouth of vial. The stock solution was then serially diluted in alcohol (2ml of stock solution to 18ml of alcohol). Testing was done by pipetting 1ml of appropriate dilution and added to 99ml of spring water to have a total volume of 100ml. Ethanol only was added to spring water for the control. The experiment was carried out at the laboratory temperature between (26°C -28°C and 70-80% RH, 12:12 (L.D).

II.2.3.2. Effect of plant powder on the developmental stages of *An. coluzzii*

For ovicidal activity, the method of Kumar *et al*. (2012) was followed for the purpose. The freshly laid eggs were collected by providing ovitraps in mosquito cages kept 2 days after the female mosquitoes were given a blood meal. Indeed, 100 gravids female mosquitoes were

placed in a screen cage where ten oviposition filter papers were introduced for oviposition. The eggs were laid on filter paper No.1 provided in the ovitrap. Sixteen cups were prepared for each test. Out of these sixteen cups, twelve were filled with test concentration of powder of the leaves of *P. crispum, O. basilicum* and *C. lusitanica* which were completed with 99ml of spring water in each cup to obtain a volume of 100ml. Four cups were used for the control (using spring water only) and the experiment was repeated three times to reduce error. After 48h, the eggs were sieved through muslin cloth for hatching assessment after counting the eggs under microscope (Su and Mulla 1998). The percentage of unhatched egg was calculated on the basis of unopened opercula (Chenniappan and Kadarkarai 2008). The hatching rate of eggs was assessed after 48 h post treatment (Rajkumar and Jebanesan 2009). The duration of each developmental stage (eggs, larvae, pupae and adult) was noted using Dempster method. Mortality at different stages was also noted. The numbers of eggs laid by each female were counted. Their fecundity was evaluated through the number of eggs laid. The duration from of eggs to adults was also noted. The development time of insects in a given stage was the time taken for two-third (2/3) of the individuals in that stage to transform into the next stage (Demster, 1961). The experiment was carried out at the laboratory temperature of 26°C -28°C and 70-80% RH (relative humidity), 12:12 (L.D).

II.2.3.3. Ovicidal test of plant methanolic extract and essential oil of *An. coluzzii*

The WHO (2005, 2013) protocol with slight modification was used for the ovicidal activity of plant methanolic extract and essential oil. Freshly laid eggs were collected by providing ovitraps in mosquito cages kept 2 days after the female mosquitoes were given a blood meal. Indeed, 100 gravids female mosquitoes were placed in a screen cage where ten oviposition filter papers were introduced for oviposition. The eggs were laid on filter paper No.1 provided in the ovitrap. The eggs were counted using a microscope and a counter (Figure 19). Sixteen cups were prepared for each test. Out of these sixteen cups (each cup containing 25 eggs), twelve were filled with test concentration of methanolic extract and essential oils of the leaves of *P. crispum, O. basilicum* and *C. lusitanica* where 1ml of each concentration was pipetted and added to 99ml of spring water (from Simbock) in each cup to obtain a volume of 100ml. Four cups were were used for the control (99 ml of spring water and 1 ml of ethanol) for both metthanolic extract and essential oil. A minimum of 100 eggs were used for each treatment, and the experiment was repeated three times. After 48h, the eggs were sieved through muslin cloth for hatching assessment after counting the eggs under microscope as shown in figure 19 below (Su and Mulla 1998). The percentage of unhatched eggs was calculated on the

basis of unopened opercula (Chenniappan and Kadarkarai 2008). The hatching rate of eggs was assessed after 48 h post treatment (Rajkumar and Jebanesan 2009). The experiment was carried out at the laboratory temperature of 26°C -28°C and 70-80% RH (relative humidity), 12:12 (L.D).

Figure 19: Eggs counting using a counter and microscope (A = Microscope, B = Eggs on a filter paper)

II.2.3.4. Larvicidal test

The larvicidal activity of the powder, methaolic extracts and essential oils of *P. crispum, O. basilicum* and *C. lusitanica* were evaluated against the major urban mosquito vector *An. coluzzii* according to the method described by WHO (2005, 2013). The experiment was carried out at the laboratory temperature between 26°C-28°C and 70-80% RH (relative humidity), 12:12 (L.D). The breeding site was prepared as follows: Plastic cups (15cm in diameter and 5cm deep) containing 99ml of spring water and 1ml of each concentration was pipetted and added to the water to obtain a volume of 100ml solution. The control consisted of 1 ml of ethanol added to 99ml spring water to have a volume of 100ml. Twenty-five of each instar (first, second, third and fourth) larvae were transferred into each concentration solutions prepared and four replicates were maintained for each concentration. Mortality for each instar was recorded after 24 h and percentage-corrected mortality was determined using Abbott's formula.

$$\% \text{ corrected mortality} = \frac{\% \, observed \; deads - \% \, control \; deads}{100 - \% \, control \; deads} \, x \; 100$$

II.2.3.5. Pupacidal test

The pupacidal effect was assessed according to the method applied by WHO (2005, 2013) with some modifications. Twenty-five (25) pupae were transferred into plastic cup (15cm in diameter and 5cm deep) containing spring water. The concentrations of methanolic extracts (0.1, 0.3 and 0.5 g/ml) and essential oils (0.01, 0.03 and 0.05mg/mL) of the plants were then pipetted and added to the spring water to make a volume of 100 ml (1:99 respectively). Ethanol (1ml) was added also to 99ml spring water to obtain 100ml volume which constituted the control. Each treatment was repeated three times with four replicates in each concentration. The number of emerged adults for each replication was recorded after 48h. The experiment was carried out under the laboratory conditions (26°C-28°C temperature and 70-80% RH (relative humidity), 12:12 (L.D).

II.2.3.6. Adulticidal test using CDC bottles.

The solutions were prepared and the bottles coated according to the CDC (2010) protocol while bioassay procedure was performed following Aizoun et al.., (2013) method

II.2.3.6.1. Preparation of stock solutions

The bottles used for the bioassay were coated inside with the diagnostic dose of the insecticide under evaluation. The diagnostic dose was a determined amount of insecticide per bottle after preliminary screening test (4 concentrations were selected for the test (5-95% mortality)). The concentrations of 0.1, 0.3, 0.5, and 0.7mg/mL for methanolic extracts and 0.01, 0.03, 0.05 and 0.07 mg/mL for essential oils were obtained using the WHO protocol 2013 with slight modificatios.

II.2.3.6.2. Procedure for cleaning, drying and coating of the bottles

The bottles were washed with warm soapy water and rinsed thoroughly with tap water at least three times. Then, the bottles were placed in an oven (50°C) for 15–20 min or were left overnight until they were thoroughly dried. To assure that the cleaning procedure was adequate, some susceptible mosquitoes were introduced into a sample of recently washed and dried bottles. After making sure that bottles and caps were completely dry the caps were removed from the bottles and then, 1 ml of stock solution of each concentration of the prepared insecticide were used to coat the bottles. Ethanol (1 ml) was used for the control bottles. The content of each bottle was swirled and inverted by gently rotating so that the sides all the way around were coated. After that, the caps were removed and continued rolling bottles on their

side until all visible signs of the liquid are gone from inside when the bottles were completely coated. The bottles were left for 24 hours on their sides and covered with aluminum foil that will keep them protected from light.

II.2.3.6.3. Bioassay procedure

The bioassay was performed with the cleaned bottles of 250 ml (Figure. 20). Using a mouth aspirator, 25 mosquitoes (laboratory and field) were introduced into each test bottle including the control bottle. The bottles were examined to count the number of mosquitoes that were knockeddown (lying with their backs). The number of mosquitoes knocked down were subsequently recorded every 60 minutes and 24 h for the insects that died. However, data were grouped such that knockeddown counts were reported for 10, 20, 30, 40, 50 and 60 mins and 24h mortality. The mortality in the control bottle was also taken into consideration after 24 hours when reporting the results of the bioassay. Abbott's formula was used to correct results if the mortality in the control bottle was between 3% and 10% (Abbot 1925). The bioassay results were discarded, if mortality in the control bottle at the end of the test was >10%. Mosquitoes were considered dead if they could no longer stand.

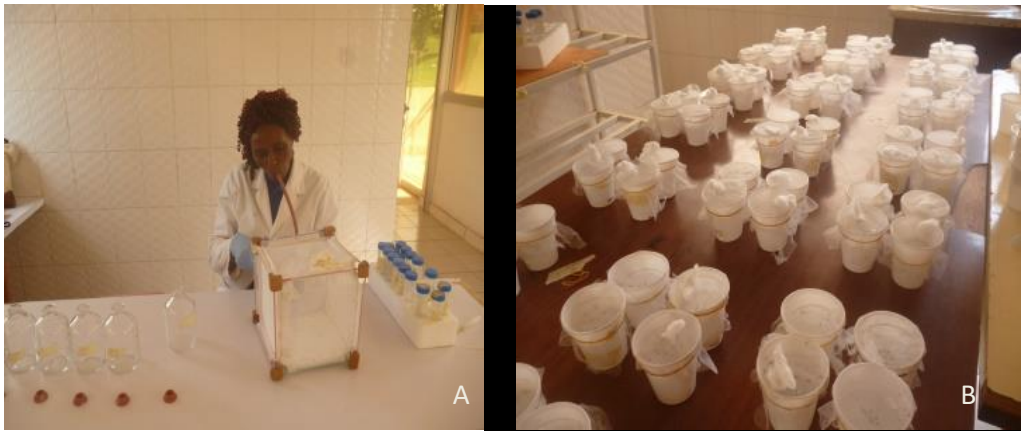

Figure 20: Knockdown test using CDC bottles (A = Cage with female mosquitoes; B = Plastic cups with adult mosquitoes for mortality test after 24h).

II.2.4. Repellent test on laboratory mosquitoes

To carry out the repellent test, the study was given an ethical approval from University of Douala Teaching Hospital by the University of Douala Ethics Review Committee. The acceptance with the reference number 2968 IEC-UD/03/2022/T (Appendix 9) was obtained after submitting the detailed proposal of the present project to the Ethics Review Committee

for proper study. Experiments were carried out against *An. coluzzii* under laboratory conditions according to the method performed by WHO (1996). A repellent effect of this essential oil was evaluated at the concentration of 0. 05 mg/mL by using human volunteers' arms (Figure. 21). Both the laboratory and field strains were tested (Appendix 10). No essential oil was used for the control. Previously, the arms of three human volunteers were washed and cleaned thoroughly with distilled water in the laboratory before application of the essential oil. Both arms were covered with rubber glove and a window area of 5 cm × 5 cm was opened on the dorsal part of the hand of the volunteers. The left hand was used for treatment and the right as control. After applying the test repellent, the volunteer was instructed not to rub, touch or wet the treated arm. The right hand, which acted as a control was treated only with ethanol and was exposed for up to 30 sec in a mosquito cage (30 cm × 30 cm × 35 cm) containing 25 nulliparous female mosquitoes (5-7 days old). If at least two mosquitoes landed on or bit the control arm, the repellency test was then continued for 3 min after every 30 min until 180 min. Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. An attempt of the mosquito to insert its stylets was considered as a bite. The experiments were repeated three times in separate cages and in each replicate different volunteer were used to nullify any effect of skin differences on repellency.

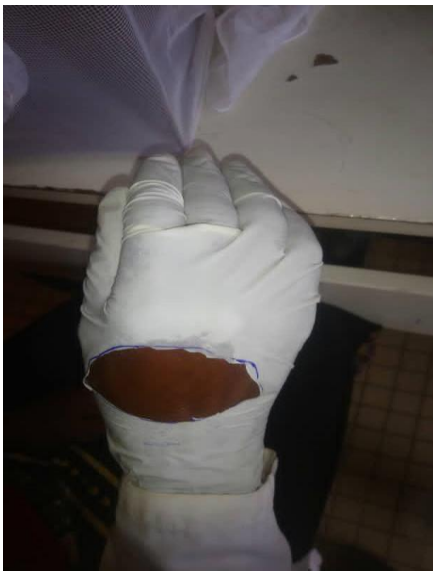

Figure 21: Laboratory repellent test using the arm

II.2.5. Acute mammalian toxicity test

The acute toxicity test on dragonfly larvae and gambusia fish was carried out in accordance with the guidelines of the Organization for Economic Cooperation and Development (Test no. 203, Acute Immobilization Test) (OECD Publishing, 2019).

II.2.5.1. Toxicity effect of plant extracts on dragonfly larvae

Dragonfly larvae (*Bracllythemis contaminata fabricius*) as show on Figure 22 were collected from the streams and stagnant water at Nkoldongo and Obobogo neighbourhoods (Yaounde). These organisms were brought to the laboratory and weighed. They were tested using *P. crispum*, *O. basilicum* and *C. lusitanica* products (powder, methanolic extracts and essential oil at different concentrations (0.1, 0.3, 0.5g/ml for powder, 0.1, 0.3, 0.5mg/mL for methanolic extracts ad 0.01, 0.03, 0.05mg/mL for essential oils). The larvae were monitored and mortality was recorded after 24hrs.

Figure 22: *Bracllythemis contaminata fabricius* (dragonfly larva)

II.2.5.2. Toxicity effect of plant extracts on gambusia fish.

The fish (*Gambusia affinis*) were collected from the streams at Messa, a quarter in Yaounde. These organisms were brought to the laboratory and weighed. They were tested using *P. crispum, O. basilicum* and *C. lusitanica* products (powder, methanolic extracts and essential oil at different concentrations (0.1, 0.3, 0.5g/mL for powder, 0.1, 0.3, 0.5mg/mL for methanolic

extracts and 0.01, 0.03, 0.05mg/mL for essential oils). The larvae were fed with tetrababy food and mortality was recorded after 24hrs.

Figure 23: *Gambusia affinis* (gambusia fish)

II.2.5.3. Acute oral toxicity: Wister rat's test

Acute toxicity studies (LD50) were measured using the method of Lorke (1989) and the OECD (Organization for Economic Corporation and Development) guideline for testing of chemicals (OECD 2012) in biochemistry laboratory of Catholic University of Cameroon Bamenda. Three groups of four rats (figure 24; 2 males and 2 females) with four replicates were orally administered separately 10% of body weight with powder (0.1, 0.3, 0.5g/m), methanolic extracts (0.1, 0.3, 0.5mg/mL) and essential oils (0.01, 0.03, 0.5mg/mL). They were observed before and after administration for any sign or symptom. Since no symptom or mortality was observed, the second phase was carried out with higher quantity, powder (0.7, 0.9, 1g/ml), methanolic extracts (0.7, 0.9, 1mg/mL) and essential oils (0.07, 0.09, 0.1mg/mL). They were observed for 24 hours then for 28 days. The numbers of deaths were recorded. The appearance and disappearance of toxic symptoms such as behavioral changes, locomotion, convulsions etc, were observed and recorded. The animals were observed constantly for the first 30 minutes after administration and thereafter 4 h up to 24 h, and subsequently once a day for 28 days.

Figure 24: Wister rats for oral toxicity

II.2.5.4. Acute dermal toxicity: Wister rat's test

The animal material chosen consisted of white rats (male and female) of Wistar strains, purchased at the Department of Animal Biology and Physiology and tested at the Biochemistry Annex Laboratory of the Department of Biochemistry University of Yaoundé I at room temperature with a 12-hour light / dark photoperiod cycle. These rats were used for the evaluation of anti-anopheles' activity. They had an average weight of 100 ± 20 g and were housed in plastic basins covered with wire netting, and received food and water at will. The litter used was sawdust, renewed twice a week to ensure the good hygienic condition of the animals. They were treated in accordance with the rules and regulations established by the European Union for Animal Safety (EUAS Council 86/609) and which have been adopted bythe

Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Operating mode: The acute dermal toxicity test (LD_{50}) was determined according to the OECD guidelines No.402 (OECD 1987). Thirty-two rats, i.e., 16 males and 16 females after an acclimatization period of 05 days were weighed, marked and distributed randomly by group of 08 rats (Figure 25 A; 04 males and 04 females with separation of sex) in basins covered with wire mesh, between treatment and control groups. Shortly before the test, approximately 10% of the fur from the body surface of the dorsal region of the trunk of the animals to be tested was shaved off (Figure 25 B). The animals were treated every day for a period of 21 days. For the

control group, the mowed dorsal region was coated with 1% of 95% ethanol, a solvent which was used to dissolve the essential oil.

Procedure:

Group I: The shaved back region was induced with an essential oil solution prepared at 0.1%;

Group II: The shaved back region was induced with an essential oil solution prepared at 0.3%

Group III: The shaved back region was induced with an essential oil solution prepared at 0.5%

Observation: Sometime before the application of the test substances, the clipped dorsal region of all animals was observed to note any particular marks present on the skin. During application of the test substance, the observations were made every day for the 21 days in order to detect any changes (skin rash: pimples, burning, redness, blackness, etc.) in comparison with the control group. The animals were observed constantly for the first 30 minutes after administration and thereafter every 4 h up to 24 h, and subsequently once a day for 21 days.

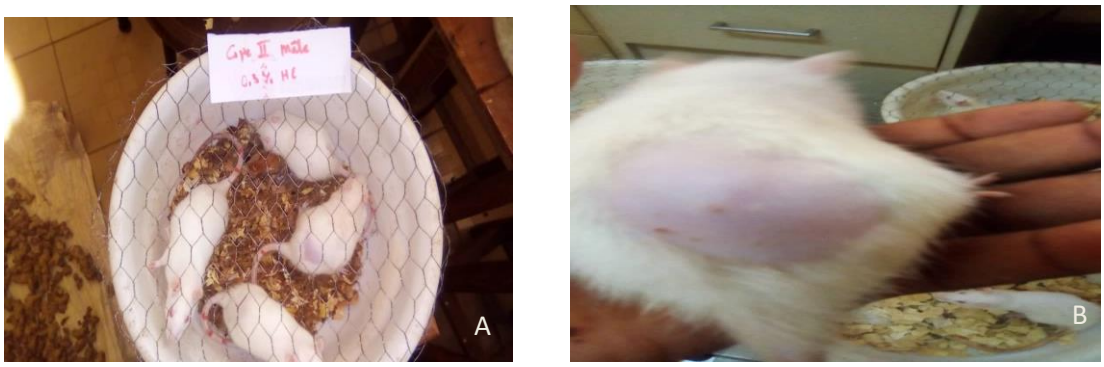

Figure 25: Dermal toxicity test using Wister rats (A = Rats feeding, B = Shaved rat)

II.2.6. Preparation of the *Petroselinum crispum* essential oils cream repellent

The cream repellent was formulated following the method used by Adeniran *et al*. (2012), • It consist of two phases: **(a)** hot phase **(**70°C water bath**):** Beeswax 20g, acetyl alcohol (Hexadecane) 10g and Shea butter 25g**. (b)** cooling phase **(-45°c):** Vitamin E 11g, *Petroselinum crispum* essential oil (0.1mg/mL) diluted in a light soya vegetable oil (1% dilution) and 10g oil colouring

Procedure: The ingredients of the hot phase were weighed and mixed in a Pyrex glass vessel. The vessel was placed in a water bath until the ingredients were completely melted. The vessel was removed from the water bath and allowed to cold at the temperature below 45 ° C. The mixture was poured into a different container and stirred slowly with a silicone spatula, then put in a plastic container.

II.2.7. Culicidian fauna sampling: nocturnal captures on human volunteers

II.2.7.1. Preliminary field test

This test was conducted at neighbourhood (Ngoa-Ekele) in Yaounde specifically at Cite des Nation environs using WHO protocol 2011 with slight modification. The experiment was carried out for three nights (from 7pm to 6am) using human baits. Twelve human volunteers were used, six applied the cream (1.5g) and six applied nothing and were used as control.

II.2.7.2. Field study

The collection sites for adult mosquitoes were chosen randomly. Collection was done from 7pm to 6 am using 36 human volunteers (Figure: 26) who willingly accepted to be part of the activity. The product was spread over the legs of volunteers, from the top of the knee to end of foot. The average amount of cream was 1.5g per volunteer; the repellent cream was spread by hand. Only outdoor mosquitoes were collected in homes. From the results of the preliminary study conducted at Ngoa Ekele, the test was further carried out in two other quarters (Olezoa and Biyem Assi). The test was done for 12days (6 days in each site) using 36 human volunteers (18 in each site) using WHO protocol 2011 with slight modification. The test was carried out for six nights each at Olezoa and Biyem Assi that were the study sites. The test was carried out from 7pm to 6am using human volunteers. For the 36 men that were used for the experiment, 18 applied the cream and 18 were used as control in Olezoa and Biyem Assi (18 men in each site). A pre-cotrol test was carried out for 3days before the test to know the natural behaviour of mosquitoes in that neighbourhood. During this testing no volunteer applied the cream. The volunteers sat on low stools 10 m equidistant from each other in a triangular formation. The mosquito reduction rate was calculated by using the Mulla formula (Mulla *et al*., 1971).

Reduction rate= $100-\frac{C1xT2}{C2xT1} \times 100$

C1= control before treatment, C2 = Ccontrol after treatment, T1 = to be treated, T2 = treated

Figure 26: Human landing catches

II.2.8. Statistical analyses

The Pearson's Chi-square and Kruskall-Wallis non-parametric tests were used to compare egg hatching and mortality rates by concentrations and formulations. The percentage of mortality, hatchability and repellent protection data were subjected to the ANOVA procedure using the Statistical Package for the Social Science (SPSS 16.0). Turkey's test at P = 0.05 was applied for mean separation. Probit analysis (Finney 1971; SPSS 16.0) was applied to determine lethal dosages causing 50% (LC50) and 90% (LC90) mortality of larvae, pupae and adults 24 h after treatment application for every stage. Abbott's formula (Abbott 1925) was used to correct for control mortality when mortality in the control were comprised between 3% and 10% before Probit analysis and ANOVA.

CHAPTER III: RESULTS AND DISCUSSION

III.1. RESULTS

III.1.1. Yield obtained from methanolic extract and essential oils

The results from the yield in methanolic extract and essential oils of each studied plant are presented in Table I. The yields obtained from the methanolic crude extract were 7.43% for *P. crispum,* 5.22% for *O. basilicum* and 8.73% for *C. lusitanica*. In the same line, the yields for essential oils were as follows, *P. crispum* (0.02%), *O. basilicum* (0.04%) and *C. lusitanica* (0.4%).

Table I: Extraction yields of the plant methanolic extracts and essential oils

Plant species	Plant products	Plant powder/ leaves used (g)	Extract/ essential oil obtained (mg)	Extraction yield (%)
Basilic (*Ocimum basilicum*)	Methanol extract	1239	64.67	5.22
	Essential oil	3275	1.31	0.04
Cypress (*Cypressus lusitanica*)	Methanol extract	913	79.71	8.73
	Essential oil	3570	14.28	0.4
Parsley (*Petroslium crispum*)	Methanol extract	1171	87.03	7.43
	Essential oil	3200	0.64	0.02

III.1.2. Qualitative phytochemical screening of plant

III.1.2.1. Qualitative phytochemical screening of plant powder and methanolic extract

The phytochemical components of the plant leaf powders and methanolic extracts are presented on Table II. The phytochemical screening of plant powder and methanolic extract revealed the presence of alkaloids, saponins and phenolic compounds in the plant powder macerated for 24 h in distilled water. In the methanolic extract of the plant, four phytoconstituents from the six targeted including alkaloids, terpenoids, saponins and phenolic compounds were present. In the plant powder, the same phytochemical compounds were present except terpenoids in parsley which were absent.

Table II: Phytochemical constituents of the plant leaf powders and methanolic extract

Plant species	Phytochemical components	Plant powder	Methanolic extract
Basilic (*Ocimum basilicum*)	Flavonoids	-	-
	Alkaloids	-	-
	Tannins	+	+
	Terpenoids	+	+
	Saponins	-	-
	Phenolic compounds	+	+
Cypress (*Cypressus lusitanica*)	Flavonoids	-	-
	Alkaloids	+	+
	Tannins	+	+
	Terpenoids	+	+
	Saponins	+	+
	Phenolic compounds	+	+
Parsley (*Petroslium crispum*)	Flavonoids	-	-
	Alkaloids	+	+
	Tannins	-	-
	Terpenoids	-	+
	Saponins	+	+
	Phenolic compounds	+	+

+ =present; - =absent

III.1.3. Chemical composition of the plant essential oils

III.1.3.1 *Petroselinum crispum* (Parsley)

Petroselinum crispum essential oil revealed the presence of 18 compounds (Table III). Among these phytoconstituents identified, myristicine was the major compounds representing 67.1% of overall constituents identified.

Table III: Chemical constituents of essential oil of *Petroselinum crispum* leaf

No.	RT (min)	Compounds	Proportion (%)
1	7.01	β-Myrcene	0.3
2	7.68	p-Isopropyltoluene	0.5
3	7.75	Limonene	0.3
4	7.81	1-Isopropyl-4-methylenebicyclo[3.1.0]hexane	0.9
5	8.79	α,p-Dimethylstyrene	0.4
6	8.89	Linalool	1.1
7	10.07	3-Thujen-2-one	0.8
8	10.27	4-Terpineol	2.1
9	10.32	m-Methylacetophenone	0.9
10	10.36	4-(1-Methylethyl)-2-cyclohexen-1-one	2.9
11	10.49	Estragole	0.8
12	12.65	α-Terpineol acetate	0.7
13	14.97	Myristicine	67.1
14	15.74	β-sesguiphelandrene	5.3
15	15.96	Bisabolene <(E)-iso-ɣ>	8.6
16	16.48	Sesguisabene hydrate	2.6
17	16.77	Apiol	3.6
18	20.37	3,4α,7,7,10α-Pentamethyl-3-vinyldodecahydro-1H-benzo[f]chromene	1.0
		Total	**99.9**

RT= retention time

III.1.3.2. *Ocimum basilicum* (Basil)

Ocimum basilicum essential oil revealed the presence of 19 compounds (Table IV). Among these phytoconstituents identified, Linalol was the major compound representing 65.7% of overall constituents identified.

Table IV: Chemical constituents of essential oil of *Ocimum basilicum* leaf

No.	RT (min)	Compounds	Proportion (%)
1	7.85	Eucalyptol	8.9
2	8.48	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	0.8
3	9.00	Linalol	65.7
4	9.79	L-camphor	0.6
5	10.10	p-Menth-1-en-8-ol	0.4
6	10.28	L-4-terpineol	1.7
7	10.53	Estragole	9.2
8	11.24	p-Allylphenol	1.9
9	11.82	Bornyl acetate	0.3
10	12.55	exo-2-Hydroxycineole acetate	0.3
11	12.75	Eugenol	3.7
12	13.32	Methyleugenol	0.3
13	13.87	2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-norpinene	0.5
14	14.25	α-Caryophyllene	0.1
15	14.55	8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	0.2
16	14.93	Myristicin	1.1
17	16.20	1-Isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydro-4a(2H)-naphthalenol	0.4
18	16.39	Methyl-(3-oxo-2-[(2Z)-2-pentenyl]cyclopentyl) acetate	0.2
19	16.50	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol	3.6

RT= retention time

III.1.3.3. *Cupresuss lusitanica* (Cypress)

Cupresuss lusitanica essential oil revealed the presence of 27 compounds (Table V). Among these phytoconstituents identified, (-)-4-Terpineol was the major compounds representing 12.7% of overall constituents identified followed by (R)-(+)-α-Citronellol (11.5%) ad 4(10)-Thujene (10.5%).

Table V: Chemical constituents of essential oil of *Cupresuss lusitanica* leaf

No.	RT (min)	Compounds	Proportion (%)
1	6.12	1R-α-Pinene	3.9
2	6.43	Camphene	0.6
3	6.80	4(10)-Thujene	10.5
4	7.03	Myrcene	1.4
5	7.68	p-Cymene	1.0
6	7.78	D-Limonene	2.1
7	7.84	Eucalyptol	2.5
8	8.26	p-Mentha-1,4-diene	1.4
9	8.73	2-Nonanone	2.7
10	8.90	Linalol	4.8
11	9.78	(+)-2-Bornanone	5.5
12	10.09	3-Thujen-2-one	4.4
13	10.29	(-)-4-Terpineol	12.7
14	10.48	Terpineol	1.7
15	10.88	(R)-(+)-α-Citronellol	11.5
16	11.81	(-)-Bornyl acetate	2.6
17	11.96	Terpinene 4-acetate	2.6
18	12.66	α-Terpineol acetate	4.7
19	13.09	Isobornyl propionate	1.6
20	13.62	Bornyl butanoate	1.3
21	14.32	(+)-Epi-bicyclosesquiphellandrene	3.4
22	14.98	1-Isopropyl-4,7-dimethyl-1,2,4a,5,8,8a-hexahydronaphthalene	1.7
23	16.50	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol	3.6
24	16.65	α-Cadinol	5.1
25	17.36	6-(p-Tolyl)-2-methyl-2-heptenol	1.2
26	20.36	3,4a,7,7,10a-Pentamethyl-3-vinyldodecahydro-1H-benzo[f]chromene	4.1
27	21.71	1-(3-Hydroxy-3-methyl-4-pentenyl)-2,5,5,8a-tetramethyldecahydro-2-naphthalenol	1.4

RT= retention time

III.1.4. Mosquitocidal activity

III.1.4.1. Effect of plant powder on the developmental stages of *An. coluzzii*

III.1.4.1.1. Effect of *Petroselinum crispum* powder on the developmental stages of *An. coluzzii*

Table VI presents the effect of *P. crispum* leaf powder on the development stages of *An. coluzii* in the laboratory conditions. Globally, the plant powder significantly affected concentration-dependent of the developmental stages of the mosquito species studied. Applied at 0.5 g/mL, the plant powder significantly (H=14.28; P=0.003) inhibited mosquito eggs hatch up to 60% compared to concentrations of 0.3 g/mL (46%), 0.1 g/ml (23%) and control (5% inhibition rate). The number of eggs hatched into larvae significantly (H=14.28; P=0.003) decreased when plant powder quantity was increased. It was low in the solution test concentration of 0.5 g/ml (9.75 larvae) compared to the control (23 larvae). The average of larval mortality rate from 1st to 4th instar did not differ significantly in all concentrations tested (ranged 12-15% mortality), but were significantly (H=9.50, P=0.023) high compared to the control (2%). The plant powder did not significantly (H=5.49, P=0.139) influence the duration of egg hatch and that average duration ranged from 1 day in control and dose 0.1 g/ml to 2 days with concentrations 0.3 and 0.5 g/mL. The number of larvae turned into pupae significantly (H=14.01 P=0.003) decreased when plant powder dose augmented. The average number of pupae obtained at 0.5, 0.3 and 0.1 g/mL were 7, 10 and 16 pupae, respectively, compared to the control (23 pupae). At 0.1 g/mL, average duration time from pupa to adult was significantly (H=8.48; P=0.037) high (approximately 3 days) compared to 2 days registered in other concentration tests and control. The average number of emergences was significantly (H=14.15; P=0.003) high in the control (22 adults) compared to those recorded in tests (16, 10 and 7 adults when plant powder was applied at 0.1, 0.3 and 0.5 g/mL, respectively). Average number of eggs laid were significantly (H=13.82; P=0.003) high in the control (91 eggs) compared to the tests with the plant powder applied at concentrations of 0.1 g/ml (61 eggs), 0.3 g/ml (40 eggs) and 0.5 g/mL (27 eggs). Average duration of *An. coluzzii* cycle was 8 days, significantly (H=8.17; P=0.042) short compared to 10, 9 and 11 days registered when plant powder was applied at 0.1, 0.3 and 0.5 g/mL, respectively.

Table VI: Effect of *Petroselinum crispum* powder on the developmental stages of *An. coluzzii* in the laboratory conditions (T=25±2°C; 75±4% RH.)

Variables	Concentration (g/L)				*H-value*	*P-value*
	0	0.1	0.3	0.5		
Number of eggs used	25	25	25	25	-	-
% Egg hatch inhibition	5.00±1.00d	23.00±3.41c	46.00±2.00b	60.00±3.41a	14.28	0.003
Number of larvae obtained	23.75±0.25a	19.25±0.85b	13.50±0.50c	9.75±0.85d	14.28	0.003
% Mortality of larvae (L_1-L_4)	2.00±1.15b	12.00±2.82a	13.00±3.00a	15.00±1.91a	9.50	0.023
Duration egg-larva (days)	1.25±0.25a	1.50±0.28a	2.00±0.40a	2.25±0.25a	5.49	0.139
Number of pupae obtained	23.25±0.25a	16.25±0.75b	9.75±0.62c	7.00±0.57d	14.01	0.003
Average % mortality of pupae	2.00±1.15a	2.00±1.91a	1.00±1.00a	2.00±1.54a	0.92	0.820
Duration Larva-Pupa (days)	4.75±0.25a	5.50±0.28a	4.75±0.25a	5.25±0.25a	5.16	0.160
Number of adults emerged	22.75±0.25a	15.5±0.86b	9.50±0.50c	6.50±0.64d	14.15	0.003
Duration Pupa-Adult	1.50±0.28a	2.75±0.25b	2.25±0.25ab	2.00±0.00a	8.48	0.037
Number of females feed	10	7	4	3	-	-
Number of eggs laid	91.50±5.18a	61.00±5.46b	40.25±2.65c	27.25±2.17c	13.82	0.003
Average duration of cycle	7.50±0.64a	9.75±0.25b	9.00±0.40ab	9.50±0.28b	8.17	0.042

Mean±SE for each developmental stage following rows followed by the same did not differ significantly according to Bonferroni test (P=0.05).

III.1.4.1.2. Effect of *Cupressus lusitanic* plant powder on the developmental stages of *An. coluzzii*

Table VII presents the effect of *C. lusitanic* leaf powder on the development stages of *An. coluzii* in the laboratory conditions. Globally, the plant powder significantly affected concentration-dependent, the development stages of the mosquito species studied. Applied at 0.5 g/ml, the plant powder significantly (H=13.99; P=0.003) inhibited mosquito eggs hatch up to 60% compared to concentrations of 0.3 g/ml (46%), 0.1 g/ml (23%) and control (5% inhibition rate). The number of eggs hatched into larvae significantly (H=13.99; P=0.003) decreased when plant powder quantity was increased. It was low in the solution test concentration of 0.5 g/mL (10/25 larvae) compared to the control 23/25 larvae. The average of larval mortality rate from 1st to 4th instar did not differ significantly in all concentrations tested (ranged 12-15% mortality), but were significantly (H=12.75 P=0.005) high compared to the control (2%). The plant powder did not significantly (H=4.52 P=0.210) influence the duration of egg hatch and that average duration ranged from 1 day in control and dose 0.1 g/ml to 2 days with concentrations 0.3 and 0.5g/ml. The number of larvae turned into pupae significantly (H=13.75 P=0.003) decreased when plant powder dose augmented. The average number of pupae obtained at 0.5, 0.3 and 0.1 g/ml were 7, 10 and 16 pupae, respectively, compared to the control (23 pupae). At 0.1 g/ml, average duration time from pupa to adult was significantly

(H=12.97; P=0.004) high (approximately 3 days) compared to 2 days registered in other concentration tests and control. The average number of emergences was significantly (H=13.95; P=0.003) high in the control (22 adults) compared to those recorded in tests (16; 10 and 7 adults when plant powder was applied at 0.1, 0.3 and 0.5 g/ml, respectively). Average number of eggs laid were significantly (H=14.32; P=0.002) high in the control (92 eggs) compared to the tests with the plant powder applied at concentrations of 0.1 g/ml (61 eggs), 0.3 g/ml (44 eggs) and 0.5 g/ml (31 eggs). Average duration of *An. coluzzii* cycle was 8 days, significantly (H=13.67; P=0.042) short compared to 9 days registered when plant powder was applied at 0.1 and 0.3 g/mL respectively. Development did not continue in 0.5g/mL concentration. *Cupressus lusitanica* powder was more potent than *Ocimum basilicum* and *Petroselinum crispum*.

Table VII: Effect of *Cupressus lusitanic* powder on the developmental stages of *An. coluzzii* in the laboratory conditions (T=25±2°C; 75±4% RH.)

Variables	Concentration (g/mL)				*H-value*	*P-value*
	0	0.1	0.3	0.5		
Number of eggs used	25	25	25	25	-	-
% Egg hatch inhibition	5.00±1.00c	14.00±1.15b	22.00±2.58a	30.00±1.91d	13.99	0.003
Number of larvae obtained	23.75±0.25a	5.50±0.64b	3.50±0.28c	0.75±0.47d	13.99	0.003
% Mortality of larvae (L_1-L_4)	2.00±1.15c	3.00±1.91c	14.00±1.15b	22.00±2.58a	12.75	0.005
Duration egg-larva (days)	1.25±0.25a	1.25±0.25a	1.50±0.28a	2.00±0.00a	4.52	0.21
Number of pupae obtained	23.25±0.25a	4.00±0.91b	2.00±0.40bc	0.00±0.00c	13.75	0.003
Average % mortality of pupae	2.00±1.15a	0.50±0.28a	0.25±0.25a	-	3	0.392
Duration Larva-Pupa (days)	4.75±0.25b	7.50±0.28a	7.25±0.25a	-	13.55	0.004
Number of adults emerged	22.75±0.25a	3.50±0.95b	1.75±0.25b	-	13.95	0.003
Duration Pupa-Adult	1.50±0.28b	3.00±0.00a	3.00±0.40a	-	12.97	0.005
Number of females feed	10	7	4	-	-	-
Number of eggs laid	91.50±5.18a	44.00±1.77b	30.50±1.32b	-	14.32	0.002
Average duration of cycle	7.50±0.64b	9.25±0.25a	9.25±0.25a	-	13.67	0.003

Mean±SE for each developmental stage following rows followed by the same did not differ significantly according to Bonferroni test (P=0.05).

III.1.4.1.3. Effect of *Ocimum basilicum* plant powder on the developmental stages of *An. coluzzii*

Table VIII presents the effect of *Ocimum basilicum* leaf powder on the development stages of *An. coluzii* in the laboratory conditions. Globally, the plant powder significantly affected concentration-dependent, the development stages of the mosquito species studied. Applied at 0.5 g/ml, the plant powder significantly (H=14.32; P=0.003) inhibited mosquito eggs hatch up to 83% compared to concentrations of 0.3 g/mL (64%), 0.1 g/ml (43%) and control (5% inhibition rate). The number of eggs hatched into larvae significantly (H=14.32; P=0.003)

decreased when plant powder quantity was increased. It was low in the solution test concentration of 0.5 g/mL (9.75 larvae) compared to the control 23/25 larvae. The average of larval mortality rate from 1st to 4th instar did not differ significantly in all concentrations tested (ranged 17-57% mortality), but were significantly (H=9.14.28; P=0.023) high compared to the control (2%). The plant powder did not significantly (H=3.24 P=0.356) influence the duration of egg hatch and that average duration ranged from 1 day in control and dose 0.1 g/ml to 3 days with concentrations 0.3 and 0.5 g/ml. The number of larvae turned into pupae significantly (H=14.17 P=0.003) decreased when plant powder dose augmented. The average number of pupae obtained at 0.5, 0.3 and 0.1 g/ml were 3, 7 and 10 pupae, respectively, compared to the control (23 pupae). At 0.1 g/ml, average duration time from pupa to adult was significantly (H=6.14; P=0.105) high (approximately 3 days) compared to 2 days registered in other concentration tests and control. The average number of emergences was significantly (H=14.28; P=0.003) high in the control (23 adults) compared to those recorded in tests (10; 7 and 3 adults when plant powder was applied at 0.1, 0.3 and 0.5 g/ml, respectively). Average number of eggs laid were significantly (H=13.95; P=0.003) high in the control (91 eggs) compared to the tests with the plant powder applied at concentrations of 0.1 g/mL (49 eggs), 0.3 g/mL (22 eggs) and 0.5 g/ml (18 eggs). Average duration of *An. coluzzii* cycle was 10 days, significantly (H=12.91; P=0.005) short compared to 10, 11 and 12 days registered when plant powder was applied at 0.1, 0.3 and 0.5 g/mL, respectively.

Table VIII: Effect of *Ocimum basilicum* powder on the developmental stages of *An. coluzzii* in the laboratory conditions (T=25±2°C; 75±4% RH.)

Variables	**Concentration (g/ml)**				***H-value***	***P-value***
	0	0.1	0.3	0.5		
Number of eggs used	25	25	25	25	-	-
% Egg hatch inhibition	5.00±1.00d	43.00±1.00c	64.00±3.26b	83.00±3.00a	14.32	0.002
Number of larvae obtained	23.75±0.25a	14.25±0.25b	9.00±0.81c	4.25±0.75d	14.32	0.002
% Mortality of larvae (L_1-L_4)	2.00±1.15d	17.00±3.00c	36.00±3.26b	57.00±1.00a	14.28	0.003
Duration egg-larva (days)	1.25±0.25a	2.25±0.25a	2.00±0.40a	1.75±0.25a	3.24	0.356
Number of pupae obtained	23.25±0.25a	9.75±0.47b	6.50±0.50c	3.25±0.75d	14.17	0.003
Average % mortality of pupae	2.00±1.15a	3.00±1.91a	1.25±0.94a	2.25±1.03a	0.59	0.904
Duration Larva-Pupa (days)	4.75±0.25ab	5.50±0.28a	5.00±0.40ab	3.75±0.25b	9.02	0.029
Number of adults emerged	22.75±0.25a	9.50±0.28b	6.25±0.47c	2.75±0.47d	14.28	0.003
Duration Pupa-Adult	1.50±0.28a	2.50±0.28a	1.75±0.25a	2.00±0.00a	6.14	0.105
Number of females feed	10	7	4	3	-	-
Number of eggs laid	91.50±5.18a	49.00±2.12	22.25±2.01	18.50±1.55	13.95	0.003
Average duration of cycle	7.50±0.64c	9.75±0.25b	10.75±0.40ab	11.75±0.28a	12.91	0.005

Mean±SE for each developmental stage following rows followed by the same did not differ significantly according to Bonferroni test (P=0.05).

III.1.4.2- Ovicidal effect of plant extracts on *An. coluzzii* egg hatch

III.1.4.2.1. Effect of plant methanolic extracts on *An. coluzzii* egg hatch

Methanolic extracts significantly inhibited *An. coluzzii* eggs to hatch into larvae (Table IX). Treated with the methanolic extract, inhibition rate of mosquito egg hatch significantly (H = 18.33; P= 0.001) ranged from 40% at 0.1 mg/mL to 79% at 0.5 mg/mL for *Petroselium crispum*, significantly ((H = 18.59; P= 0.001) ranged from 21% at 0.1 mg/mL to 46% at 0.5 mg/mL for *Ocimum basilicum* and significantly (H = 18.29; P= 0.0001) ranged from 23% at 0.1 mg/mL to 59% at 0.5 mg/mL for *Cupresuss lusitanica*. No mosquito egg hatched was recorded with the positive control (Dichlovos) while no egg hatch inhibition was recorded in the negative control. Among the three plant extracts tested on *An. coluzzii* eggs, *P. crispum* extract at 0.5mg/mL was revealved to be the most potent (LC50 = 0.18%) as compare to *C. lusitanica* (LC50 = 0.16%) and *O. basilicum* (LC50 = 0.24%).

Table IX: Mosquito egg hatching Inhibition rate and LC_{50} as well as LC_{95} values (in %) of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselium crispum* methanolic extracts after 24h against *An. coluzzii* with Dichlovos as a positive control in the laboratory conditions (T=25±2°C; 75±4% RH).

Plant products	Conc (mg/mL)	% Inhibition of egg hatch	Slope ± SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	0	0.00±0.00e	1.44±0.13	0.66	0.24 (0.20-0.30)	2.40 (1.41-6.16)	14.73 ns
	0.1	21.00±2.51d					
	0.3	37.00±3.41c					
	0.5	46.00±1.19b					
	Dichlovos	100.0±0.00a					
	H-value	18.59					
	P-value	0.001					
Cupresuss lusitanica	0	0.00±0.00d	0.97±0.09	0.77	0.61 (0.61-0.81)	29.67 (13.48-95.39)	14.03 ns
	0.1	23.00±3.41c					
	0.3	40.00±1.91b					
	0.5	59.00±2.58b					
	Dichlovos	100.0±0.00a					
	H-value	18.29					
	P-value	0.001					
Petroselium crispum	0	0.00±0.00e	1.32±0.12	0.67	0.18 (0.13-0.22)	3.15 (1.55-13.46)	40.55 ***
	0.1	40.00±2.82d					
	0.3	51.00±3.41c					
	0.5	79.00±1.00b					
	Dichlovos	100.0±0.00a					
	H-value	18.33					
	P-value	0.001					

Mean±SE for each developmental stage following rows followed by the same letter did not differ significantly according to Bonferroni test (P=0.05).

III.1.4.2.2. Effect of plant essential oils on *An. coluzzii* eggs hatch

The plant essential oil significantly inhibited mosquito egg hatch (Table X) and varied significantly(H = 18.53; P= 0.001) ranged from 78% at 0.1 mg/mL to 100% at 0.5 mg/mL for *Petroselium crispum,* significantly ((H = 18.66; P= 0.001) ranged from 29% at 0.1 mg/mL to 77% at 0.5 mg/mL for *Ocimum basilicum* and significantly (H = 18.29; P= 0.0001) ranged from 43% at 0.1 mg/mL to 83% at 0.5 mg/mL for *Cupresuss lusitanica*. No mosquito egg hatched was recorded with the positive control (Dichlovos) while no egg hatch inhibition was recorded in the negative control. Among the three plant essential oils tested on *An. coluzzii* eggs, *P. crispum* essential oil at 0.5mg/mL was revealved to be the most potent (LC50 = 0.004%) as compare to *C. lusitanica* (LC50 = 0.014%) and *O. basilicum* (LC50 = 0.025%).

Table X: Mosquito egg hatch Inhibition rate and LC_{50} as well as LC_{95} values (in %) of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselium crispum* essential oils with Dichlovos as the positive control after 24h against *An. coluzzii* in the laboratory conditions (T=25±2°C; 75±4% RH).

Plant products	Conc (mg/mL)	% Inhibition of egg hatch	Slope ± SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	0	0.00±0.00e	1.66±0.13	0.71	0.025 (0.020-0.30)	0.241 (0.141-0.620)	40.17***
	0.1	29.00±1.91d					
	0.3	44.00±2.30c					
	0.5	77.00±1.00b					
	Dichlovos	100.0±0.00a					
	H-value	18.66					
	P-value	0.001					
Petroselinum cripsum	0	0.00±0.00d	1.53±0.17	0.36	0.004 (0.001-0.006)	0.042 (0.029-0.088)	46.26***
	0.1	78.00±2.58c					
	0.3	86.00±1.15b					
	0.5	100.00±0.00a					
	Dichlovos	100.0±0.00a					
	H-value	18.53					
	P-value	0.001					
Cupressus lusitanica	0	0.00±0.00e	1.50±0.13	0.60	0.014 (0.011-0.017)	0.174 (0.116-0.326)	21.12ns
	0.1	43.00±1.00d					
	0.3	64.00±3.26c					
	0.5	83.00±3.00b					
	Dichlovos	100.0±0.00a					
	H-value	18.65					
	P-value	0.001					

Mean±SE for each developmental stage following rows followed by the same did not differ significantly according to Bonferroni test (P=0.05).

III.1.4.3. Larvicidal activity of plant extracts on *Anopheles coluzzii* larvae

III.1.4.3.1. Toxicity of plant methanolic extracts on *An. coluzzii* larvae

The methanolic extract of *P. crispum* leaves significantly caused high mortality in *An. coluzzii* 1st, 2nd, 3rd and 4th instar larvae (Figure 27). The larvicidal effect of the methanolic extract increased with the increasing concentrations applied. No larval mortality was noticed in the negative control while 100% mortality was recorded against the all-development stages of *An. coluzzii* when the positive control (Dichlovos) was applied.

The mortality of the 1st instar *An. coluzzii* larvae in *P. crispum* varied significantly (H=14.24; P=0.003) from 48 to 81% when the plant methanolic extract was applied at 0.1 and 0.5 mg/mL, respectively (Appendix 1).

For the test against 2nd instar larvae of *An. coluzzii*, the methanolic extract of *P. crispum* caused a mortality rate that varied significantly (H=14.01; P=0.003) from 43% (at 0.1 mg/mL) to 79% (at 0.5 mg/mL), while that mortality rate ranged significantly (H=14.43; P=0.002) from 41% to 75% against the 3rd instar mosquito larvae. Against 4th instar larvae of *An. coluzzii*, the methanolic extract of *P. crispum* caused larval mortality Globally, LC_{50} and CL_{95} (mg/mL) values of the methanolic extract of *P. crispum* varied with the mosquito developmental stages.

The mortality of the 1st instar *An. coluzzii* larvae in *O. basilicum* varied significantly (H=16.38; P=0.003) from 51 to 99% when the plant methanolic extract was applied at 0.1 and 0.5 mg/mL, respectively.

For the tested against 2nd instar larvae of *An. coluzzii*, the methanolic extract of *O. basilicum* caused a mortality rate that varied significantly (H=16.53; P=0.002) from 36% (at 0.1 mg/mL) to 79% (at 0.5 mg/mL), while that mortality rate ranged significantly (H=16.52; P=0.002) from 31% to 60% against the 3rd instar mosquito larvae. Against 4th instar larvae of *An. coluzzii*, the methanolic extract of *O. basilicum* caused larval mortality varying significantly (H=16.57; P=0.002) from 38% (at 0.1 mg/mL) to 70% (at 0.5 mg/mL). Globally, LC_{50} and CL_{95} (mg/mL) values of the methanolic extract of *O. basilicum* varied with the mosquito developmental stages.

The mortality of the 1st instar *An. coluzzii* larvae in *C. lusitanica* varied significantly (H=17.68; P=0.001) from 55 to 100% when the plant methanolic extract was applied at 0.1 and 0.5 mg/mL, respectively. For the tested against 2nd instar larvae of *An. coluzzii*, the methanolic extract of *C. lusitanica* caused a mortality rate that varied significantly (H=17.55; P=0.001) from 39% (at 0.1 mg/mL) to 82% (at 0.5 mg/mL), while that mortality rate ranged significantly (H=17.61; P=0.001) from 36% to 65% against the 3rd instar mosquito larvae. Against 4th instar

larvae of *An. coluzzii*, the methanolic extract of *C. lusitanica* caused larval mortality varying significantly (H=17.35; P=0.002) from 26% (at 0.1 mg/mL) to 51% (at 0.5 mg/mL). Globally, LC_{50} and CL_{95} (mg/mL) values (Table XI) of the methanolic extract of *C. lusitanica* varied with the mosquito developmental stages.

The plant methanolic extract was most toxic against the 1st instar larvae (LC_{50}=0.12 mg/mL; LC_{95}=2.97 mg/mL) followed by 2nd (LC_{50}=0.15 mg/mL; LC_{95}=2.83 mg/mL), 3rd (LC_{50}=0.17 mg/mL; LC_{95}=5.44 mg/mL) and 4th (LC_{50}=0.20 mg/mL; LC_{95}=6.54 mg/mL) instar larvae. *Petroselinum crispum* was more effective than *O. basilicum* and *C. lusitanica*. Egg inhibition was due to egg contact with the products while larva mortality was due to contact and ingestion of the products

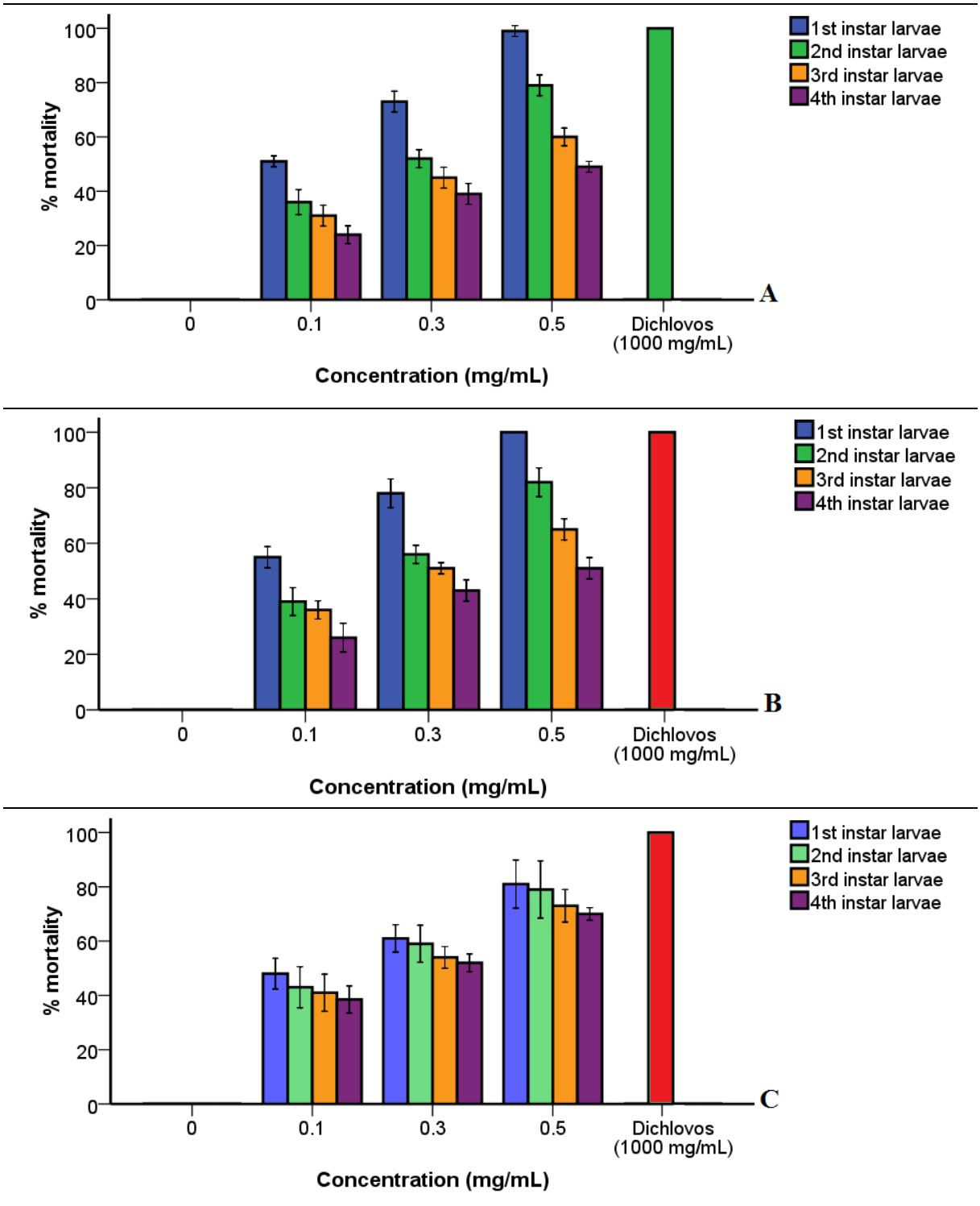

Figure 27: Mortality rate of *An. coluzzii* larvae exposed for 24h to methanolic extracts of *Ocimum basilicum* (A), *Cupresuss lusitanica* (B) and *Petroselinum crispum* (C) with Dichlovos as a positive control in the laboratory conditions.

Table XI: LC_{50} and LC_{95} (mg/mL) of the methanolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* instar larvae.

Plant species	Instar larvae	Slope±SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	1st	2.18±0.15	0.98	0.10(0.06-0.14)	0.61(0.41-1.31)	62.65***
	2nd	1.50±0.13	0.94	0.19(0.14-0.24)	2.42(1.32-7.76)	28.95**
	3rd	1.02±0.12	0.93	0.32(0.27-0.39)	12.97 (5.90-47.05)	8.35ns
	4th	1.00±0.11	0.69	0.53(0.43-0.70)	23.68(10.48-83.35)	4.56ns
Cupresuss lusitanica	1st	2.25±0.15	0.97	0.09(0.05-0.12)	0.51(0.31-1.02)	60.26***
	2nd	1.53±0.13	0.93	0.17(0.12-0.21)	2.02(1.14-6.14)	30.86**
	3rd	1.02±0.12	0.95	0.23(0.20-0.28)	9.69(4.65-30.09)	6.23ns
	4th	0.93±0.13	0.84	0.46(0.36-0.62)	24.13(9.19-126.23)	7.72ns
Petroselinum crispum	1st	1.18±0.12	0.83	0.12(0.06-0.17)	2.97(1.30-24.44)	34.04***
	2nd	1.28±0.13	0.79	0.15(0.08-0.20)	2.83(1.25-23.53)	41.61***
	3rd	1.10±0.12	0.86	0.17(0.12-0.23)	5.44(2.23-38.79)	22.32*
	4th	1.09±0.12	0.69	0.20(0.17-0.23)	6.54(3.52-17.27)	12.53ns

*P< 0,05 ; **P<0,01 ; ***P<0,001 ; CI= Confidence Interval ; LC= Lethal Concentration ; R^2= coefficient of determination ; χ^2= chi square ; SE = Standard Error

III.1.4.3.2. Lethal time of methanolic extract against mosquito larvae

The lethal time 50 of methanolic extracts against mosquito larvae were recorded in Appendix 2. It was observed that the lethal time 50 decreases with increasing concentrations, from 0.1mg/mL to 0.5mg/mL. For the concentration of 0.1mg/mL, the lethal time ranged from 3.4 days to 6.5 days. For the concentration of 0.3mg/mL, it ranged from 0.6 day to 5.8 days and for the concentration 0.5mg/mL it was from 0.2 day to 4.2 days. In the case of laboratory-reared mosquito larvae, the lethal time of parsley varied from 3.4 days to 6.1 days for the lowest concentration (0.1mg/mL). The lethal time was between 0.6 day to 5.3 days in the case of laboratory-reared mosquito larvae and 0.8 days to 5.8 days. The lethal time was between 0.2 days to 3.0 days in the concentration of 0.5mg/mL. Thus, the methanolic extracts had a significanth effect on laboratory-reared mosquito larvae ($p \leq 0.05$).

For the concentration 0.1mg/mL, the first instar was statistically the most affected in 2 cases out of 4 ($p < 0.05$). It was followed by the second, third instar and the fourth instar. For the concentrations 0.3mg/mL and 0.5mg/mL, the first instar was still the most affected ($p < 0.05$), followed by the third instar and the fourth instar. Parsley methanolic extract was the most effective extract followed by cypress methanolic extract and then basil methanolic extract on the different larvae stages at the concentrations 0.1mg/mL, 0.3mg/mL and 0.5mg/mL ($p < 0.05$).

III.1.4.3.3. Toxicity of plant essential oils on *An. coluzzii* larvae

Essential oil of *P. crispum* caused a significant dose-dependent larvicidal and pupicidal activities against 1st, 2 nd, 3rd and 4th instar larvae and pupae of *An. coluzzii* (Figure 28). The commercial insecticide Dichlovos caused 100% mortality of all mosquito developmental stages tested while no mortality of all stages was observed in the negative control. The mortality of

1st instar *An. coluzzii* larvae in *P. crispum* ranged significantly (H=18.09; P=0.001) from 58% (0.01 mg/mL) to 86% (at 0.05 mg/mL) (Appendix 3). When tested against 2nd instar larvae of *An. coluzzii*, larval mortality increased significantly (H=16.99; P=0.002) from 50% to 83% at concentrations of 0.01 and 0.05 mg/mL, respectively. Against 3rd instar larvae of *An. coluzzii*, larval mortality rate ranged significantly (H=17.80; P=0.002) from 47% (at 0.01 mg/mL) to 77% (at 0.05 mg/mL). On 4th instar larvae of *An. coluzzii*, a significant (H=17.05; P=0.002) larval mortality activity ranging from 26 to 58% was recorded when *P. crispum* was applied at doses of 0.01 and 0.05 mg/mL, respectively. Generally, the values of LC_{50} and LC_{95} (mg/mL) of *P. crispum* essential oil increased with mosquito instar larvae.

1st instar *An. coluzzii* larvae in *O. basilicum* ranged significantly (H=18.41; P=0.001) from 61% (0.01 mg/mL) to 99% (at 0.05 mg/mL). Tested against 2nd instar larvae of *An. coluzzii* larval mortality increased significantly (H=18.66; P=0.001) from 49% to 92% at concentrations of 0.01 and 0.05 mg/mL, respectively. Against 3rd instar larvae of *An. coluzzii*, larval mortality rate ranged significantly (H=18.48; P=0.001) from 41% (at 0.01 mg/mL) to 74% (at 0.05 mg/mL). On 4th instar larvae of *An. coluzzii*, a significant (H=18.35; P=0.001) larval mortality activity ranging from 37 to 63% was recorded when *O. basilicum* was applied at doses of 0.01 and 0.05 mg/mL, respectively. Generally, the values of LC_{50} and LC_{95} (mg/mL) of *O. basilicum* essential oil increased with mosquito instar larvae.

1st instar *An. coluzzii* larvae in *C. lusitanica* ranged significantly (H=18.09; P=0.001) from 63% (0.01 mg/mL) to 100% (at 0.05 mg/mL). Tested against 2nd instar larvae of *An. coluzzii* larval mortality increased significantly (H=16.99; P=0.002) from 53% to 99% at concentrations of 0.01 and 0.05 mg/mL, respectively. Against 3rd instar larvae of *An. coluzzii*, larval mortality rate ranged significantly (H=17.80; P=0.002) from 43% (at 0.01 mg/mL) to 79% (at 0.05 mg/mL). On 4th instar larvae of *An. coluzzii*, a significant (H=17.05; P=0.002) larval mortality activity ranging from 39 to 66% was recorded when *C. lusitanica* was applied at doses of 0.01 and 0.05 mg/mL, respectively. Generally, the values of LC_{50} and LC_{95} (mg/mL) of *C. lusitanica* essential oil increased with mosquito instar larvae.

Parsley essential oil was the most effective extractfollowed by cypress essential oil lastly basil. *Petroselinum crispum* essential oil was most effective against the 1st instar larvae (LC_{50}=0.007 mg/mL; LC_{95}=0.146 mg/mL) followed by 2nd (LC_{50}=0.010 mg/mL; LC_{95}=0.156 mg/mL), 3rd (LC_{50}=0.011 mg/mL; LC_{95}=0.257 mg/mL) and lastly 4th (LC_{50}=0.014 mg/mL; LC_{95}=0.123 mg/mL) instar larvae.

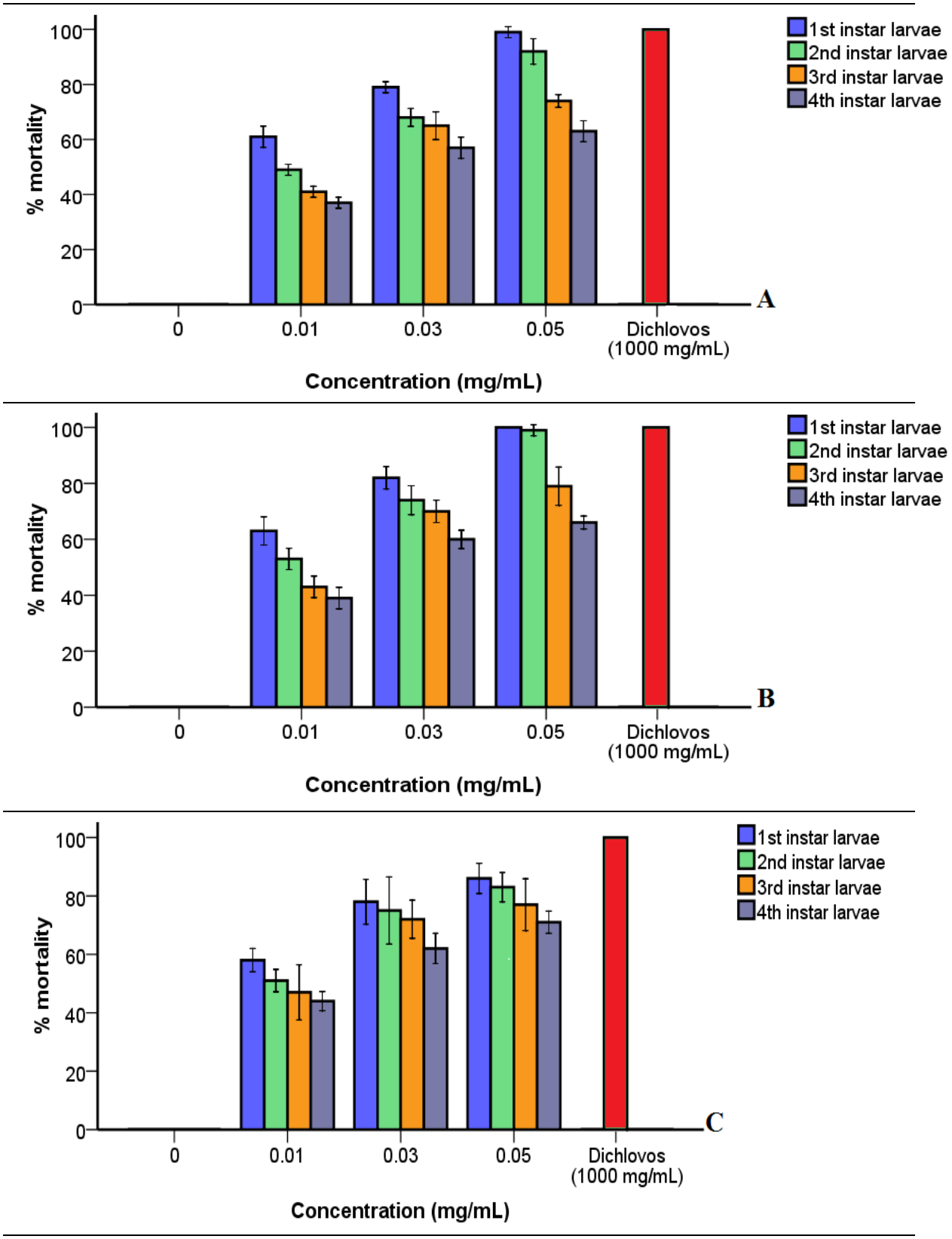

Figure 28: Mortality rate of *An. coluzzii* larvae exposed for 24h to essential oils of *Ocimum basilicum* (A), *Cupresuss lusitanica* (B) and *Petroselinum crispum* (C) with Dichlovos as a positive control in the laboratory conditions.

Table XII: LC_{50} and LC_{95} (mg/mL) of the essential oils of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* against An. coluzzii instar larvae

Plant species	Instar larvae	Slope±SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	1st	1.94±0.15	0.97	0.008(0.004-0.011)	0.055(0.038-0.115)	47.20***
	2nd	1.72±0.13	0.96	0.011(0.007-0.015)	0.102(0.066-0.234)	35.61***
	3rd	1.25±0.12	0.89	0.015(0.013-0.018)	0.310 (0.198-0.601)	4.70ns
	4th	0.96±0.12	0.85	0.021(0.018-0.025)	1.070(0.489-3.979)	4.68ns
Cupresuss lusitanica	1st	2.07±0.16	0.95	0.008(0.004-0.011)	0.047(0.033-0.094)	50.87***
	2nd	2.12±0.15	0.96	0.010(0.006-0.014)	0.060(0.037-0.137)	64.67***
	3rd	1.41±0.13	0.85	0.013(0.011-0.015)	0.190(0.135-0.308)	12.74ns
	4th	1.01±0.12	0.86	0.018(0.015-0.022)	0.778(0.387-2.437)	4.45ns
Petroselinum crispum	1st	1.24±0.13	0.79	0.007(0.004-0.010)	0.146(0.087-0.397)	19.02*
	2nd	1.33±0.13	0.61	0.010(0.005-0.013)	0.162(0.091-0.545)	28.18**
	3rd	1.20±0.13	0.66	0.011(0.006-0.016)	0.257(0.120-1.620)	32.24***
	4th	1.45±0.13	0.60	0.014(0.011-0.017)	0.123(0.061-1.349)	76.63***

*P< 0,05; **P<0,01; ***P<0,001; CI= Confidence Interval; CL= lethal Concentration; R^2= coefficient of determination; χ^2= chi square; SE = Standard Error

III.1.4.3.4. The lethal time 50 of essential oils against mosquito larvae

The lethal time 50 of essential oils against mosquito larvae were recorded in Appendix 4. For the concentration of 0.01mg/mL, the lethal time ranged from 2.1 days to 3.8 days. For the concentration of 0.03mg/mL, it ranged from 0.4 day to 5.6 days and for the concentration 0.5% it was from 0.2 day to 5.8 days. It was observed that the lethal time 50 decreases with increasing concentrations; from 0.01mg/mL to 0.05mg/mL. Concentration 0.03mg/mL and 0.5mg/mL had almost the same lethal effect ($p \leq 0.05$).

In the case of laboratory-reared mosquito larvae, the lethal time of parsley essential oil varied from 2.1 days to 3.7 days for the lowest concentration (0.01mg/mL), whereas for field-reared mosquito larvae it was from 2.3 days to 3.2 days. The lethal times for parsley and cypress essential oils were between 0.4 day to 3.0 days in the case of laboratory-reared mosquito larvae and 0.7 days to 5.6 days in the case of field-reared mosquito larvae for the concentration of 0.3%. The lethal time was between 0.2 day to 2.9 days in the case of laboratory-reared mosquito larvae and 0.2 days to 5.8 days in the case of field-reared mosquito larvae for the concentration of 0.05mg/mL. Thus, the essential oils were statistically more effective on laboratory-reared than on field-reared mosquito larvae ($p \leq 0.05$).

For the concentration 0.01mg/mL, the first instar was statistically the most affected in 2 cases out of 4 ($p < 0.05$). The third instar and fourth instar had almost the same susceptibility

with mortality higher in the third. For the concentrations 0.03mg/mL and 0.05mg/mL, the first instar was still the most susceptible ($p < 0.05$), followed by the third and the fourth instars.

Parsley essential oil was the most effective extract. It was followed by cypress essential oil and then basil essential oils on the different larvae stages at the concentrations 0.01mg/mL 0.03mg/mL 0.05mg/mL ($p < 0.05$).

III.1.4.4 Pupicidal Activity of plant extracts on pupae of *An. coluzzii*

III.1.4.4.1. Toxicity of plant methanolic extracts on pupae of *An. coluzzii*

Methanolic extract of *Cupresuss lusitanica* caused a significant pupal mortality varying from 37% (at 0.1 mg/mL) to 61% (at 0.5 mg/mL). The values of LC_{50} and LC_{95} (in %) of the plant extract registered were 0.27% and 8.17%, respectively against *An. coluzzii* pupae (Table XIII). Cypres methanolic extract was the most effective extract (Figure 29).

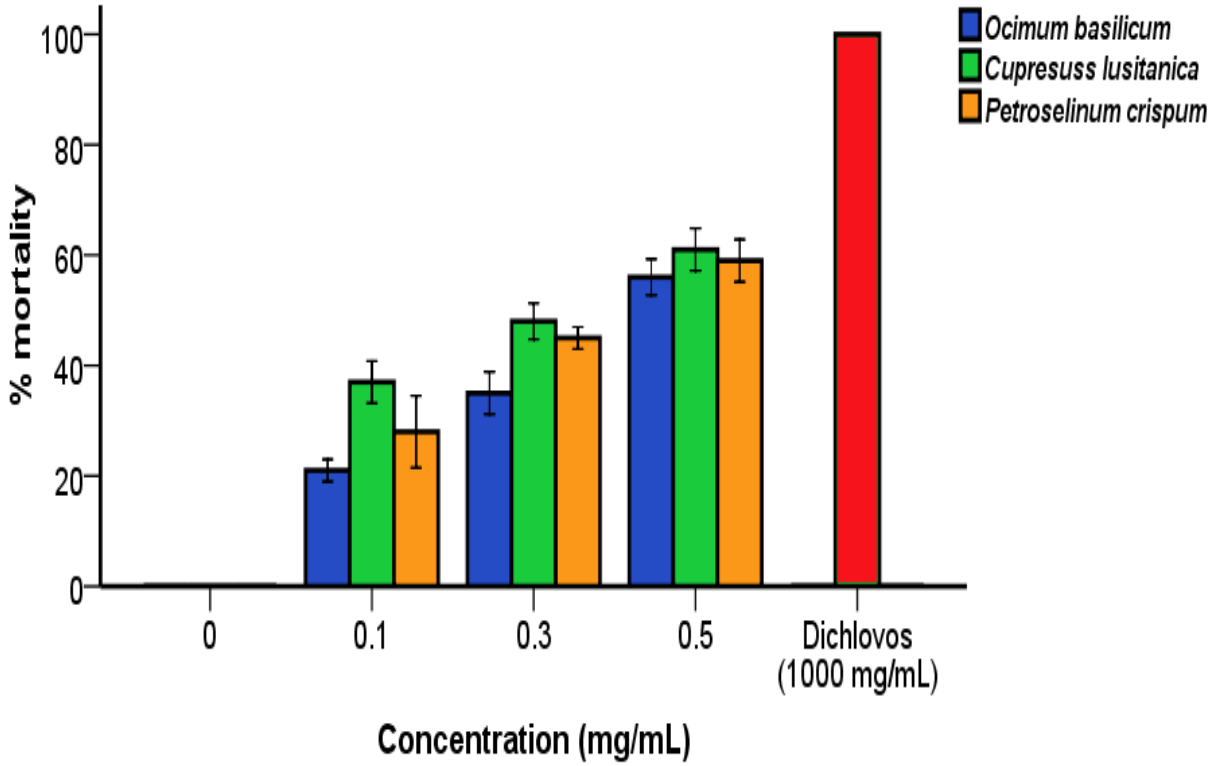

Figure 29: Mortality rate of *An. coluzzii* pupae exposed for 24h to methanolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* with Dichlovos as a positive control in the laboratory conditions.

Table XIII: LC_{50} and LC_{95} (mg/mL) of the methanolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* against *An. Coluzzii* pupae

Plant species	Slope±SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	1.31±0.13	0.95	0.45(0.39-0.55)	8.13(4.53-19.49)	12.15ns
Cupresuss lusitanica	0.82±0.12	0.90	0.27(0.22-0.33)	8.17(8.85-192.29)	7.95ns
Petroselinum crispum	1.14±0.12	0.90	0.33(0.29-0.40)	9.20(4.74-25.86)	11.44ns

CI= Confidence Interval; LC= lethal Concentration; R^2= coefficient of determination; χ^2= chi square; SE = Standard Error

III.1.4.4.2 Toxicity of plant essential oils on pupae of *An. coluzzii*

Against pupae of *An. coluzzii*, essential oil of *Cupresuss lusitanica* caused a significant pupal mortality ranging from 34% (at 0.01 mg/mL) to 58% (at 0.05 mg/mL), Cypres LC_{50} and LC_{95} (in mg/mL) values of the plant essential oil recorded were 0.0036 mg/mL and 3.851 mg/mL, respectively against pupae of the mosquito species assessed (Table XIV). Cypres essential oils was the most effective essential oils. It was followed by persley essential oils and then basil essential oils.

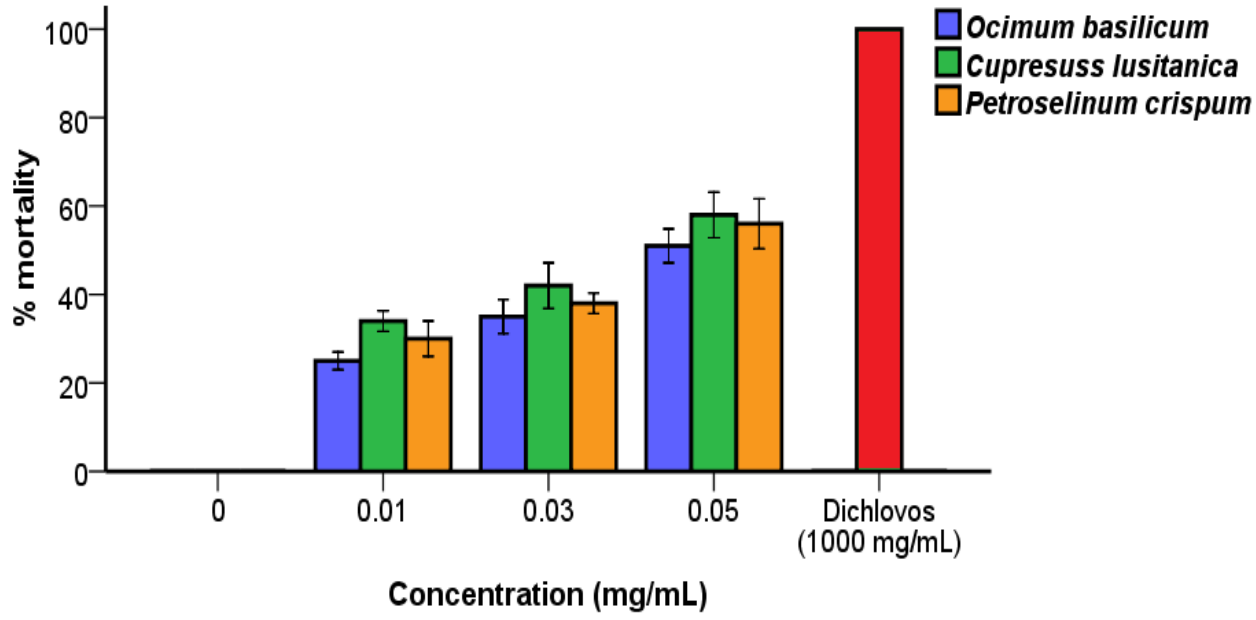

Figure 30: Mortality rate of *An. coluzzii* pupae exposed for 24h to essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* with Dichlovos as a positive control in the laboratory conditions.

Table XIV: LC_{50} and LC_{95} (mg/mL) of the essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* pupae

Plant species	Slope±SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Ocimum basilicum	0.95±0.13	0.91	0.057(0.045-0.081)	3.062(1.095-18.203)	9.75ns
Cupresuss lusitanica	0.81±0.12	0.84	0.036(0.030-0.049)	3.851(1.169-35.902)	14.11ns
Petroselinum crispum	0.89±0.12	0.85	0.044(0.033-0.073)	3.054(0.753-81.97)	16.07ns

CI= Confidence interval; LC= lethal Concentration; R^2= coefficient of determination; χ^2= chi square; SE = Standard Error

III.1.4.5. Adulticidal activity

III.1.4.5.1 Knockdown effect of plant methanolic extracts against *An. coluzzii* adults

Figure 31 presents the knockdown effect after 10, 20, 30, 40, 50 and 60 min of *An. coluzzii* adults (laboratory and field strain) exposed to different concentrations of methanolic extracts of *C. lusitanica, O. basilicum* and *P. crispum* in the laboratory conditions. Generally, the knockdown effect of the three plant methanolic extracts augmented with the increasing concentrations and the exposure period.

Tested with the methanolic extract of *C. lusitanica*, the knockdown activity after 10 min varied significantly (H=21.22; P=0.001) from 13% (at 0.1 mg/mL) to 45% (at 0.7 mg/mL) and after 60 min, the knockdown rate ranged significantly (H=21.67; P=0.001) from 41% (at 0.1 mg/mL) to 79% (at 0.7 mg/mL).

Similarly with *O. basilicum* methanolic extract, the knockdown effect ranged significantly (H=22.18; P<0.001) after 10 min from 6% (at 0.1 mg/mL) to 22% (at 0.7 mg/mL) and after 60 min, that activity varied significantly (H=22.16; P<0.001) from 33% (at 0.1 mg/mL) to 58% (at 0.7 mg/mL).

In the same way, the methanolic extracts of *P. crispum* caused a significant knockdown activity varying significantly (H=22.56; P<0.001) from 17% (at 0.1 mg/mL) to 60% (at 0.7 mg/mL) after 10 min and significantly (H=22.37; P<0.001) from 59% (at 0.1 mg/mL) to 100% (at 0.7 mg/mL) after 60 min.

Table XV presents KdC_{50} and KdC_{90} (mg/mL/bottle) values of the methanolic extracts of *C. lusitanica, O. basilicum* and *P. crispum* against *An. coluzzii* adults after 10, 20,30, 40,50 and 60 min post-exposure in the laboratory conditions. Comparing the efficacy of the three

plant essential oils, *P. crispum* (KdC_{50}=0.65 and 0.08 mg/mL/bottle after 10 and 60 min, respectively) was revealed to caused more knockdown effect on *An. coluzzii* adults compared to *C. lusitanica* (KdC_{50}=1.46 and 0.16 mg/mL/bottle after 10 and 60 min, respectively) and *O. basilicum* (KdC_{50}=3.62 and 0.47 mg/mL/bottle after 10 and 60 min, respectively).

The KdT_{50} and KdT_{90} (minutes) values of the essential oils of *C. lusitanica, O. basilicum* and *P. crispum* against *An. coluzzii* adults at 0.1, 0.3, 0.5 and 0.7 mg/mL/bottle in the laboratory conditions are presented in (Table XVI). Among the three plant essential oils tested against *An. coluzzii,* once again *P. crispum* (KdT_{50}=61.49 and 9.19 min after 10 and 60 min, respectively) achieved knockdown effect on *An. coluzzii* adults in short time compared to knockdown effect induced by *C. lusitanica* (KdT_{50}=106.58 and 17.44 min after 10 and 60 min, respectively) and *O. basilicum* (KdT_{50}=142.70 and 62.97 min after 10 and 60 min, respectively).

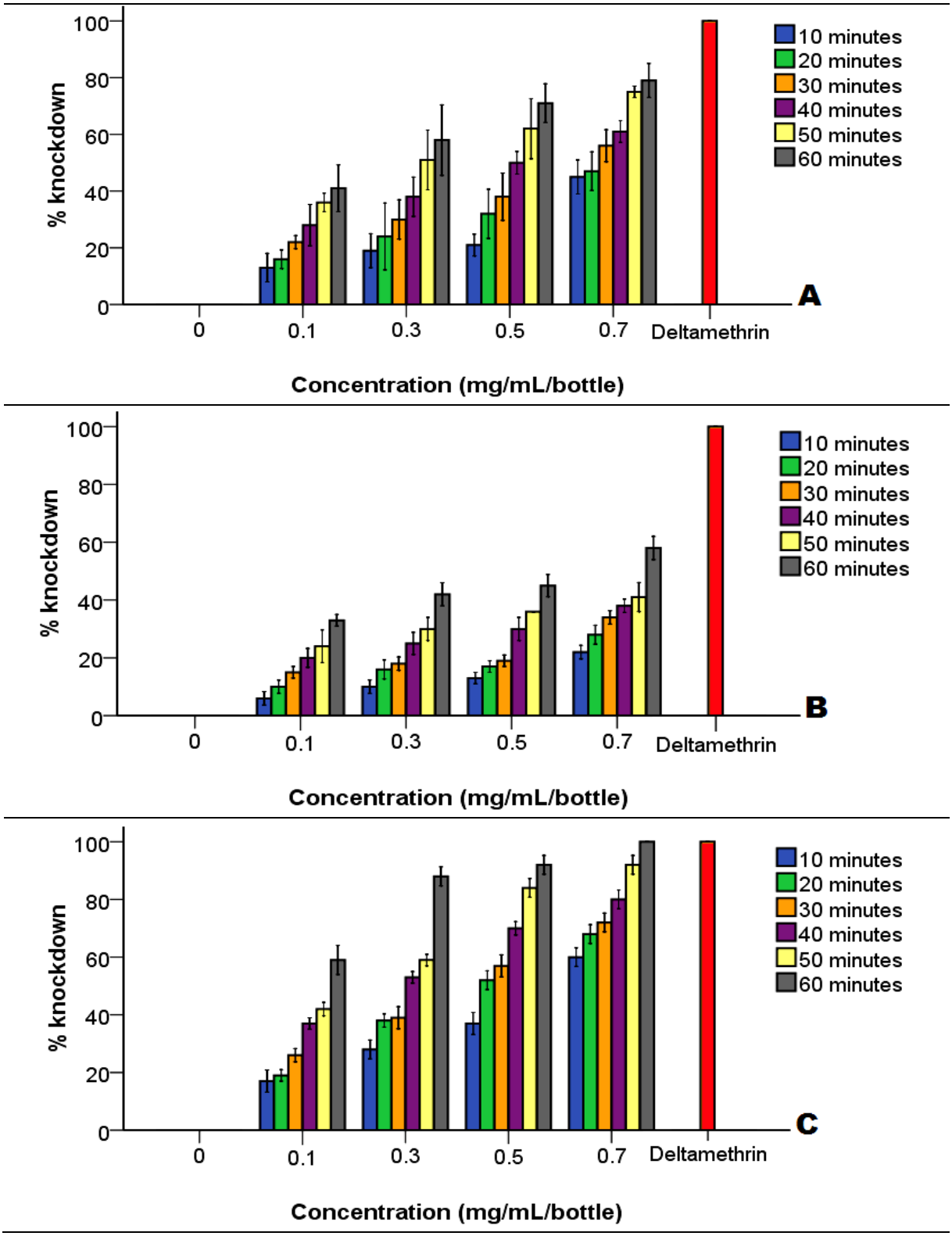

Figure 31: Knockdown effect after 10, 20, 30, 40, 50 and 60 mins of *An. coluzzii* adults exposed to different concentrations of methanolic extracts of *Ocimum basilicum* (A), *Cupresuss lusitanica* (B) and *Petroselinum crispum* (C) with Deltamethrin as a positive control in the laboratory.

Table XV: KdC_{50} and KdC_{90} (mg/mL/bottle) values of the methanolic extracts of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* adults after 10, 20, 30,40,50 and 60 mins post-exposure in the laboratory conditions.

Plant species	Time (min)	Slope±SE	R^2	KdC_{50} (CI 95%)	KdC_{90} (CI 95%)	χ^2
Cupressuss	10	1.10±0.09	0.82	1.46(0.97-3.01)	21.01(7.71-146.09)	58.83***
lucitanica	20	1.07±0.09	0.77	1.07(0.76-1.87)	16.75(6.66-92.50)	51.00***
	30	1.03±0.09	0.69	0.72(0.56-1.04)	12.70(5.77-49.19)	38.26**
	40	1.00±0.09	0.60	1.45(0.37-0.56)	8.64(4.60-23.40)	25.82ns
	50	1.13±0.10	0.42	0.23(0.18-0.28)	3.11(1.85-7.58)	38.46**
	60	1.18±0.01	0.33	0.16(0.12-0.21)	2.02(1.27-4.46)	44.86***
Ocimum	10	1.14±0.08	0.96	3.62(2.69-5.29)	48.43(26.77-105.54)	18.27ns
basilicum	20	1.03±0.06	0.93	2.76(2.12-3.83)	48.70(27.64-101.08)	21.38ns
	30	0.95±0.06	0.90	2.28(1.62-3.58)	50.66(23.66-149.38)	32.37*
	40	0.88±0.85	0.85	1.47(1.18-1.92)	41.48(23.76-84.97)	21.28ns
	50	0.84±0.05	0.80	1.10(0.90-1.41)	37.20(21.28-76.66)	23.17ns
	60	0.81±0.06	0.64	0.47(0.39-0.57	17.82(10.20-37.99)	17.26ns
Petroselium	10	1.31±0.11	0.67	0.65(0.53-0.85)	6.13(3.44-15.61)	34.18*
crispum	20	1.53±0.10	0.54	0.41(0.37-0.46)	2.84(2.16-4.06)	11.80ns
	30	1.38±0.10	0.49	0.34(0.31-0.38	2.90(2.15-4.30)	23.66ns
	40	1.33±0.10	0.36	0.20(0.17-0.22)	1.82(1.41-2.56)	13.90ns
	50	1.77±0.11	0.23	0.15(0.12-0.17)	0.80(0.64-1.07)	40.15**
	60	2.07±0.13	0.11	0.08(0.06-0.09)	0.33(0.28-0.39)	31.89*

KdC= knockdown concentration; CI= confident Interval; SE= Standard error; R^2= Coefficient of determination. nsP>0.05; *P<0.05; **P<0.01 and ***P<0.001. χ^2=Chi-square.

Table XVI: KdT_{50} and KdT_{90} (minutes) values of the methanolic extracts of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* adults at 0.01, 0.03, 0.05 and 0.07 mg/mL/bottle post-exposure in the laboratory conditions.

Plant species extracts	Concentrations (mg/mL/bottle)	slope±SE	R^2	KdT_{50} (CI 95%)	KdT_{90} (CI 95%)	χ^2
Ocimum basilicum	0.1	1.22±0.11	0.81	106.58(81.12-165.59)	1175.11(558.04-4129.02)	38.45*
	0.3	1.42±0.10	0.72	54.27(43.79-76.50)	432.05(221.90-1592.18)	96.46***
	0.5	1.71±0.10	0.87	35.49(31.28-40.80)	197.82(139.01-340.15)	64.45***
	0.7	1.18±0.10	0.85	17.44(13.29-21.05)	211.29(134.39-459.47)	53.44***
Cupressuss lucitanica	0.1	1.44±0.13	0.89	142.70(112.89-198.91)	1098.72(639.49-2401.26)	19.28ns
	0.3	1.32±0.12	0.88	113.21(92.26-150.49)	1044.42(615.65-2223.50)	26.75ns
	0.5	1.32±0.11	0.92	94.62(79.24-120.49)	878.43(538.81-1749.09)	25.83ns
	0.7	1.08±0.10	0.86	62.97(52.49-81.95)	948.02(489.81-2736.03)	30.41ns
Petroselium crispum	0.1	1.49±0.11	0.91	61.47(52.09-77.65)	443.43(270.28-964.96)	47.15**
	0.3	1.70±0.10	0.87	29.21(24.40-34.73)	164.65(109.44-337.72)	113.59***
	0.5	1.95±0.10	0.96	17.85(15.21-20.27)	80.99(66.44-106.75)	60.41***
	0.7	1.71±0.11	0.94	9.19(5.82-12.11)	51.92(41.49-73.78)	94.99***

KdT= knockdown time; CI= confident Interval; SE= Standard error; R^2= Coefficient of determination. nsP>0.05; *P<0.05; **P<0.01 and ***P<0.001. χ^2=Chi-square.

III.1.4.5.2. Knockdown effect of essential oils against *An. coluzzii* adults

Knockdown effect after 10, 20, 30, 40, 50 and 60 mins of *An. coluzzii* adults (laboratory strains) exposed to different concentrations of essential oils of *O. basilicum, C. lusitanica* and *P. crispum* is presented in Figure 32. Generally, the knockdown effect of the three plant essential oils increased with the increasing concentrations and the exposure period.

Tested with the essential oils of *C. lusitanica*, the knockdown activity after 10 min varied significantly (H=22.63; P<0.001) from 13% (at 0.01 mg/mL) to 59% (at 0.07 mg/mL) while after 60 min, the knockdown rate ranged significantly (H=22.11; P<0.001) from 72% (at 0.01 mg/mL) to 100% (at 0.07 mg/mL). With *O. basilicum* essential oil, the knockdown effect ranged significantly (H=22.01; P=0.001) after 10 min from 9% (at 0.01 mg/mL) to 28% (at 0.07) and after 60 min, that activity varied significantly (H=22.58; P<0.001) from 47% (at 0.01 mg/mL) to 94% (at 0.07 mg/mL). In the same way, the essential oils of *P. crispum* caused a significant knockdown activity varying significantly (H=22.60; P<0.001) from 18% (at 0.01 mg/mL) to 68% (at 0.07 mg/mL) after 10 min and significantly (H=22.83; P<0.001) from 77% (at 0.01 mg/mL) to 100% (at 0.05 mg/mL) after 60 min.

Table XVII presents KdC_{50} and KdC_{90} (mg/mL/bottle) values of the essential oils of *C. lusitanica, O. basilicum* and *P. crispum* against *An. coluzzii* adults after 10, 20, 30,40,50 and 60 min post-exposure in the laboratory conditions. Comparing the efficacy of the three plant essential oils, *P. crispum* (KdC_{50}=0.045 and 0.004 mg/mL/bottle after 10 and 60 min, respectively) was revealed to cause more knockdown effect on *An. coluzzii* adults compared to *C. lusitanica* (KdC_{50}=0.062 and 0.005 mg/mL/bottle after 10 and 60 min, respectively) and *O. basilicum* (KdC_{50}=0.406 and 0.013 mg/mL/bottle after 10 and 60 min, respectively) shown on Table XVII.

The KdT_{50} and KdT_{90} (minutes) values of the essential oils of *C. lusitanica, O. basilicum* and *P. crispum* against *An. coluzzii* adults at 0.01, 0.03, 0.05 and 0.07 mg/mL/bottle in the laboratory conditions are presented in Table XIII. Among the three plant essential oils tested against *An. colizzii,* once again *P. crispum* (KdT_{50}=30.77 and 7.87 min after 10 and 60 min, respectively) achieved knockdown effect on *An. coluzzii* adults in short time compared to knockdown effect induced by *C. lusitanica* (KdT_{50}=43.12 and 9.81 min after 10 and 60 min, respectively) and *O. basilicum* (KdT_{50}=87.04 and 26.81 min after 10 and 60 min, respectively).

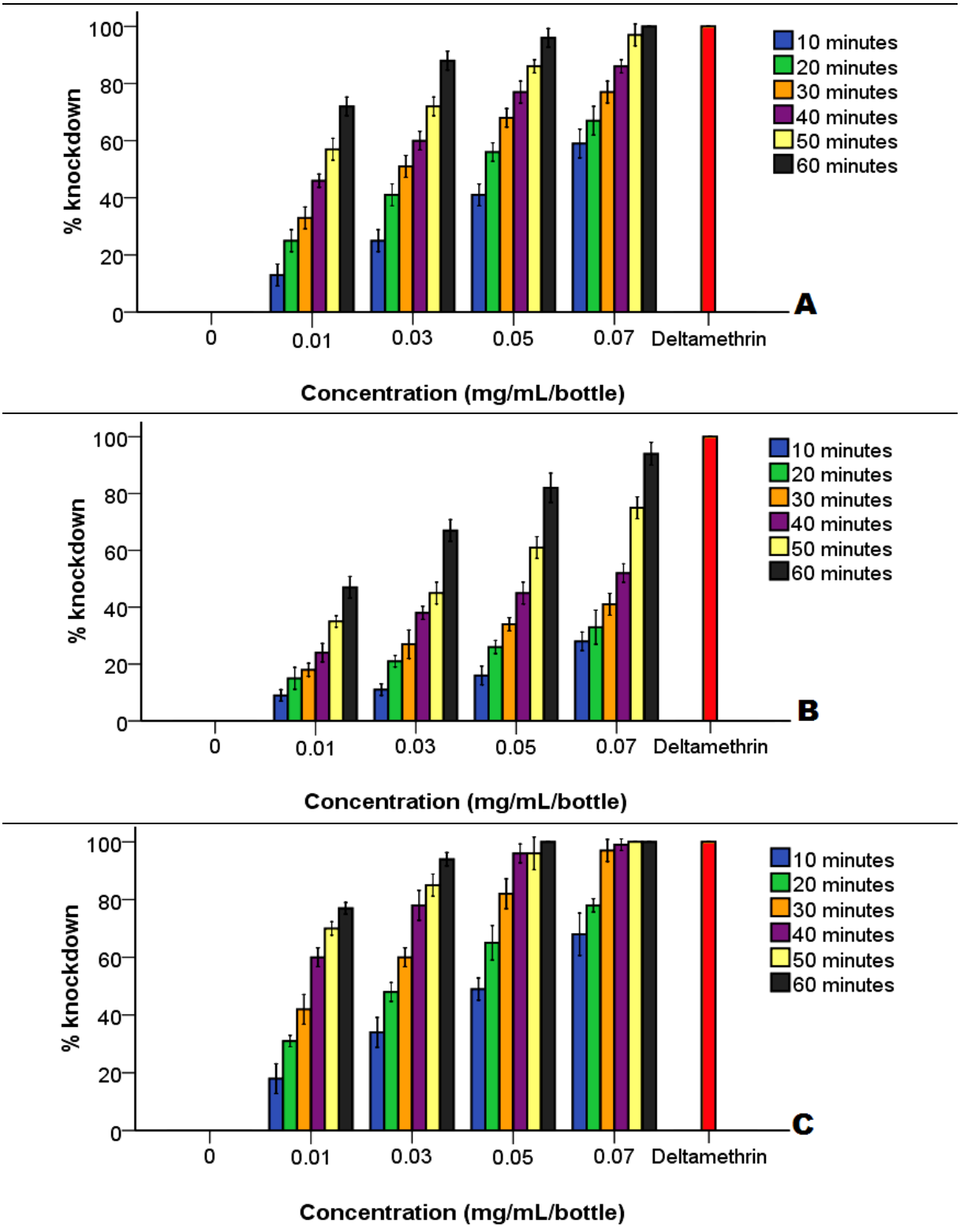

Figure 32: Knockdown effect after 10, 20, 30, 40, 50 and 60 mins of *An. coluzzii* adults exposed to different concentrations of essential oils of *Cupresuss lusitanica* (A), *Ocimum basilicum* (B) and *Petroselinum crispum* (C) with Deltamethrin (2.5 mg/mL) as a positive control in the laboratory.

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Table XVII: KdC_{50} and KdC_{90} (mg/mL/bottle) values of the essential oils of *Cupresuss lusitanica*, *Ocimum basilicum* and *Petroselinum crispum* against *An. Coluzzii* adults after 10, 20, 30-, 40-, 50- and 60-min post-exposure in the laboratory conditions.

Plant species	Time (min)	slope±SE	R^2	KdC_{50} (CI 95%)	KdC_{90} (CI 95%)	χ^2
Cupressuss lucitanica	10	1.58±0.12	0.66	0.062(0.054-0.075)	0.403(0.269-0.725)	27.56ns
	20	1.29±0.10	0.53	0.037(0.033-0.042)	0.362(0.257-0.577)	14.30ns
	30	1.37±0.10	0.39	0.023(0.020-0.026)	0.199(0.154-0.280)	14.10ns
	40	1.31±0.10	0.27	0.014(0.011-0.016)	0.130(0.104-0.176)	20.41ns
	50	1.56±0.11	0.16	0.009(0.006-0.012)	0.059(0.047-0.081)	42.63**
	60	1.81±0.14	0.09	0.005(0.003-0.007)	0.026(0.022-0.032)	30.87*
Ocimum basilicum	10	0.93±0.09	0.93	0.406(0.270-0.729)	9.427(3.862-34.770)	22.22ns
	20	0.81±0.07	0.89	0.256(0.185-0.397)	9.681(4.278-30.250)	14.70ns
	30	0.84±0.08	0.82	0.141(0.107-0.209)	4.627(2.054-15.996)	9.12ns
	40	0.88±0.09	0.69	0.065(0.054-0.084)	1.819(0.882-5.543)	5.87ns
	50	1.16±0.10	0.44	0.026(0.022-0.041)	0.327(0.210-0.644)	25.61ns
	60	1.65±0.11	0.21	0.013(0.010-0.015)	0.074(0.060-0.100)	36.38**
Petroselium crispum	10	1.56±0.11	0.55	0.045(0.039-0.053)	0.296(0.202-0.522)	33.56*
	20	1.43±0.10	0.40	0.025(0.023-0.028)	0.200(0.156-0.276)	19.56ns
	30	1.91±0.11	0.21	0.015(0.012-0.019)	0.071(0.055-0.102)	70.72***
	40	1.98±0.13	0.12	0.008(0.006-0.011)	0.037(0.031-0.048)	53.24***
	50	1.82±0.14	0.09	0.006(0.003-0.008)	0.029(0.024-0.038)	54.43***
	60	2.05±0.18	0.07	0.004(0.003-0.005)	0.018(0.016-0.021)	16.47ns

KdC= knockdown concentration; CI= confident Interval; SE= Standard error; R^2= Coefficient of determination. nsP>0.05; *P<0.05; **P<0.01 and ***P<0.001. χ^2=Chi-square.

Table XVIII: KdT50 and KdT90 (minutes) values of the essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* against *An. Coluzzii* adults at 0.01, 0.03, 0.05 and 0.07 mg/mL/bottle in the laboratory conditions.

Plant species extracts	Concentrations (mg/mL/bottle)	slope±SE	R^2	KdT_{50} (CI 95%)	KdT_{90} (CI 95%)	χ^2
Cupressuss lucitanica	0.01	2.30±0.12	0.95	43.12(39.17-48.25)	137.01(109.56-186.41)	56.79***
	0.03	2.05±0.11	0.96	24.87(22.37-27.38)	104.34(85.59-136-37)	50.51***
	0.05	2.06±0.10	0.97	15.09(12.94-17.06)	62.82(54.00-76.58)	48.59**
	0.07	2.08±0.12	0.94	9.81(6.98-12.27)	40.50(34.03-51.62)	94.35***
Ocimum basilicum	0.01	1.61±0.12	0.91	87.04(72.58-112.63)	541.82(339.78-1083.65)	33.91*
	0.03	1.99±0.11	0.93	50.39(45.18-57.73)	220.91(163.81-337.49)	46.42**
	0.05	2.23±0.11	0.94	36.40(32.50-41.21)	136.17(104.21-202.30)	83.47***
	0.07	2.16±0.10	0.91	26.81(22.24-31.70)	104.83(76.25-181.29)	170.32***
Petroselium crispum	0.01	2.19±0.11	0.96	30.77(28.69-32.98)	118.08(100.63-144.34)	30.33ns
	0.03	2.28±0.11	0.95	18.28(16.06-20.36)	66.41(57.01-81.15)	58.03***
	0.05	2.76±0.13	0.85	11.80(9.62-13.73)	34.27(30.00-40.56)	91.59***
	0.07	2.92±0.17	0.73	7.87(5.91-9.56)	21.58(18.93-25.15)	80.78***

KdT= knockdown time; CI= confident Interval; SE= Standard error; R^2= Coefficient of determination. P>0.05; *P<0.05; **P<0.01 and ***P<0.001. χ^2=Chi-square

III.1.4.5.3 Mortality of *An. coluzzii* adults caused by plant extracts

Figure 33 and Appendix 8 presents the mortality after 24 h of *An. coluzzii* adults exposed to different concentrations of methanol extracts (A) and essential oils (B) of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum.* Globlly, all the plant extracts and essential oils caused a significant mortality of *An. colizzii* adults and that mortality increased with the increasing concentrations.

For the plant extracts (Figure 33), the mortality of *An. coluzzii* adults varied significantly (H=21.83; P=0.001) from 48% (at 0.1 mg/mL/bottle) to 87% (at 0.7 mg/mL/bottle) with methanolic extract of *O. basilicum*; significantly (H=22.04; P=0.001) from 40% (at 0.1 mg/mL/bottle) to 69% (at 0.7 mg/mL/bottle) with methanolic extract of *C. lusitanica* and significantly (H=22.02; P=0.001) from 47% (at 0.1 mg/mL/bottle) to 77% (at 0.7 mg/mL/bottle) with methanolic extract of *P. crispum*.

In the same way, the mortality of *An. coluzzii* adults varied significantly (H=22.74; P<0.001) from 48% (at 0.01 mg/mL/bottle) to 100% (at 0.07 mg/mL/bottle) with essential oils of *O. basilicum*; significantly (H=22.58; P<0.001) from 42% (at 0.01 mg/mL/bottle) to 84% (at 0.07 mg/mL/bottle) with essential oils of *C. lusitanica* and significantly (H=22.78; P<0.001) from 60% (at 0.01 mg/mL/bottle) to 100% (at 0.7 mg/mL/bottle) with essential oils of *P. crispum* (Figure 33).

LC_{50} and LC_{90} (mg/mL/bottle) values of the methanolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* adults after 24 h post-exposure in the laboratory conditions is presented in Figure33. Among the three plant methanolic extracts, *O. basilicum* (LC_{50}= 0.11 mg/mL/bottle and LC_{90}= 2.15 mg/mL/bottle) and *P. crispum* (LC_{50}= 0.11 mg/mL/bottle and LC_{90}= 12.93 mg/mL/bottle) seemed to be more toxic against mosquito species assessed compared to *C. lusitanica* (LC_{50}= 0.25 mg/mL/bottle and LC_{90}= 35.44 mg/mL/bottle) methanolic extract. Similarly, between the three plant essential oils tested, *P. crispum* (LC_{50}= 0.008 mg/mL/bottle and LC_{90}= 0.029 mg/mL/bottle) essential oil was revealed to be the most potent against *An. coluzzii* adults compared to *O. basilicum* (LC_{50}= 0.012 mg/mL/bottle and LC_{90}= 0.055 mg/mL/bottle) and *C. lusitanica* (LC_{50}= 0.017 mg/mL/bottle and LC_{90}= 0.197 mg/mL/bottle) essential oils.

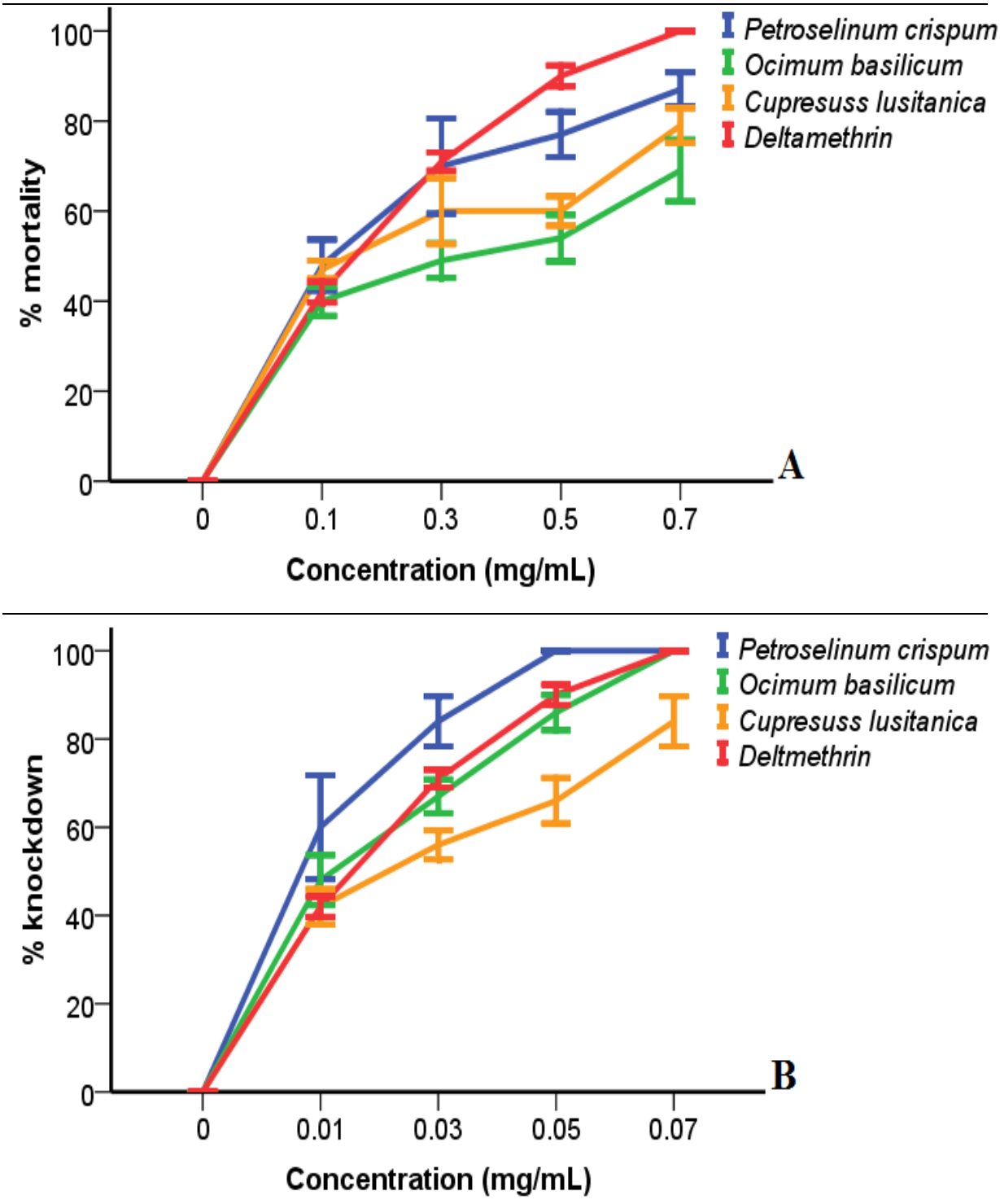


Figure 33: Mortality after 24 h of *An. coluzzii* adults exposed to different concentrations of methanolic extracts (A) and essential oils (B) of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* with Deltamethrin as a positive control in the laboratory.

Table XIX: LC_{50} and LC_{95} (mg/mL/bottle) values of the methanolic extracts and essential oil of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* against *An. coluzzii* adults after 24 h post-exposure in the laboratory conditions.

Products	**Plant species**	**Slope±SE**	**R^2**	**LC_{50} (CI 95%)**	**LC_{90} (CI 95%)**	**χ^2**
Methanol extracts	*Petroselinum crispum*	1.28±0.11	0.80	0.11(0.07-0.14)	2.15(1.36-4.59)	31.88**
	Ocimum basilicum	0.76±0.10	0.82	0.25(0.18-0.33)	35.44(9.72-76.40)	24.67*
	Cupresuss lusitanica	0.83±0.10	0.79	0.14(0.07-0.19)	12.93(4.39-47.50)	32.32**
Essential oils	*Cupresuss lusitanica*	1.98±0.12	0.17	0.012(0.009-0.016)	0.055(0.044-0.077)	73.33***
	Ocimum basilicum	1.21±0.10	0.34	0.017(0.013-0.021)	0.197(0.131-0.384)	35.40**
	Petroselinum crispum	2.37±0.14	0.10	0.008(0.006-0.011)	0.029(0.024-0.037)	69.35***

III.1.5. Mortality of dragonfly larvae and gambusia fish in plant extracts

III.1.5.1. Mortality of dragonfly larvae in plant extracts

III.1.5.1.1. Mortality of dragonfly larvae in plant powder

Dragonfly larvae live together with mosquito larvae and feed on them, thereby maintaining their population in the ecosystem. The effect of plant powder against dragonfly is summarized in Figure 34 for mortality and Table XX in terms of lethal dose. The LD_{50} of powder varied from 0.8% to 22.7% for dragonfly. Cypress powder was the most active with LD_{50} from O.8%. Parsley powder was the second more active with LD_{50} from 1.9% for dragonfly. Basil powder was the most tolera with LD_{50} from 22.7%. In the non-lethal concentrations, basil was the most tolerant having almost no effect on the dragonfly larvae (Table XXI).

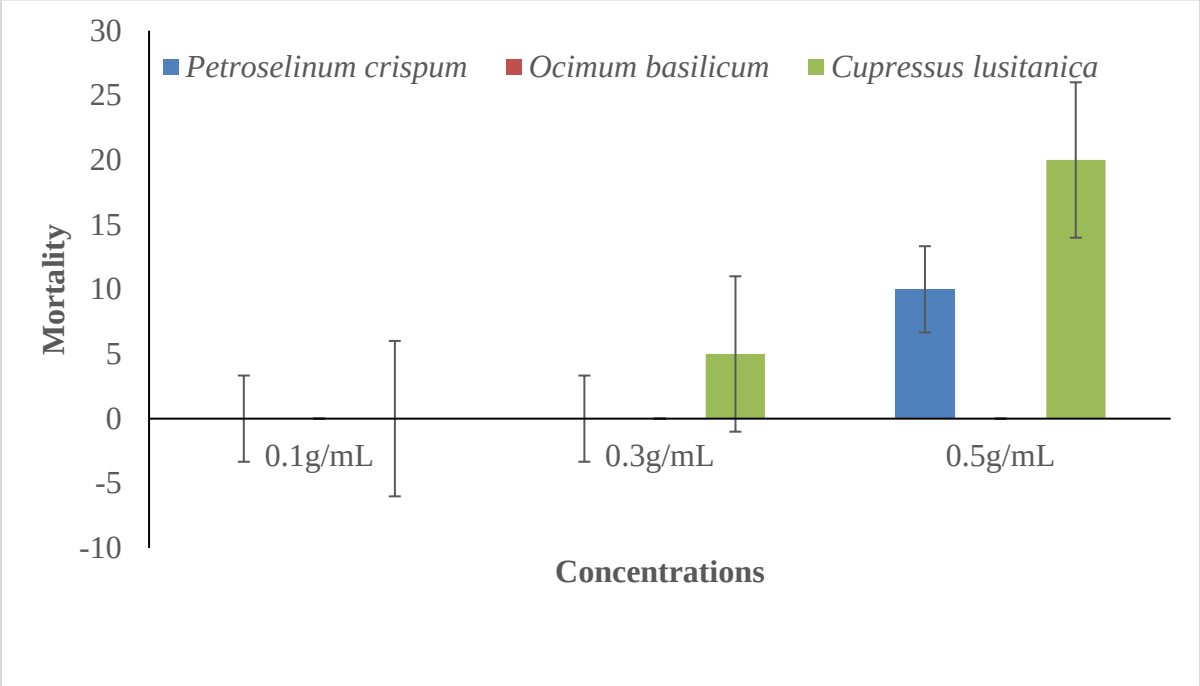


Figure 34: Mortality of dragonfly larvae in *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* powder

III.1.5.1.2. Mortality of dragonfly larvae in plant methanolic extracts

The effect of methanolic extract against dragonfly is summarized in Figure 35 for mortality and Table XX in terms of lethal dose. The LD_{50} of methanolic extracts varied from 5.1% to 22.2% for dragonfly. Parsley methanolic extract was the most active with LD_{50} from

5.1%. Cypress methanolic extract was the second more active with LD_{50} of 8.2% for dragonfly. Basil methanolic extracts was the least active with LD_{50} from 22.2%. In the non-lethal concentrations, basil was the most effective having almost less effect on the dragonfly larvae (Table XXI).

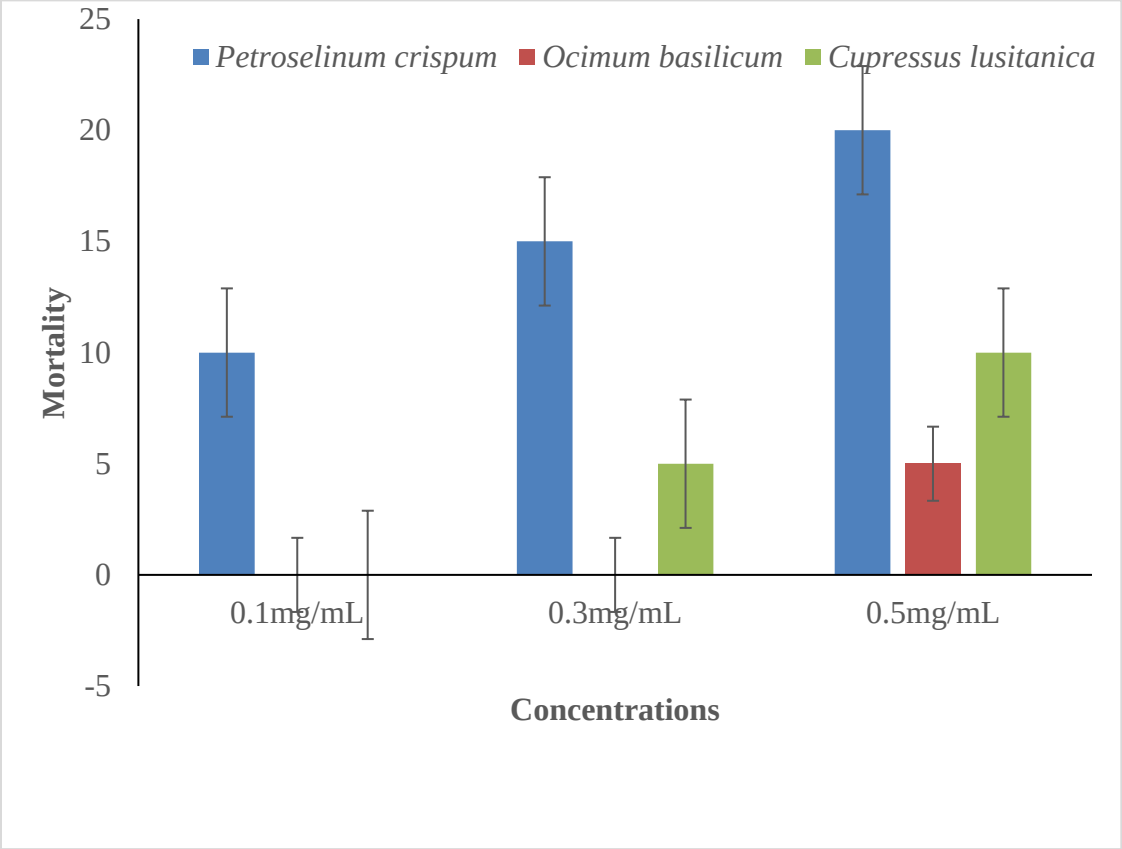


Figure 35: Mortality rate of dragonfly larvae in *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* methanolic extracts

III.1.5.1.3. Mortality of dragonfly larvae in plant essential oils

The effect of essential oil against dragonfly was summarized in Figure 36 for mortality and Table XX in terms of lethal dose. The essential oils were the most active with LD_{50} varying from 0.2% to 1.5%. Parsley essential oil s was the most active with LD_{50} from 0.2%. Cypress essential oils was the second more active with LD_{50} from 0.3% for dragonfly. Basil extracts were the least active with LD_{50} from 1.5%. In the non-lethal concentrations, basil was the most effective having almost no effect on the dragonfly larvae (Table XXI).

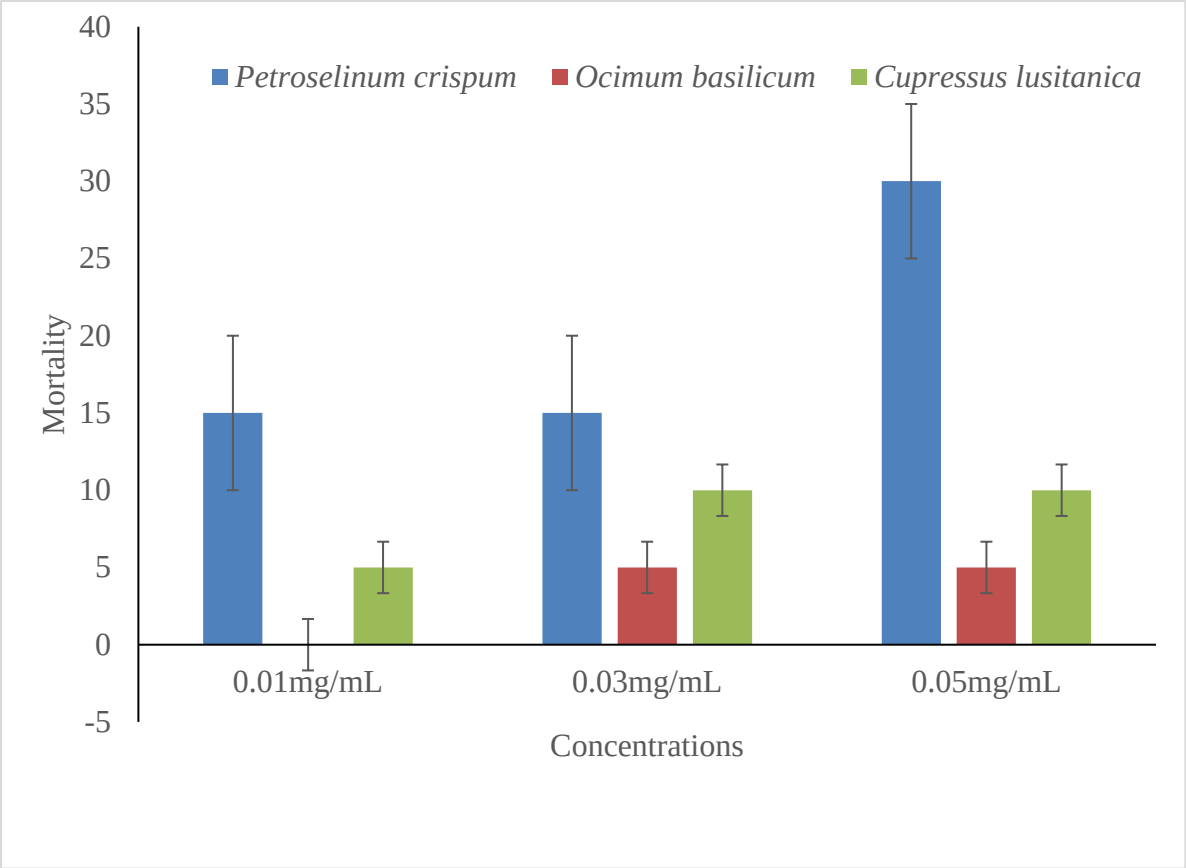


Figure 36: Mortality of dragonfly Larvae in *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* essential oil.

III.1.5.2. Mortality of gambusia fish in plant extracts

III.1.5.2.1. Mortality of gambusia fish in plant powder

Gambusia affinis (fish) live together with mosquito larvae and prey on them, thereby maintaining their population in that ecosystem. The effect of plant powder against fish is summarized in Figure 37 for mortality and Table XX in terms of lethal dose. The LD_{50} of powder varied from 2.3% to 8% for fish. Cypress powder was the most active with LD_{50} from 2.3%. Parsley powder was the second more active with LD_{50} from 3.9% for fish. Basil powder was the least active with LD_{50} from 8%. In the non-lethal concentrations, basil was the most effective having almost no effect on the (Table XXI).

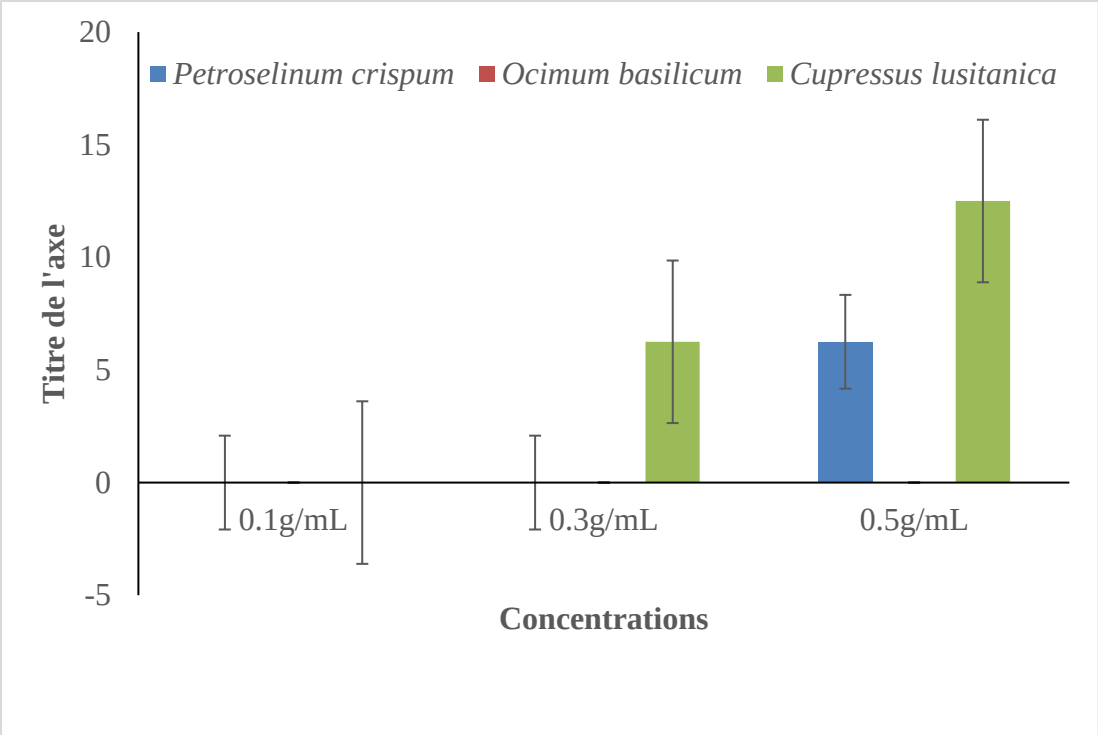


Figure 37: Mortality rate of gambusia fish in *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* powder

III.1.5.2.2. Mortality of gambusia fish in plant methanolic extracts

The effect of methanolic extract against fish is summarized in Figure 38 for mortality and Table XX in terms of lethal dose. The LD_{50} of methanolic extracts varied from 3.1% to 4.5% for fish. Parsley methanolic extract was the most active with LD_{50} from 3.1mg/mL. Cypress methanolic extract was the second more active with LD_{50} of 4.0% for fish. Basil powder was the least active with LD_{50} from 4.5%. In the non-lethal concentrations, basil was the most tolerant having almost no effect on the fish in all the products (Table XXI).

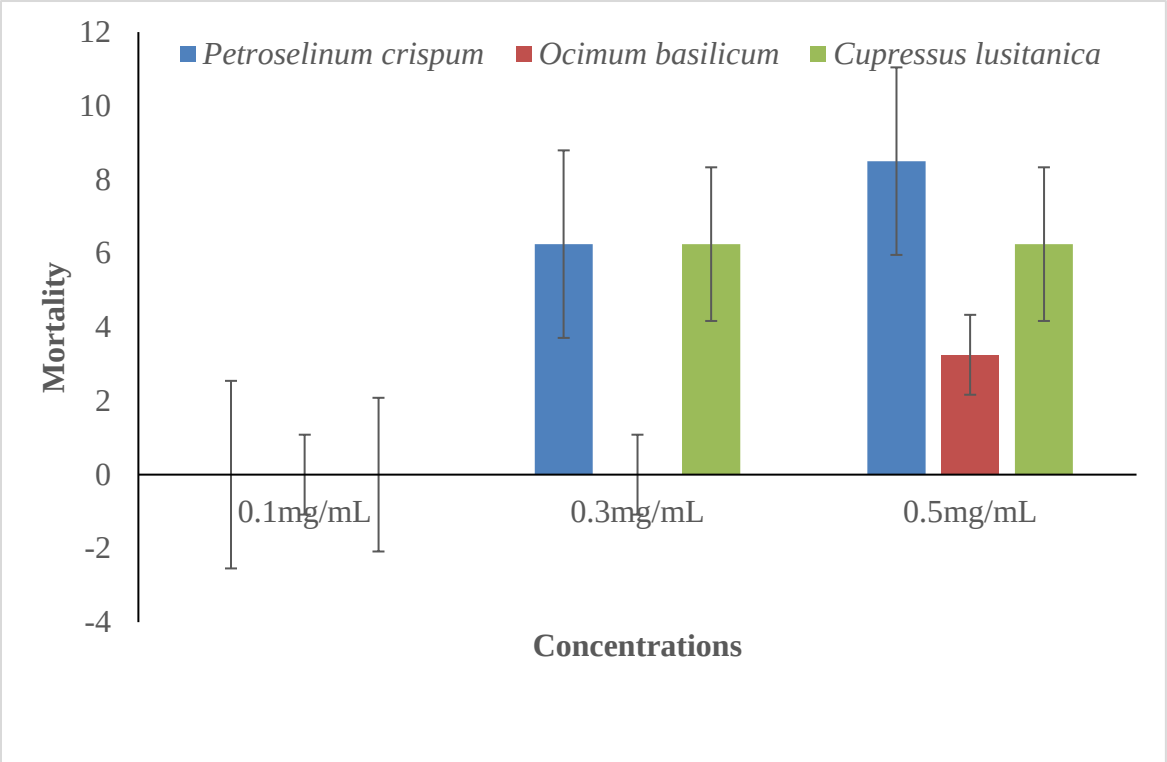


Figure 38: Mortality rate of gambusia fish in *Petroselinum crispum*, *Ocimum basilicum* and *Cupressus lusitanica* methanolic extracts

III.1.5.2.3. Mortality of gambusia fish in plant essential oils

The effect of essential oil against fish is summarized in Figure 39 for mortality and Table XX in terms of the lethal dose. The essential oils were the most active with LD_{50} varying from 0.1% to 1.5%. Parsley essential oil was the most active with LD_{50} from 0.1%. Cypress essential oils was the second more active with LD_{50} from 0.3%. Basil extracts were the least active with LD_{50} from 0.5%. In the non-lethal concentrations, basil was the most tolerant having less effect on the fish (Table XXI).

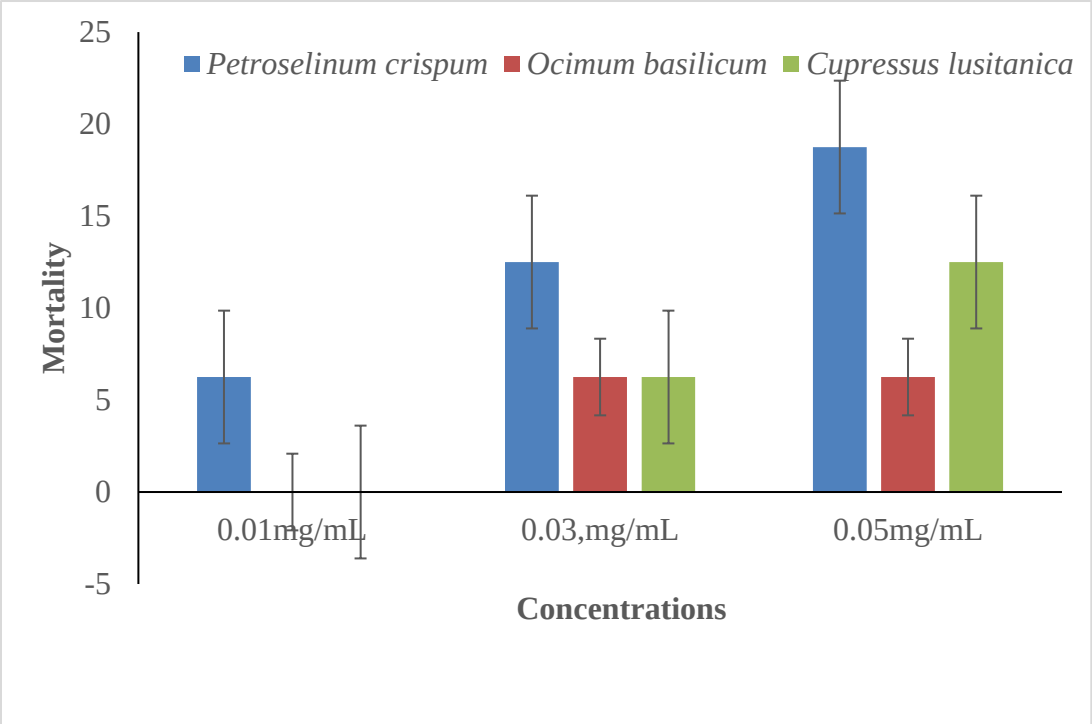


Figure 39: Mortality rate of gambusia fish in *Petroselinu crispum*, *Ocimum basilicum* and *Cupressus lusitanica* essential oils

Table XX: Lethal dose (LD_{50}) of essential oils, methanolic extracts and powder at different concentrations against dragonfly larvae and fish.

Plant	**Form (conc.)**	**LD_{50} (%)**	
		Dragonfly	**Fish**
Parsley	Powder (g/mL)	1.9 ± 0.0^{c}	3.9 ± 0.0^{c}
	Methanolic extract (mg/mL)	5.1 ± 0.1^{e}	3.1 ± 0.1^{e}
	Essential oils (mg/mL)	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
Cypress	Powder (g/mL)	0.8 ± 0.0^{b}	2.3 ± 0.0^{d}
	Methanolic extract (mg/mL)	8.2 ± 0.2^{f}	4.0 ± 0.0^{f}
	Essential oils (mg/mL)	0.3 ± 0.0^{ab}	0.3 ± 0.0^{a}
Basil	Powder (g/mL)	22.7 ± 0.0^{d}	8.0 ± 0.0^{b}
	Methanolic extract (mg/mL)	22.2 ± 0.2^{g}	4.5 ± 0.1^{f}
	Essential oils (mg/mL)	1.5 ± 0.0^{ab}	0.5 ± 0.0^{a}

Values carrying different letter in the same column are statistically significant ($p \leq 0.05$);

III.1.5.2.4. Non-lethal concentrations of diefferent extracts

The non-lethal concentrations of the different extracts against dragonfly and fish were summarized in Table XXI. Parsley and basil powders had a non-lethal dose on dragonfly and fish. Cypress powder had lethal doses dragonfly and fish. Methanolic extracts of cypress and basil had non-lethal dose of 0.1mg/mL. Parsley methanolic extract had a non-lethal concentration less than 0.1mg/mL. Essential oils of all the 3 plants had less lethal concentrations than 0.01mg/mL.

Table XXI: Non-lethal dose of powder, methanolic extracts and essential oils against dragonfly larvae and fish.

Plant	Form	Non-lethal concentrations	
		Dragonfly	**Fish**
Parsley	Powder (g/mL)	0.1,0.3	0.1,0.3
	Methanolic extract (mg/mL)	0.1,0.3	<0.1,0.3
	Essential oils (mg/mL)	0.01	0.01
Cypress	Powder (g/ml)	0.1	0.1
	Methanolic extract (mg/mL)	0.1	0.1
	Essential oils (mg/mL)	0.01	0.01
Basil	Powder (g/ml)	0.1,0.3,0.5	0.1,0.3,0.5
	Methanolic extract (mg/mL)	0.1,03	0.1,0.3,0.5
	Essential oils (mg/mL)	0.01, 0.03	0.01, 0.03

Concentrations = 0.1, 0.3, 0.5 (g/mL powder); 0.1, 0.3, 0.5 (mg/mL, extract); 0.01, 0.03, 0.05 (mg/mL essential oil).

III.1.6. Acute mammalian toxicity test

III.1.6.1- Oral toxicity

The results of the acute oral toxicity test of plausing rat models are presented in Table XXII. The low doses of 0.1, 0.3, 0.5mg/mL for powder 0.1, 0.3, 0.5mg/mL, methanolic extract and 0.01, 0.03, 0.05mg/mL essential oil in phase I administered orally to a group of 3 rats led to no mortality. As no mortality was observed in phase I, the doses were increased to 0.7, 0.9, 1g/mL powder, 0.7, 0.9, 1mg/mL methanolic extract, ańd 0.07, 0.09 and 0.1 mg/mL essential oil. Still, no mortality was recorded even at higher doses. Adverse reactions like increased motor activity, blinking eyes, tremors, convulsion, lacrimation, stimulation, muscle weakness, sedation, urination, salivation, lethargy, sleep, tremors, arching and rolling and coma were not noticed within 28 days.

Table XXII: Acute oral toxicity of *Petroselinum crispum, Ocimum basilicum* and *Cupressus lusitanica* powder using rats as animal models

Groups	Concentrations (g),mg /mL	*Petroselinum crispum*			*Ocimum basilicum*			*Cupressus lusitanica*		
		PP	ME	EO	PP	ME	EO	PP	ME	EO
Phase I										
Group1	0.1, 0.1, 0.01	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 2	0.3, 0.3, 0.03	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 3	0.5, 0.5, 0.05	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Phase II										
Group 1	0.7, 0.7, 0.07	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 2	0.9, 0.9, 0.09	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 3	1, 1, 0.1	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Group 4	control	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

PP=plant powder; ME=methanolic extract; EO=essential oil

III.1.6.2. Dermal toxicity

Table XIII represents the safety profile of essentials oil of *Petroselinum crispum* oil on the skin of rats. The oil was safe up to the dose of 0.1mg/mL. In general, no signs of dermal toxicity were observed in the skin of rats treated with each concentration of essential oil for 21 days post-exposure. There were no changes in fur, eyes and behavior of treated animals as well as no toxic reactions such as erythema, edema, dermal lesions, necrosis and desquamaton during the 21 days observation period.

Table XXIII: Acute dermal toxicity of *Petroselinum crispum* essential oil formulation at differet concentrattions, on rat skin

Days	***P. crispum***		***O. basilicum***		***C. lusitanica***	
	Methanol extract	Essential oil	Methanol extract	Essential oil	Methanol extract	Essential oil
1	NE	NE	NE	NE	NE	NE
7	NE	NE	NE	NE	NE	NE
14	NE	NE	NE	NE	NE	NE
21	NE	NE	NE	NE	NE	NE

NE: No Effect related to erythema, edema, dermal lesions, necrosis, desquamaton, etc. EO = essential oil.

III.1.7. Culicidian fauna sampling (Field RepellentTest)

III.1.7.1. *Petroselinum crispum* cream

The cream produced from essential oil of *P. crispum* is shown on Figure 40. This cream was used to carry out field repellent test on human volunteers.

Figure 40: Parsley cream

III.1.7.2. Field Pilot Study

The pilot test was conducted at Ngoa Ekele (a quarter in Yaounde). A total of 516 mosquitoes belonging to three genera were collected when 0.09mg/mL of the active ingredient was used in preparing the cream. In general, the majority of these mosquitoes (76.55%) were collected from the untreated persons (control) while 23.45% were collected from the treated persons (Table XXIV). *Culex* species was the most predominants of all the mosquito species collected. Considering only the later, 78% were collected from the control persons and 22% from those treated.

When 0.1mg/mL of the active ingredient was used for the preparation of the test cream, the total number of mosquitoes collected were 530 belonging to the three mosquito genera. The collection from the control group was 81.2% while 19.8% were collected from the persons that were treated (Table XXIV). With the *Culex* spp. only, 81% were collected from the control group and 19% from the treated group. A total of 1046 mosquitoes were collected, 820 (78.4%) from control group and 226 (21.6%) from the treated group.

Table XXIV: Mosquito abundance in the field pilot study

Mosquito species	**Product concentration**					
	Persil 0.09mg/mL			**Persil 0.1mg/mL**		
	Treated (%)	**Control (%)**	**Total 1**	**Treated (%)**	**Control (%)**	**Total 2**
***Aedes* spp.**	2 (100%)	0 (0%)	2 (100%)	2 (33.3)	4 (66.7)	6 (100%)
***Culex* spp.**	110 (22%)	390 (78%)	500 (100%)	96 (19%)	410 (81%)	506 (100%)
***Mansonia* spp.**	9 (64.3%)	5 (35.7%)	14 (100%)	7 (38.9%)	11 (61.1%)	18 (100%)
Total	**121 (23.45%)**	**395 (76.55%)**	**516 (100%)**	**105 (19.8%)**	**425 (81.2%)**	**530 (100%)**

In the same way, when 0.09mg/mL active ingredient was used, the aggressivity of *Culex* spp. which was more predominant in the study site was 9.16 bites per person per night for the treated group against 32.5 bites per person per night for the control group (Table XXV). With 0.1mg/mL active ingredient, the number of bites per person per night for the treated group was 8 bites against 34.17 bites for the control group (Table XXVI).

Table XXV: Mosquito biting rate

Mosquito species	**Persil 0.09mg/mL**					
	Treated			**Control**		
	N mosq	Man-night	B/M/N	N mosq	Man-night	B/M/N
***Aedes* spp.**	2	12	0.16	0	12	0
***Culex* spp.**	110	12	9.16	390	12	32.5
***Mansonia* spp.**	9	12	0.75	5	12	0.41

Table XXVI: Mosquito biting rate

Mosquito species	**Persil 0.1mg/mL**					
	Treated			**Control**		
	N mosq	Man-night	B/M/N	N mosq	Man-night	B/M/N
***Aedes* spp.**	2	12	0.16	4	12	0.33
***Culex* spp.**	96	12	8	410	12	34.17
***Mansonia* spp.**	7	12	0.58	11	12	0.92

N mosq=Number of mosquitoes, B/M/N=Bite/man/ night

III.1.8. Field Study sites

III.1.8.1. Mosquito abundance in study sites

When the field trial carried out at Ngoa Ekele was effective, the test was further conducted in other neighbourhoods in Yaounde (Olezoa and Biyem Assi). The test was carried out for 12 nights and mosquito abundance collected from Olezoa and Biyem Assi neighbourhoohs are shown on Table XXVII. A total of 3,936 mosquitoes of the genera *Culex* and *Anopheles* were collected. Majority of mosquitoes, that is 62% (n= 2442) were collected at Biyem Assi. The genus *Culex* was far more abundant with 92% (n=3632) of the total collection. The anopheline fauna was more abundant in Olezoa. The number of mosquitoes collected at Olezoa and Biyem Assi on trated and untreated persons is shown on Table XXVIII and Figure

41. The different species of mosquitoes collected at Olezoa and Biyem Assi are displayed on Figure 42.

Table XXVII: Mosquito abundance in the study sites

	Collection Site		
Species	**Olézoa**	**Biyem-assi**	**Total**
Aopheles gamiae sl.	269 (18.0%)	35 (1.4%)	**304 (7.7%)**
Culex spp.	1225 (82.0%)	2407 (98.6%)	**3632 (92.3%)**
Total	**1494 (100%)**	**2442 (100%)**	**3936 (100%)**

Table XXVIII: Mosquitoes collected at Olezoa and Biyem Assi from the control and treated persons

	Olezoa			**Biyem Assi**			
Species	Pre-control	Control	treated	Pre-control	Control	Treated	**Total**
Anopheles	(4.9%)196	(1.3%)50	(0.6%)23	(0.5%)19	(0.3%)10	(0.2%)6	(7.7%)304
Culex	(19.2%)755	(8.3%)328	(3.6%)142	(39.7%)1207	(22.5%)884	(8.0%)316	(92.3%)3632
Total	(24.2%)951	(9.6%)378	(4.2%)165	(31.1%)1226	(22.7%)894	(8.1%)322	(100%)**3936**

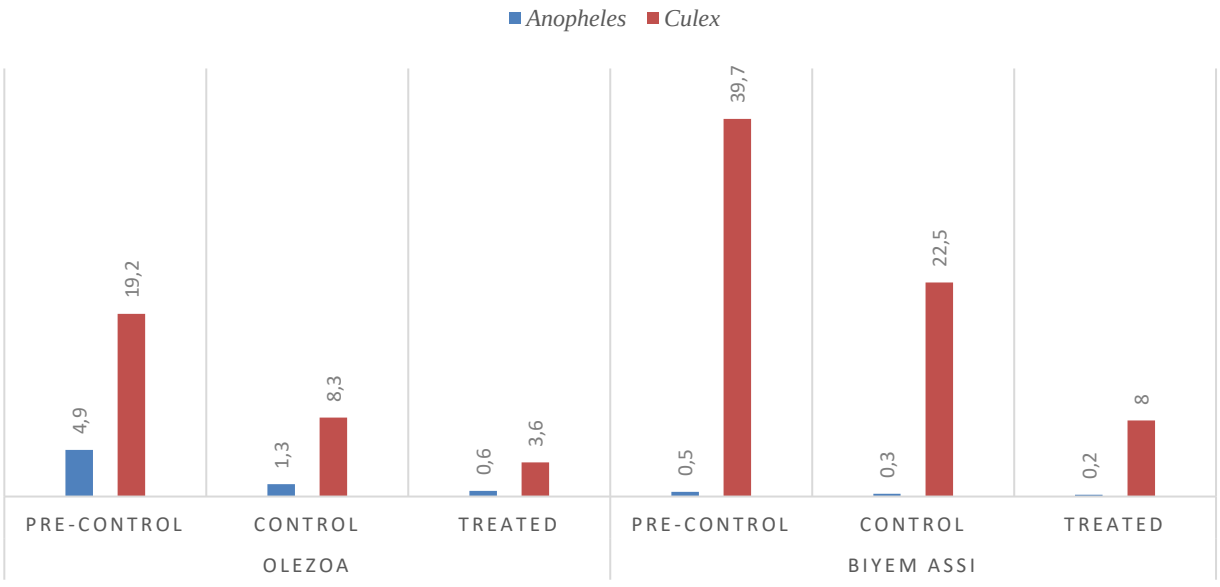


Figure 41: Different collections at the study sites

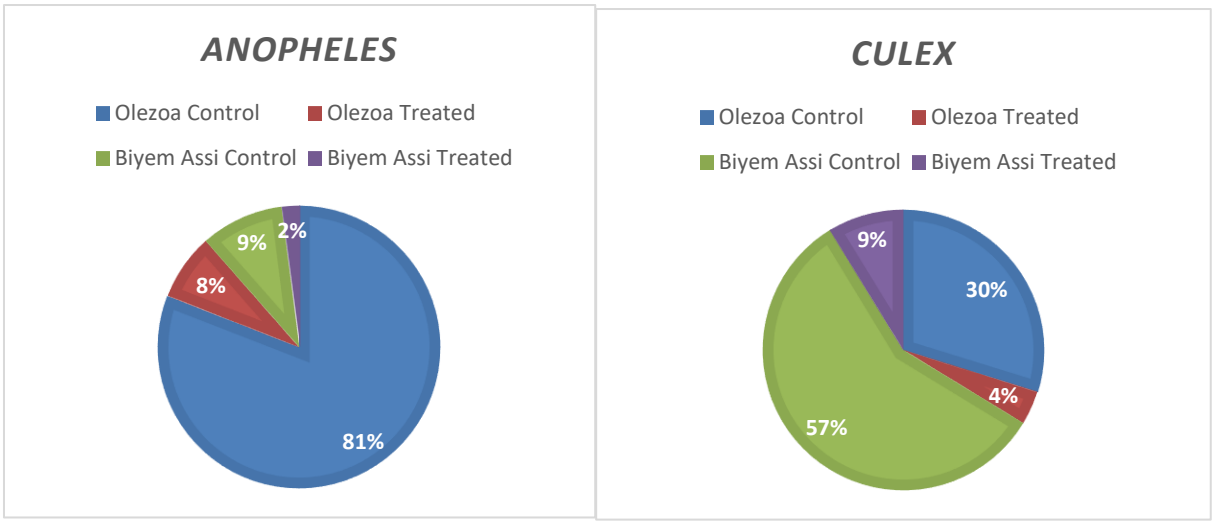


Figure 42: Abundance of *Anopheles* and *Culex* at the study sites

III.1.8.1.1. Reduction rate of mosquitoes aggressiveness

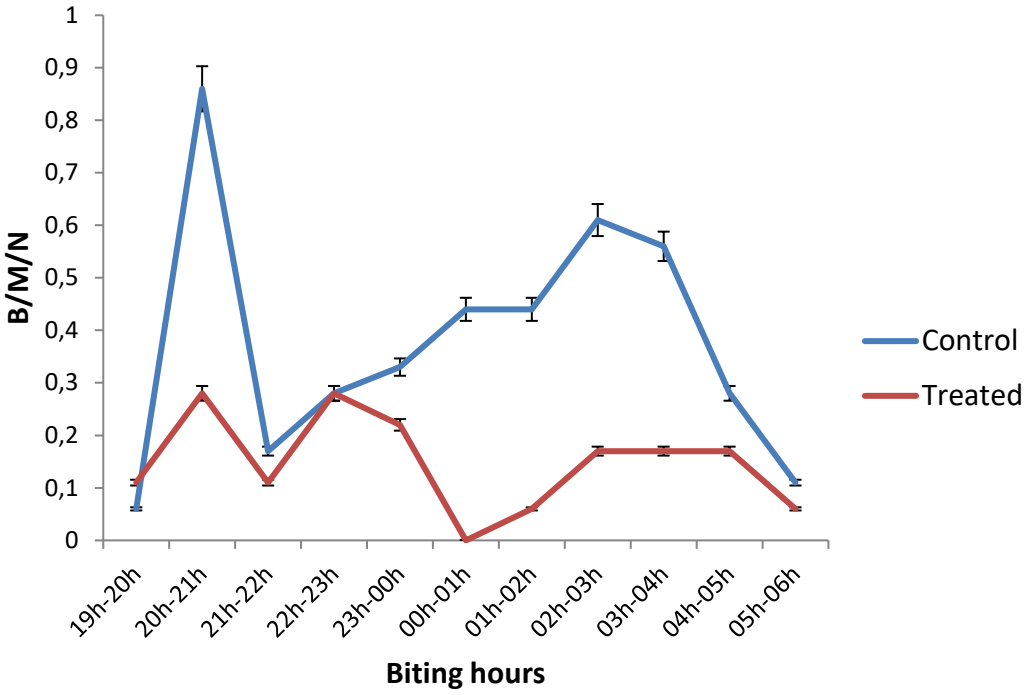
The impact of the *P. crispum* cream was evaluated on the aggressiveness of mosquitoes in the two study areas. It was noted that the application of *P. crispum* cream led to density reduction of mosquitoes on humans (56.3% at Olezoa and 64.3% at Biyem Assi). There was a general density reduction of 61.74%. This reduction was 62.21% for mosquitoes of the genus *Culex* and 52.24% for the genus *Anopheles* (Table XXIX).

Table XXIX: The reduction rate of culiciline aggresiveness

	Pre-intervention		**Intervention**		
Species	**Control**	**To be treated**	**Control**	**Treated**	**Percent reduction**
Anopheles gambiae sl.	11,94	11,94	6,7	3,2	**52,24%**
Culex spp.	109	109	134,7	50,9	**62,21%**
Total	**120,94**	**120,94**	**141,4**	**54,1**	**61,74%**

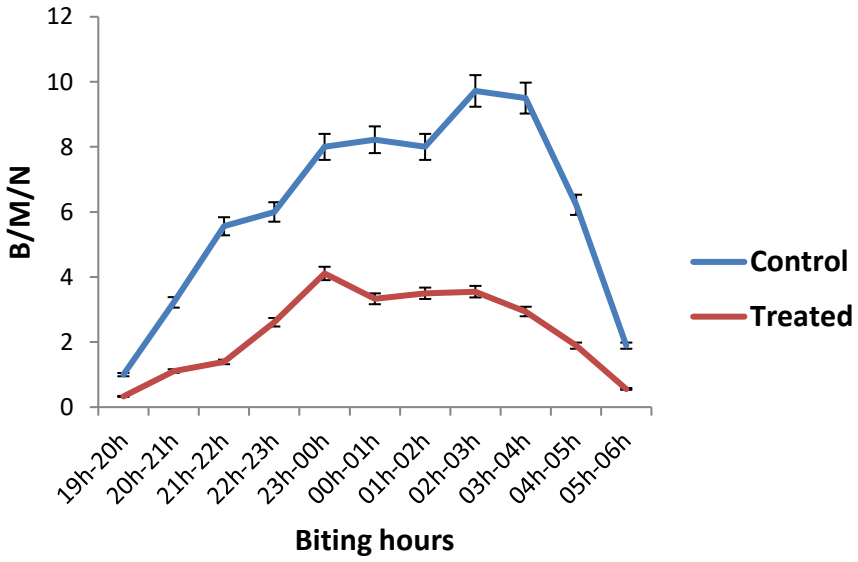
III.1.8.1.2. Biting cycles of mosquitoes

For the two mosquito genera (*Culex* and *Anopheles*) the nocturnal biting cycles were compared in treated and control groups. Figure 43 showed that anopheles' mosquito in both treated and control groups were aggressive throughout the night except between 00h-01h in treated group when there was no mosquito bite. On the other hand, *Culex* mosquitoes were aggressive throughout the night in both treated and control groups but far higher in the control group as shown on Figure 44.



B/M/N = Bite per man per night

Figure 43: Night biting cycle of *Anopheles gambiae sl*



B/M/N = Bite per man per night

Figure 44: Night biting cycle of *Culex spp*

III.1.8.1.3. Biting rate in the first and second half of the night

Mosquito biting rate was compared during the two halves of the night for the two mosquito genera. The Figure 45 below showed that *Culex* spp. was more aggressive in the second half of the night (00h-06h) in both control and the treated groups. But higher in the control group than the treated group. Concerning *Anopheles*, similar pattern was observed. Nevertheless, it was noted that in the treated group aggressiveness was higher during the first half of the night (19h-00h) than the second as presented in Figure 46 contrary to *Culex*.

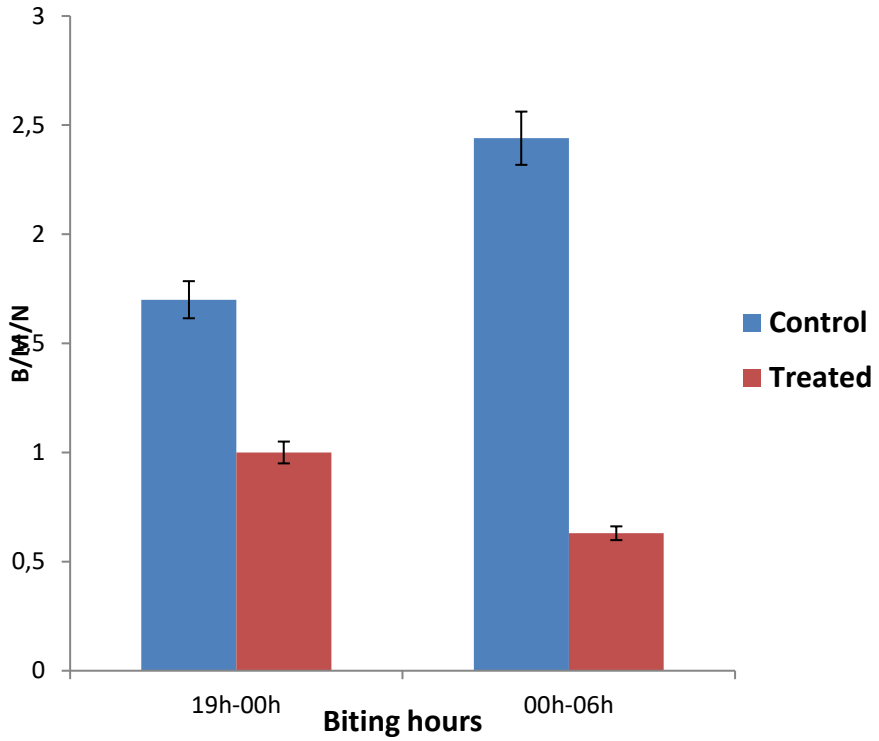


Figure 45: Biting rate of *Anopheles gambiae* sl. in the first and second half of the night

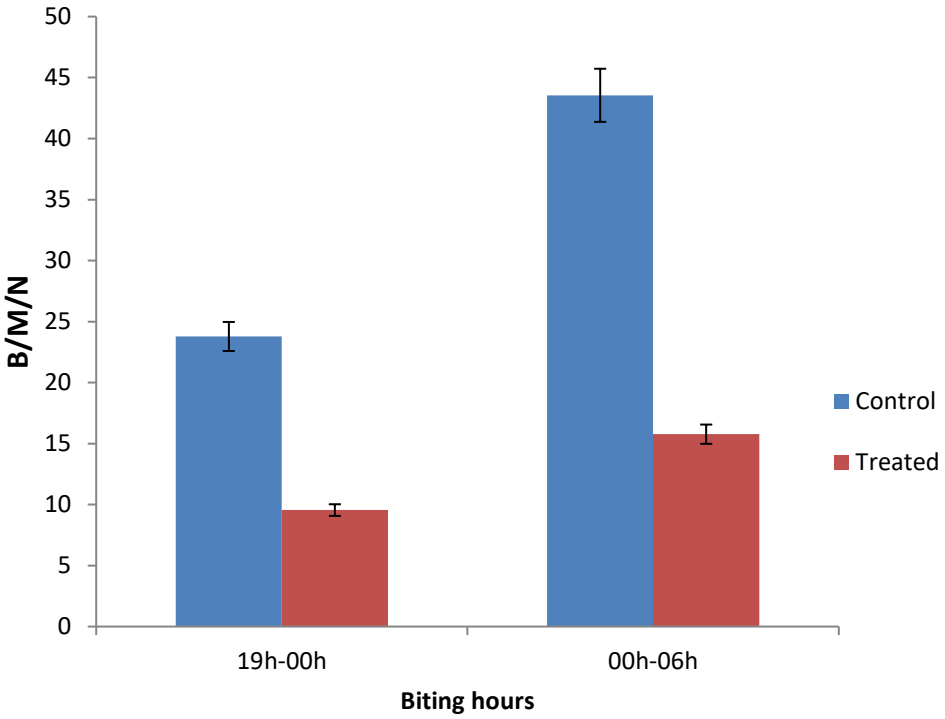


Figure 46: Biting rate of *Culex* spp. in the first and second half of the night

III.2. DISCUSSION

The main goal of this study was to investigate the anti-mosquito potential of the leaf powder, methanolic extracts and essential oils of *Cupresuss lusitanica, Ocimum basilicum* and *Petroselinum crispum* against different development stages of one of the major malaria vectors (*Anpheles coluzzii*) in Cameroon.

For the past years, protection against mosquitoes was generally done by the use of synthetic chemical products which were usually associated with resistance to the vector and toxicity to man and the environment (Tripathi *et al.,* 2003). Nevertheless, natural products provided useful future alterntive means for mosquito control. Some plant extracts or phytochemical products are known to contain ovicidal, larvicidal, adulticidal and insecticidal activities against various insect species (Isman, 2015). Secondary plant methabolic compounds (polyphenols) are known to have adverse effects on the midgut epithelia of Lepidoptera and Orthoptera larvae (Barbeherm and Martin, 1994). Yang *et al*., (2005) showed that tepenoids and alkaloids destroy eggs and female of *Pediculus humanus capitis* (head lice).

The powder, methanolic extracts and essential oils of plant species have a complex mixture of chemical elements; these secondary metabolites have been used empirically in vector control and other causal agents of desease (Leyva *et al*., 2012). The use of plant extracts as repellents is an ancient knowledge and this practice is basically done with compounds that have odour or irritant effects such as chili and garlic (Leyva *et al*., 2012). This study agrees with the previous work carried out by Foko *et al.,* 2007 in demonstrating the ovicidal, larvicidal, pupicidal and adultiidal activity of plant extracts on *Anopheles gambiae*. Also, Ndung'u *et al.,* 2004 worked on the laboratory evaluation of some eastern African Meliaceae as sources of larvicidal botanicals for the control *Anopheles gambiae*. Previous studies have likewise been carried out in different parts of Cameroon to investigate the anti-mosquito potential of the leaf methanolic extracts and essential oils of plants against *Anopheles gambiae,* the major mosquito vector species in the Sub-Saharan Africa that causes malaria (Djoueche *et al.,* 2011). Other works in Cameroon include that of Akono *et al.,* 2012 on the chemical composition and insecticidal effects of *Ocimum basilicum* essential oil on *Anopheles funestus* in Yaounde (Central region of Cameroon) and Valentine *et al.,* 2017 worked on Mosquito larvicidal activity of *Cassia tora* seed powder in Kumba (South West region).

The cold maceration extraction process of the leaves of *P. crispum O. basilicum and C. lusitanica* with 95% methanol solvent gave a yield of 8.73% for *P. crispum,* 5.22% for *O.*

basilicum and 7.43% for *C. lusitanica*. These results are more or less comparable to the yield obtained with methanol absolute extracts of *Aloe barbadensis* leaves (8.25%), *Eugenia jambolana* bark (10.10%), *Moringa oleifera* leaves (10.30 %), *Azadirachta indica* bark (11.10 %) and *Terminalia arjuna* bark (12.20 %), extracted using the same process (Sultana *et al*., 2009). Conversely to the results of the present study, low methanol extraction yields were reported in some previous studies (Anwar *et al*., 2006; Sultana *et al*., 2007). Amongst other contributing factors affecting efficiency of the extraction are part, structure and plant species as well as chemical composition, polarity of solvents used for extraction, method and procedure of plant extraction (Waksmundzka–Hajnos *et al*., 2007; Sultana *et al*., 2007). This difference may aslo be due to various phytochemical constituents which are in the form of secondary metabolites usually for the protection of the plants (Seyoum *et al* (2002). Various constituents such as saponins, phenols, alkaloids, flavonoids and terpenoids among others have been extracted from plants. Phenols, tannins; saponins were conspicuously present in all the plant extracts tested. Accortig to Hsu *et al.,* 2006, this particular difference might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants. The present study supports the rapid evolution of plants effects on vector management (Maud *et al*., 2013). The effectiveness of *P. crispum O. basilicum and C. lusitanica* proves they can be used for malaria control. These botanicals are biopesticide which have little or no effect on the environment as compare to synthetic insecticides (Seyoum *et al*., (2002). Phytochemical constituents of methanolic extract were found to be more potent than aqueous extracts (powder) which agrees with the work of Govindarajan *et al.,* (2011). However, methanolic extracts showed lower LD_{50} values as compared to aqueous extracts. This could be due to the presence of toxic substances in the methanolic extracts which were not extracted due to their polarity characters (Maud *et al.,* 2013). Therefore, if these extracts are well managed, they will go a long way to improve the health of the Cameroonians and the world at large by increasing life span of individuals since malaria is one of the world's killer diseases especially in the sub-saharan Africa where conditions are favourable for the growth and multiplication of this vector.

Essential oils obtained by the process hydrodistillation in the present study presented a low extraction yield of 0.02% for *P. crispum* which was lower compare to that obtained by Jonathan and William (2017) on *P. crispum var. crispum* and *P. crispum var. neapolitanum* (yieds, 0.193 and 0.606% respectively). In this work, the essential oil of *P. crispum* with Myristicin (67.1%) as a major compound caused a significant insecticidal activity against all

tested immature stages of *An. coluzzii* in the laboratory conditions. These results are same with those reported by Seghier *et al*., (2020) in which, chemical analysis of *P. crispum* essential oils revealed the presence of 25 compounds among which Pulegone (51.06%) and D-Limonene (18.77%) as major compounds exhibited high mortality of larvae of *Culex pipiens* and *Culiseta longiareolata***.** Anuluck *et al*., (2021) identified also 17 compounds in the *P. crispum* essential oils and thymol (74.57%), *p*-cymene (10.73%), and γ-terpinene (8.34%) were the abundant compounds which caused high mortality on *Ae. aegypti* larvae. Essential oil of *P. crispum* with thymol (42.41 %), p-cymene (27.71 %), and γ-terpinene (20.98 %) as major constituents also caused high larvicidal activity with LC_{50} value of 0.023% (43.22 ppm) against *Ae. aegypti* larvae (Intirach *et al.*, 2016). The 0.04% yield of *O. basilicum* obtained in this work was lower compared to 0.11% for *O. basilicum* and 4.6% for *Eucalyptus citriodora* (Mejdoub and Katsiotis 1998); 0.75% for *Lippia adoensis* and 1.24% for *Piper nigrum* (Rmili *et al*., 2014) isolated by the same process for 3 h. According to Malebo *et al.,* 2013, the yields of up to 0.60%, 0.25% and 0.80% were obtained from *Ocimum basilicum, Ocimum. tenuiflorum* and *Ocimum gratissimum,* respectively, which were higher than that from the present study (0.04%). Nurzyńska-Wierdak, 2013 worked on *O. basilicum* and had a yield of 1.09% which was very high compare to this work. This could be as the result high content of linalool present in the former. The yield of *C. lusitanica* (0.4%) in this study was same with some previous studies carried out by Philip *et al.,* 2016 who worked on *C. lusitanica* essential oil against major insect pest of stored grain with the same yield. Hassanzadeh *et al.,* (2010) reported that the major components of *C. lusitanica* leaf oil in Tanzania were α-pinene (40 to 82%), limonene (4 to 18%), *cis*-muurola-4 (14%) and isobornyl acetate (up to 10%) which were effective in vector management. It was different in this study, where the major components of *C. lusitanica* leaf oil was (-)-4-Terpineol (12.7%), 4(10)-Thujene (10.5%), (R)-(+)-α-Citronellol (11.5%) but were also effective in control of immature stages of *An. Coluzzii*. Therefore, the chemical composition of plants differs in different regions due to the time of harvest (Maud *et al.,* 2013). Thus, plant cells subjected to thermal stress exceed their capacity for expansion, and causes their dislocation more rapidly leading to the release of their contents in the middle of extraction (Paré and Bélanger., 1997).

The low yields of *Petroselinum crispum* and *Ocimum basilicum* essential oils in this study might be attributed to seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and post-harvest storage (Marotti *et al*., 1994; Hussain *et al*., 2008). Factors also influencing essential oil yield include plant species, duration

of distillation, chemical composition and extraction procedure (Renedo *et al*., 1990; Mejdoub and Katsiotis 1998).

Chemical compounds were identified through Gas Chromatography coupled with to Mass Spectrometry (GC-MS) analyses for these plants. Eighteen elements were identified from *P. crispum* in this work and myristicin was more predominant (67.1%). Lechtenberg and co-workers examined two samples of curly leaf parsley from different sources (Lechtenberg *et al*., 2007). One sample was not very rich in myristicin (59.4%) as compared to that of our study but the other sample was dominated by myristicin (82.3%). A leaf oil sample from Rockdale, New South Wales, Australia, was rich in 1,3,8-*p*menthatriene, apiole, and β-phellandrene, but was devoid of myristicin (Lechtenberg *et al*., 2007). Jonathan D Craft and William N Setzer ((2017) worked on the same plant and 38 compounds were revealed with myristicin still the most predominant but having 38% compared to that of this work with 67.1%. The results of this work also differ from those of Enam *et al*., (2013) in Egypt who obtained among the 21 compounds of the EO of *P. crispum*, β- Myrcene, β- Phellandrene and Myristcine as major compounds, and γ- Terpinene, (-)-Isolongifolol and p- Cymen-8-ol as minority compounds; those of Zhang *et al.* (2006) in the United States of America and in China for whom the essence of *P. crispum* revealed the presence of 15 terpene compounds, the main compounds of which were: Myristicin (32.75%), Apiol (17.54 %), and a-Pinene (16.64%), b-Pinene (11.54%) and 1-Allyl-2,3,4,5-tetrameth-oxy-Benzene (10%); and as minority compounds unknown 1 (0.17%), unknown 2 (0.22%), P-Cymenene (0.26%). Ninteen elements were revealed in the chemical composition of *Ocimum basilicum* and the element with the highest percentage was Linalol (65.7%). This was different from the work of Akono *et al.,* 2012 who worked on the same plant, 14 elements were revealed and Limonène had the highest percentage (30,9%) with Linalol having a lower percentage (18.9%) compared to that of this study. The work of other researchers on *O. americanum* oil had 72 compounds with geraniol (18.72%) being the highest (Bett *et al.,* 2022). The difference may due to the fact that the plants were harvested in different regions of the country (Cameroon). Similar results were gotten by Ntezurubanza *et al.* (1985) in Rwanda on *Ocimum canum* with Linalol having the highest percentage (60%). *Cupressus lusitanica* essential oils were analyzed by GC-MS and 27 compounds were identified. The work of Bett *et al.,* 2022 on *C. lusitanica* oil, 91 compounds were identified with the highest compound being α-pinene (13.8%) which was different from our findings with 27 compounds with the predominant element (-)-4-Terpineol (12.7%). This was also different from the work carried out by Philip *et al.,* 2016; Tapondjou *et al.,* 2005 on stored products. Caroll *et al.,* worked-on

C. funebris as mosquito repellent and obtained 30 chemical composition elements with α-cedrene (16.9%) being the most predominant which was different from this work having 27 elements and (-)-4-Terpineol (12.7%) with the highest percentage. This difference may be due to the fact that the plant was harvested in different geographical regions at different time and method of extraction.

Insecticidal impact of plant powder on the developmental stages of *Anopheles coluzzii*

In the present study, the powder (aqueous extract) of *P. crispum O. basilicum and C. lusitanica* inhibited growth and development of *An. Coluzzii* at different growth stages. Similar work was done by Foko Dadji *et al*., 2007 on *Capsicum annuum* powder, red and yellow varieties against immature stages of *Anopheles gambiae*. The results revealed the embryonic mortality to be 100% when treated with yellow variety. This study also showed a significant insecticidal efficacy on the embryonic development, egg hatching, larval and pupal development. Embryonic duration development was 48h in all the plants powder, the duration of larva development was approximately 8 days in *C. lusitanica* similar to the work of Foko Dadji *et al.,* 2007; Mouchet *et al.,*1961. The pupal development duration in *P. crispum* and *O. basilicum* powder were 48h and that of *C. lusitanica* was 72h which was different from that of Foko Dadji *et al.,* 2007. The total developmental cycle duration was approximately 11.5days in parsley similar to the work carried out by Foko Dadji *et al.,* 2007 on *Capsicum annuum*. *Cupressus lusitanica* exhibited a long larval duration of 13 days with a pupal period of 2days. Similarly, *Cx. Pipiens* eggs treated with *Peganum harmala* extract exhibited a long larval period up to 12 days compared to the control 10 days and pupal period lasted for 3 days in the test groups and 2 days in the control. The plant powder might inhibit growth regulator enzymes causing morphological and physiological disorders which interfere with total development of insect (Adanan *et al.,* 2005; Kamaraj *et al*., 2008). The total mortality was greatly correlated to the quantity of plant extract in the breeding medium (Foko *et al.,* 2007). Plant powder can be considered a good material for the control of malaria vector with no poisonous effect on man or environment.

The effects of methanolic extract and essential oil of *P. crispum O. basilicum and C. lusitanica* against the aquatic stages of *An. coluzzii*

The aquatic immature larvae stage was recognized as the most vulnerable and best control strategy to effectively reduce mosquito population in our study. This finding was confirmed my WHO (2016). The methanolic extracts from *P. crispum O. basilicum and C. lusitanica* tested in the present investigation significantly inhibited egg hatching and caused

high mortality of *An. coluzzii* larvae. Similarly, methanolic extract of *Hyptis suaveolens* inhibited completely *An. gambiae* egg hatching (Ivoke *et al.,* 2009). Methanolic extract of *Coccinia indica* induced zero egg hatchability for *Cx. quinquefasciatus* (Govindarajan *et al.,* 2011) which was different from this work with no zero-egg hatchability. *Petroselinum crispum Ocimum basilicum and Cupressus lusitanica* methanolic extracts were tested for the ovicidal activity against *An. coluzzii* with LC50; 0.18%, 0.61% and 0.24% respectively. Acording to Govindarajan *et al.,* 2013, methanolic extract of *Cassia fistula* was tested for ovicidal activity against *An. stephensi* in India; the LC50 obtained was 0.021% similar to that of *P. crispum* in the present study. Methanolic extracts of the leaves of these botanicals applied at 5% showed each a high increase in larval and pupal mortality similar to the plant *Pseudocalymma alliaceum* which also showed high increase in larval and pupal mortality (Granados-Echegoyen *et al*., 2014). The methanolic extract from *Atlantia monophylla* was also reported to induce high larvicidal properties against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Sivagnaname and Kalyanasundar, 2004). Generally, plant methanolic extracts contain phyto-constituents such as flavonoids, terpenoids, steroids, alkaloids, saponins, tannins, phenols, etc., with pesticidal properties against mosquito vectors (Yang *et al.,* 2005). In the different proportion of each plant, these phyto-constituents act singly or in combination to cause mortality of mosquito especially the immature stages (Yang *et al.,* 2005). These phytochemicals might deactivate acetylcholinesterase enzyme causing neurotoxic symptoms including convulsion, hyper-excitation leading to the death of the insect (Ryan *et al*., 1992; Kostyukovsky *et al.,* 2002). Phytochemicals in methanolic extracts primarily affect the midgut epithelium and also affect the gastric caeca and the malpighian tubules in mosquito larvae (David *et al.,* 2000). The mortality rate of *An. coluzzii* at different developmental stages in this work was higher with increase concentration of *P. crispum O. basilicum* and *C. lusitanica* with the highest at 0.5mg/mL and lowest when the concentration was 0.1mg/mL. This was similar to the work carried out by Govindarajan *et al*., (2008). Egg hatching Inhibition rate was higher in *P. crispum* than *O. basilicum* and *C. lusitanica* at 0.03mg/mL concentration with LC50 of 0.18% in methanolic extracts. Hadjiakhoondi *et al.,* 2005 reported the efficacy of the methanolic extract of *Tagetes minuta* L. on the *An. stephensi* larvae in which, the LC_{50} and LC_{90} values were obtained as 2.5 mg/l and 11.0 mg/l, respectively Ovicidal effects of essential oils have often been linked to the presence of oxygenated monoterpenes (Marimithu., 2010). *P. crispum* had the highest LC50 0.004% at 0.03mg/mL concentration. A high ovicidal effect was recorded with the eggs of *Anopheles coluzzii* when *Zingiber officinale* essential oil was used for testing (Foko *et al*., 2018). Work carried out in Congo with the plants *Cyperus articulatus*, *Cyperus*

rotundus, *Cyperus esculentus*, *Aframomum stipulatum*, *Aframomum giganteum*, *Zingiber officinale*, *Chenopodium ambrosioides*, *Lippia multiflora*, *Cymbopogon citratus*, *Hyptis suaveolens* and *Guibourtia demeusei* essential oils demonstrated remarkable ovicidal activity with the eggs of *Anopheles gambiae* with several levels of toxicity and all of these oils showed 100% egg inhibition at a concentration of 0.4 g L^{-1} (Ghislain *et al.*, 2022).

Larval mortality rate was the highest in *P. crispum* essential oils with LC50 of 1st, 2nd, 3rd and 4th instars (0.12, 0.15, 0.17 and 0.20%) at 0.03mg/mL concentration with increase mortality from 1st to 4th instars. Similar results were obtained on 2nd and 3rd instars of *An. gambiae* in *Piper nigrum* with LC50 of 0.027 and 0.214% respectively (Kehinde *et al.*, 2018). In the same way, eight plant essential oils were screened for larvicidal and ovicidal activity against *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* and they revealed high mortality (Manimaran *et al.*, 2012). Essential oil of *Kelussia odoratissima* with LC_{50} value of 0.49 mg/mL (4.88 ppm) was reported to be effective against the fourth instar larvae of *An. stephensi* (Vatandoost *et al.*, 2012). The essential oil of *Piper nigrum* against *An. gambiae* larvae showed 100% mortality (Khinde *et al.*, 2017). The larvicidal activity of *Tagetes patula* oil has been reported to have a high effect on the larvae of *An. stephensi* with the LC_{50} and LC_{90} values of 12.08 mg/l and 57.62 mg/l, respectively (Dharmagadda *et al.*, 2005). Results of this study revealed *P. crispum*, essential oil was very potent and significantly inhibited egg hatching, caused high larvicidal and pupalcidal mortality on *An. coluzzii*. There was 100% mortality on aphids with *O. basilicum* essential oil (Pikassale *et al.*, 2020). Similarly, Foko Dadji *et al*., 2016 worked on ovicidal, larvicidal and pupicidal activities of *O. basilicum* essential oil against *An. gambiae* sl with a high insecticidal activity at various stages after 24h exposure at a concentration of 0.003% (30ppm). *Ocimum basilicum* essential oil contains many compounds which demonstrated an efficient toxic effect against *An. gambiae* sl (Pikassale *et al.*, 2020). The finding of the current study showed a high mosquitocidal effect of *O. basilicum* essential oil on the different stages of *An. coluzzii*. When maize weevil was treated with *C. lusitanica* essential oil, there was 98-100% mortality (Pikassale *et al.*, 2020). Essential oils of this work showed low LC50 values (more toxic) as compared to methanolic extracts of the same plants. Previous works have demonstrated the same effect (Akono *et al.*, 2015). This could be attributed to their complex nature since they are normally a mixture of various compounds. The toxic effects of these plant products were concentration-dependent and the effect augmented with increasing concentrations (Jeyabalan *et al.*, 2003). These variations highlighted, would be due to the variation in the chemical composition of the soils which affect the biochemical composition of

the plants from one geographical area to another. In addition, it is established that soil salinity would influence the extraction yield and the percentage of compounds of an essential oil (Enam *et al.*, 2013).

Knockdown and mortality effects of methanolic extract and essential oil of *P. crispum O. basilicum and C. lusitanica* on the adults *An. coluzzii*

Knockdown and repellent effects are the keys to the prevention of mosquito bite. In the present study, three essential oils registered knockdown and adulticidal effects. The literature is available with regard to bioefficacy of volatile oils against vector mosquitoes. These findings are comparable to those of Vartak and Sharma. 1993 who reported the knockdown effect of terpenoids volatile oils against *A. aegypti* adult females. The knockdown was observed for 60 minutes and it increased with increase concentration similar to that of this work (0.01 to 0.07mg/mL for essential oil and 0.1 to 0.7mg/mL methanolic extract). At 60 min and 24h exposure of female *An. coluzzii* in the laboratory, there was 100% knockdown and mortality in parsley leaves essential oil at 0.05mg/mL. Semilar results were obtained by Foko Dadji *et al.,* 2018 using *Clausena anisata* leaves essential oil on *An. gambiae* in laboratory. The essential oil of *C. lusitanica* and *O. basilicum* also had high repellent effect on adult An. co*Tribolium castaneum, Acanthoscelides obtectus* and *Sitophilus zeamais* applied at 0.20% to wheat and bean grains and stored for 30–120 days caused a mortality up to 65.0% (Philip *et al.,* 2017). This was in contrast to this where *O. basilicum* had a low effect on adult *An. coluzzii*. Reports have shown that essential oils have biological properties in repelling insect pests and in contact with the insect by inhibiting enzymes, leading to deformities and mortality observed in different development stages of insects (Parthiban *et al.*, 2020; Senthil-Nathan., 2020). Repellent properties of several EO appear to be associated with the presence of monoterpenoids and sesquiterpenes monoterpenes such as a-pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of EO as presenting mosquito repellent activity (Vatandoost *et al.*, 2012), this agrees with the present study.

Mammalia toxicity

Powder, methanolic extracts and essential oils had no effect on white rats when tested for 21 days. Also from the present investigation, no signs of dermal toxicity were observed in the skin of persons and rats treated with each essential oil of *P. crispum, O. basilicum* and *C. lusitanica* after 21 days post-exposure. Similarly**,** Panneerselvam *et al.*, (2012) reported that the essential oil of *Artemisia nilagirica* gave protection against mosquito bites without any allergic reaction to the test person. Also, the methanol extracts of *Cassia tora* crude extracts gave

protection against mosquito bites without any allergic reaction to the test person (Amerasan *et al*., 2012). Conversely to the present study, there have been reports of side effect on men and rats after the use of some tested products such as diethyltoluamide and dimethylphthalate creams formulated as mosquito repellents caused signs of dermatitis such as thin epidermis, in folded thick dermis with more intercellular spaces filled with fluid and more of elastic and collagen fibers on rabbit's skin after a month (Sammar *et al*., 2001). Similarly, undiluted oil of lemon eucalyptus at high doses (5000 mg/kg), dermal irritation was noted at the site of test material application, which included erythema, edema, dermal lesions, necrosis, and desquamation after day 7 of test (Chemical Watch Factsheet, 2010). Dragonfly and gambusia fish were more tolerant to of basil then cypress and parsley. Mortality was highest in parsley and lowest in basil. Powder was the most effective followed by methanolic extract and essential oil in the form of toxicity. Basil was more effective for the management of the ecosystem. In this study, non-target organisms were preserved while the target organisms were affected.

Field Test

In the present investigation, cream formulated with essential oils of *P. crispum* and applied at 0.09mg/mL active ingredient repelled *An. gambiae* female from biting for more than 90 mins. Similarly, the essential oil of *Tagetes minuta*, provided repellency up to 90% protection for 2h against *An. stephensi, Cx. quinquefasciatus* while less than 60% repellency against *Ae. aegypti*, was reported by Tyagi *et al*., (1997). Three quarters (Ngoa Ekele, Olezoa and Biyem Assi) in Yaounde Urban area were tested for vector control using this cream. A pilot study was first carried out and the results were encouraging with 71.4% reduction density. A preliminary study had been done in Yaounde before with density reduction of 55% (Hougard *et al*., 1993). Similar studies had also been carried out in other parts of Africa such as in Bukina Faso which gave a reduction density of 55-82% and 55-92% in Ouagadougou for the control of *Culex quinquefasciatus* (Ouedraogo. 2011). The results we obtained from this work showed a variation of effectiveness of the cream in the different neighbourhoods where the testing was conducted. There was higher reduction density in Olezoa than Biyem Assi. This might be due to availability of mosquito breeding sites present in that area. Large species diversity of *Culex* spp was recorded in Yaounde urban area during this study. Similar results have been obtained in Cameroon and other parts of Africa showing the high adaptability of *Culex* spp especially *Cx quinquefasciatus* in urban environments (Bessaud *et al*., 2006; Nklinkebeng *et al*., 2008; Yadouleton *et al*., 2010, Antonio-Nkondjio *et al*., 2011; Talipouo *et al*., 2017; Nchoutpouen *et al*., 2019). The high density of *Culex* spp recorded could also be due to the presence of diverse

environmental activities including vegetations used for agriculture and also the presence of lakes. In general, the cream formulated from the essential oil of *P. crispum* caused a significant concentration-dependent repellent activity against female adults of *Culex quinquefasciatus* which declined with time post-exposure contrary to *Anpheles gambiae sl* that increased with time post-exposure. Similar remark was observed by (Kovendan *et al.*, 2013). Despite the fair results obtained from the field study, our main target was *Anopheles gambiae*, the vector of malaria.

Biting cycle of mosquitoes

The biting cycle which was nocturnal, consisted of 2 mosquito genera made up of treated and control groups. There was higher aggressiveness in the control group than the treated group in both mosquitose genera. The reduction rate in mosquito bite was different in the mosquitoes general, being 52.2% for anopheles and 62.4% for culex. These results were similar to those carried out by Ntonifor *et al*., 2006 used plants as traditional anti-mosquito method in the SW region of Cameroon, of the 179 respondents, 88 (49. 16%) used traditional anti-mosquito for malaria control. Carnevale *et al*., 1992 in Yaounde- Cameroon with reduction rate of *Anopheles gambiae* to be 62%. In the entire experimental area, the total reduduction of female mosquitoe bites was 61.74%. The total number of mosquitoes collected from the pilot study was 1046, 80% for those who did not apply the cream and 20% on those who applied. Total number of mosquitoes collected from the study area were 3936 (79%) was on control group and 21% on the treated group. Several works have been carried out for the past years on mosquito repellent in Edea, Cameroon (Robert *et al*., 1993). Carnevale *et al*., 1992 carried out similar work in Yaounde and out of the 2125 mosquitoes collected, 72% was from the control and 28% from the treated individuals. A semi-field experiment was conducted in Mbingu village, Tanzania, the total mosquitoes collected were 4.844 and the risk of being bitten by mosquitoes was reduced by 91.8% (Sangoro *et al*., 2014). *Ocimum forskolei* reduced biting by over 50% against *An. arabiensis* under field conditions (Dekker *et al*., 2011; Waka *et al.*, 2004).

The results obtained from this study could be used to develop safer alternatives to fight other vector species. Based on the available literature, it is evident that plant extracts are biodegradable and thus will not cause similar environmental risks as many of the widely used synthetic agrochemicals. Biodegradability of botanicals may be an important factor which will increase demand for plant-based chemical products (Kari *et al*., 2011). It is very difficult to obtain data indicating the spread of botanicals in the environment, because all the measurable components break down in soil within a few days. There is no single active ingredient or

decomposition product which can be used as an indicator of the leaching risk (Kari *et al*., 2011). Other challenges include: the fact that most botanicals are slow in action and lack the rapid knockdown affect, brings about doubt as to whether they are as effective as the synthetic products. The issue of genetic variability of plant species in different localities is instability of the active ingredients when exposed to direct sunlight. There is competition with synthetic pesticides through aggressive advertisement by commercial pesticides dealers but commercially formulated botanicals are more expensive than synthetic insecticides and are not as widely available (Yallappa *et al*., 2012).

Essential oil is abundant in nature and apart from its medicinal and flavour value, its use in repelling mosquito can be considered as sustainable and biocompatible delivery device as fruitful alternative.

CONCLUSION, RECOMMENDATIONS AND PERSPECTIVES

CONCLUSION

The present study investigated the mosquitocidal activities of three local plants *P. crispum, O. basilicum and C. lusitanica* against malaria vector *Anopheles coluzzii*, under ambient laboratory conditions and the knockdown effect of leaf methanolic extracts and essential oils on adult mosquitoes using the laboratory and field strains. These three plants extracts were used on different mosquitoe stages under laboratory conditions. *Petroselium crispum* was the most potent among the three plants extracts tested and first instar larva was the most subceptible instar. Among the six phytochemical classes (alkaloids, flavonoids, saponins, tannins, phenolic group, steroids and terpenoids) known for insecticidal activity, phenolic group was present in all the plants. The essential oil of *P. crispum* was showen to be more active against differet mosquito stages compared to *O. basilicum and C. lusitanica*. *An. coluzzii* first ad second instars were generally more susceptible to plat extracts than the other instars.

The adult mosquitoes presented a knockdown effect when exposed to various concentrations of methanolic extracts and essential oil of using methanolic extracts and essential oil of the different plants. The lethal effect of these plants extracts was also tested on non-target organisms such as rats, dragonfly and fish and was found to be harmless. All the extracts assessed in this study were found to have weak acute toxicity properties. These weak acute toxicity properties could be the reason why these plants have for long been used by local communities as storage pesticides. The oil has strong repellent action against mosquitoes in general and particularly against *An. coluzzii*, which is responsible for 70–75% of the malaria transmission in Cameroon.

The result of our work revealed that the plant extracts of *P. crispum, O. basilicum and C. lusitanica* were promising ovicidal, larvicide and adulticide to control mosquitoesespecially *An. coluzzii*. *Petroselinum crispum* methanolic extracts and essential oil showed highest larvicidal and adulticidal activity within the range of 0.3-0.5 mg/mL methanolic extracts and 0.03-0.05mg/mL essential oil concentrations. The results reported in the present study open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extracts as ovicidal, larvicides and adulticides to control not only *Anopheles coluzzii* but also other mosquito species. Our results also revealed that biopesticides from these plants had effects.

RECOMMENDATIONS

- Government should subsidize research on botanicals for more studies to carried out on other plants
- The public authorities should implement large-scale vector control measures, Especially in large cities in order to reduce culicidian nuisance and diseases;
- The government should multiply sanitation infrastructure in order to reduce Anpheles population
- The public should be educated on the danger of malaria and the importance of environmental sanitation.
- People living in malaria endemic regions should be encouraged to apply *Petroselinum crispum* cream which is an excellent mosquito repellent product with no side effect especially for outdoor application
- Repellent creams formulated with essential oils of parsley plant should by apply as botanical repellent. (With no side effect).

PERSPECTIVES

- Determination of the toxic effect of methanolic leaf extract and essential oils of *P. crispum, C. lusitanica* and *O. basilicum* should be done by combining two or the three these plant extracts or essential oils
- Evaluation of the toxic effect of powder, methanolic extracts and essential oils of *P. crispum, C. lusitanica* and *O. basilicum* on other mosquito species and even on other noxious insects
- Small-scale field trials concerning the mosquitocidal efficacy of the powder, methanolic extract and the essential oils of these plant products in aquatic environments against eggs, larvae and pupae, as well as in impregnated mosquito net, and indoors and outdoor spraying against the adult mosquitoes
- Enhancing the efficacy of cream repellents formulated from *P. crispum* essential oil, by purification of the active fraction, increase in shelf life and increase in the duration of repellency
- Producing other creams from *Cupressus lusitanica* and *Ocimum basilicum* essential oils will improve malaria prevention.

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APPENDICES

Appendix 1. Mortality after 24 h of *An. coluzzii* larvae exposed to different concentrations of methanolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum*

Plant species	Conc (mg/mL)	Instar larvae				*H-value*	*P-value*
		1st	2nd	3rd	4th		
Ocimum basilicum	0	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	51.00±1.00cA	36.00±2.30dB	31.00±1.91dBC	24.00±1.63dC	13.13	0.004
	0.3	73.00±1.91bA	52.00±1.63cB	45.00±1.91cBC	39.00±1.91cC	13.30	0.004
	0.5	99.00±1.00aA	79.00±1.91bB	60.00±1.63bC	49.00±1.00bD	14.32	0.002
	Dichlovos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	16.38	16.53	16.52	16.57		
	P-value	0.003	0.002	0.002	0.002		
Cupresuss lusitanica	0	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	55.00±1.91cA	39.00±2.51dB	36.00±1.63dB	26.00±2.58dC	12.64	0.005
	0.3	78.00±2.58bA	56.00±1.63cB	51.00±1.00cB	43.00±1.91cC	13.71	0.003
	0.5	100.00±1.00aA	82.00±2.58bB	65.00±1.91bC	51.00±1.91bD	14.37	0.002
	Dichlovos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	17.68	17.55	17.61	17.35		
	P-value	0.001	0.001	0.001	0.002		
Petroselinum crispum	0	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	**48.**00±2.82dA	43.00±3.78dA	41.00±3.41dA	38.00±2.00dA	4.94	0.176
	0.3	61.00±2.51cA	59.00±3.41cA	54.00±2.00cA	52.00±1.63cA	6.97	0.079
	0.5	**81**.00±4.43bA	79.00±5.25bA	73.00±3.00bA	70.00±1.15bA	3.80	0.283
	Dichlovos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	14.24	14.01	14.43	16.52		
	P-value	0.003	0.003	0.002	0.002		

Appendix 2: Lethal time (days) of mosquitoes (larvae) using plant methanolic extract at three concentrations

Larvae stage	Methanolic extract (0.5mg/ml)					
	Laboratory mosquito			Field mosquito		
	Parsley	Basil	Cypress	Parsley	Basil	Cypress
1st instar	0.5 ± 0.1a*	0.2 ± 0.1a*	0.2 ± 0.1a*	0.2±0.2^{b*}	0.5 ± 0.1^{a**}	0.2 ± 0.1a*
2nd instar	0.3 ± 0.1^{a*}	0.2 ± 0.1^{a*}	0.2 ± 0.1^{a*}	0.3±0.2^{b*}	0.7 ± 0.1^{a**}	0.2 ± 0.1^{a*}
3th instar	2.0 ± 0.1^{b*}	2.5 ± 0.3^{b*}	2.0 ± 0.1^{b*}	2.4 ±0.2^{b*}	3.2 ± 0.2^{b**}	2.9 ± 0.1^{b**}
4th instar	2.6 ± 0.0^{c**}	3.0 ± 0.2^{b**}	3.0 ± 0.1^{b**}	2.4 ±0.0^{a*}	4.1 ±0.2^{c***}	4.2 ±0.4^{c***}
	Methanolic extract (0.3mg/ml)					
1st instar	0.4 ± 0.1^{a*}	1.5 ± 0.4^{b**}	1.2 ± 0.3^{b**}	0.7 ±0.4^{b*}	2.0 ±0.6^{b***}	3.2 ±0.3^{b***}
2nd instar	0.6 ± 0.1^{a*}	1.7 ± 0.4^{b**}	1.3 ± 0.3^{b**}	0.8 ±0.4^{b*}	3.0 ±0.6^{b***}	2.4 ±0.3^{b***}
3th instar	2.1 ± 0.0^{b**}	4.9 ± 0.5^{a*}	4.3 ± 0.4^{a*}	3.8 ±0.0^{a*}	ND	ND
4th instar	4.0 ± 0.5^{c*}	5.3 ± 0.5^{a**}	4.4 ± 0.2^{a*}	3.9 ±0.7^{a*}	5.7 ± 0.4^{a**}	5.8 ± 0.3^{a**}
	Methanolic extract (0.5mg/ml)					
1st instar	**2.4** ± 0.6^{b*}	ND	ND	2.4 ±0.3^{b*}	ND	ND
2nd instar	3.4 ± 0.6^{b*}	ND	ND	3.6 ±0.3^{b*}	ND	ND
3th instar	4.9 ± 0.3^{ab*}	4.3 ± 0.2^{a*}	ND	ND	ND	ND
4th instar	6.1 ± 0.3^{a*}	6.5 ± 0.4^{b*}	ND	ND	ND	ND

LT_{50}: lethal time required to kill 50% of the population exposed; ND: not determined. Values carrying the same letter in the same column for the same extract weight are not statistically significant ($p \geq 0.05$) *values carrying the superscript in laboratory and field for the same plant are not statistically significant ($p \geq 0.05$).

Appendix 3. Mortality after 24 h of *An. coluzzii* larvae exposed to different concentrations of essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum*

Plant species	**Conc (mg/mL)**	**Instar larvae**				***H-value***	***P-value***
		1st	2nd	3rd	4th		
Ocimum basilicum	0	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	61.00±1.91cA	49.00±1.00dB	41.00±1.00dBC	37.00±1.00dC	14.03	0.003
	0.3	79.00±1.00bA	68.00±1.63cB	65.00±2.51cB	57.00±1.91cC	12.80	0.005
	0.5	99.00±1.00aA	92.00±2.30bA	74.00±1.15bB	63.00±1.91bC	14.03	0.003
	Dichlovos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	18.41	18.66	18.48	18.35		
	P-value	0.001	0.001	0.001	0.001		
Cupresuss lusitanica	0	0.00±0.00d	0.00±0.00d	0.00±0.00e	0.00±0.00e		
	0.1	63.00±2.51cA	53.00±1.91cB	43.00±1.91dC	39.00±1.91dC	12.82	0.005
	0.3	82.00±2.00bA	74.00±2.58bAB	70.00±2.00cB	60.00±1.63cC	12.53	0.006
	0.5	100.00±1.00aA	99.00±1.00aA	79.00±3.41bB	66.00±1.15bC	13.98	0.003
	Dichlovos	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	17.74	17.38	17.31	17.36		
	P-value	0.001	0.002	0.002	0.002		
Petroselinum crispum	0	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00e		
	0.01	**58.**00±2.00cA	51.00±1.91cAB	47.00±4.72cB	44.00±1.63dB	7.88	0.048
	0.03	78.00±3.82bA	75.00±5.74bAB	72.00±3.26bAB	62.00±2.58cB	7.99	0.046
	0.05	**86**.00±2.58bA	83.00±2.51abA	77.00±4.43bAB	71.00±1.91bB	7.87	0.049
	Dichlovos	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	18.09	16.99	17.80	17.05		
	P-value	0.001	0.002	0.001	0.001		

Appendix 4: Lethal time (days) of mosquitoes (larvae) using plant essential oil at three concentrations

Larvae stage	Essential oil (0.05mg/ml)					
	Laboratory mosquito			Field mosquito		
	Parsley	Basil	Cypress	Parsley	Basil	Cypress
2nd instar	$0.2 \pm 0.0^{a*}$	$0.4 \pm 0.1^{a*}$	$0.2 \pm 0.0^{b*}$	0.2 ± 0.1^{b}	$2.0 \pm 0.4^{b**}$	$0.6 \pm 0.1^{a*}$
3th instar	$1.9 \pm 0.1^{b*}$	$2.5 \pm 0.2^{b**}$	$2.4 \pm 0.1^{a**}$	$2.0 \pm 0.2^{a*}$	ND	$2.7 \pm 0.1^{b**}$
4th instar	$2.1 \pm 0.2^{b*}$	$2.9 \pm 0.1^{b*}$	$2.5 \pm 0.1^{a*}$	$2.5 \pm 0.1^{a*}$	$5.8 \pm 0.2^{a***}$	$3.4 \pm 0.2^{c**}$
	Essential oil (0.03mg/ml)					
2nd instar	$0.4 \pm 0.0^{b**}$	$1.0 \pm 0.1^{c*}$	$0.8 \pm 0.1^{b*}$	$0.7 \pm 0.1^{b*}$	ND	$2.0 \pm 0.1^{c***}$
3th instar	$2.4 \pm 0.2^{a*}$	$2.7 \pm 0.0^{b*}$	$2.5 \pm 0.1^{a*}$	$3.0 \pm 0.2^{a*}$	ND	$4.2 \pm 0.0^{b**}$
4th instar	$2.7 \pm 0.3^{a*}$	$3.5 \pm 0.2^{a*}$	$3.0 \pm 0.3^{a*}$	$3.0 \pm 0.0^{a*}$	ND	$5.6 \pm 0.4^{a**}$
	Essential oil (0.05mg/ml)					
2nd instar	**2.1** $\pm 0.3^{b*}$	$3.4 \pm 0.6^{a**}$	$2.9 \pm 0.3^{b**}$	$2.3 \pm 0.1^{a*}$	ND	ND
3th instar	$3.7 \pm 0.2^{a*}$	ND	ND	$3.2 \pm 0.1^{a*}$	ND	ND
4th instar	**3.1** $\pm 0.2^{ab*}$	$3.8 \pm 0.2^{a**}$	$4.4 \pm 0.3^{a**}$	$3.1 \pm 0.3^{a*}$	ND	ND

LT_{50}: lethal time required to kill 50% of the population exposed; ND: not determined. Values carrying the same letter in the same column for the same essential oil weight were not statistically significant ($p \geq 0.05$),*values carrying the superscript in laboratory and field for the same plant are not statistically significant ($p \geq 0.05$)

Appendix 5. Mortality after 24 h of *An. coluzzii* pupae exposed to different concentrations of methanolic extracts and essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum*

Plant products	Comcentration (mg/mL)	Plant species			*H-value*	*P-value*
		Ocimum basilicum	*Cupresuss lusitanica*	*Petroselinum crispum*		
Methanol Extracts	0	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	21.00±1.00dB	37.00±1.91dA	28.00±3.26dA	7.92	0.019
	0.3	35.00±1.91cB	48.00±1.36cA	45.00±1.00cA	6.61	0.013
	0.5	56.00±1.63bA	61.00±1.91bA	59.00±1.91bA	3.11	0.211
	Dichlovos (1000 mg/mL)	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	18.65	18.6	18.65		
	P-value	0.001	0.001	0.001		
Essential oils	0	0.00±0.00e	0.00±0.00e	0.00±0.00		
	0.01	25.00±1.00dB	34.00±1.15dA	30.00±2.00dAB	7.58	0.023
	0.03	35.00±1.91cA	42.00±2.58cA	38.00±1.15c A	4.15	0.125
	0.05	51.00±1.91bA	58.00±2.58bA	56.00±2.82bA	3.67	0.159
	Dichlovos (1000 mg/mL)	100.00±0.00a	100.00±0.00a	100.00±0.00a		
	H-value	18.65	18.41	18.66		
	P-value	0.001	0.001	0.001		

Appendix 6. Knockdown effect after 10, 20, 30, 40, 50 and 60 min of *An. coluzzii* adults exposed to different concentrations of methnolic extracts of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* in the laboratory.

Plant species	Conc (mg/mL)	Time in minutes						*H-value*	*P-value*
		10	20	30	40	50	60		
Cupresuss lusitanica	0	0.00±0.00e	0.00±0.00e	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00e		
	0.1	13.00±2.51deD	16.00±1.63dCD	22.00±1.15eCD	28.00±3.65eBC	36.00±1.63eAB	41.00±4.12dA	19.35	0.002
	0.3	19.00±3.00dD	24.00±5.88cdCD	30.00±3.46dCD	38.00±3.46dBC	51.00±5.25dAB	58.00±6.21cA	17.86	0.003
	0.5	21.00±1.91cD	32.00±4.32cCD	38.00±4.16cC	50.00±2.00cB	62.00±5.29cA	71.00±3.41bA	20.5	0.001
	0.7	45.00±3.00bC	47.00±3.41bC	56.00±2.82bB	61.00±1.91bB	75.00±1.00bA	79.00±3.00bA	20.04	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	21.22	21.18	21.73	22.07	21.45	21.67		
	P-value	0.001	0.001	0.001	0.001	0.001	0.001		
Ocimum basilicum	0	0.00±0.00f	0.00±0.00e	0.00±0.00e	0.00±0.00f	0.00±0.00e	0.00±0.00e		
	0.1	6.00±1.15eD	10.00±1.15dD	15.00±1.00dC	20.00±1.63eB	24.00±2.82dB	33.00±1.00dA	21.17	0.001
	0.3	10.00±1.15dD	16.00±1.63cC	18.00±1.15cC	25.00±1.91dB	30.00±2.00cB	42.00±2.00cA	21.26	0.001
	0.5	13.00±1.00cE	17.00±1.00cD	19.00±1.00cD	30.00±2.00cC	36.00±0.00bC	45.00±1.91cA	21.98	0.001
	0.7	22.00±1.15bE	28.00±1.63bD	34.00±1.15bC	38.00±1.15bBC	41.00±2.51bB	58.00±2.00bA	21.29	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.18	22.01	21.63	21.86	22.23	22.16		
	P-value	<0.001	0.001	0.001	0.001	<0.001	<0.001		
Petroselinum crispum	0	0.00±0.00e	0.00±0.00e	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00d		
	0.1	17.00±1.91eE	19.00±1.00eE	26.00±1.15eD	37.00±1.00eC	42.00±1.15eB	59.00±2.51cA	21.98	0.001
	0.3	28.00±1.63dE	38.00±1.15dD	39.00±1.91dD	53.00±1.00dC	59.00±1.00dB	88.00±1.63bA	21.93	0.001
	0.5	37.00±1.91cF	52.00±1.63cE	57.00±1.91cD	70.00±1.15cC	84.00±1.63cB	92.00±1.63bA	22.10	0.001
	0.7	60.00±1.63bE	68.00±1.63bD	72.00±1.63bD	80.00±1.63bC	92.00±1.63bB	100.0±0.00aA	22.04	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.56	22.67	22.64	22.70	22.60	22.37		
	P-value	0.001	0.001	0.001	0.001	0.001	0.001		

Appendix 7. Knockdown effect after 10, 20, 30, 40, 50 and 60 mins of *An. coluzzii* adults exposed to different concentrations of essential oils of *Ocimum basilicum, Cupresuss lusitanica* and *Petroselinum crispum* in the laboratory.

Plant species	Conc (mg/mL)	Time in minutes						*H-value*	*P-value*
		10	20	30	40	50	60		
Ocimum basilicum	0	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00e	0.00±0.00d		
	0.1	13.00±1.91eF	25.00±1.91eE	33.00±1.91eD	46.00±1.15eC	57.00±1.91dB	72.00±1.63cA	22.226	<0.001
	0.3	25.00±1.91dF	41.00±1.91dE	51.00±1.91dD	60.00±1.63dC	72.00±1.63cB	88.00±1.63bA	22.391	<0.001
	0.5	41.00±1.91cF	56.00±1.63cE	68.00±1.63cD	77.00±1.91cC	86.00±1.15bB	96.00±1.63aA	22.4	<0.001
	0.7	59.00±2.51bE	67.00±2.51bD	77.00±1.91bC	86.00±1.15bB	97.00±1.91aA	100.0±0.00aA	21.973	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.63	22.56	22.56	22.65	22.39	22.11		
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Cupresuss lusitanica	0	0.00±0.00e	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00f		
	0.1	9.00±1.00dE	15.00±1.91eD	18.00±1.15eD	24.00±1.63eC	35.00±1.00eB	47.00±1.91eA	21.66	0.001
	0.3	11.00±1.00dF	21.00±1.00dE	27.00±2.51dD	38.00±1.15dC	45.00±1.91dB	67.00±1.91dA	22.12	<0.001
	0.5	16.00±1.63cF	26.00±1.15cE	34.00±1.15cD	45.00±1.91cC	61.00±1.91cB	82.00±2.58cA	22.46	<0.001
	0.7	28.00±1.63bE	33.00±3.00bE	41.00±1.91bD	52.00±1.63bC	75.00±1.91bB	94.00±2.00bA	21.74	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.01	22.15	22.26	22.39	22.66	22.58		
	P-value	0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Petroselinum crispum	0	0.00±0.00f	0.00±0.00f	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d		
	0.1	18.00±2.58eF	31.00±1.00eE	42.00±2.58dD	60.00±1.63cC	70.00±1.15cB	77.00±1.00cA	22.5	<0.001
	0.3	34.00±2.58dF	48.00±1.63dE	60.00±1.63cD	78.00±2.58bC	85.00±1.91bB	94.00±1.15bA	22.18	<0.001
	0.5	49.00±1.91cD	65.00±3.00cC	82.00±2.58bB	96.00±1.63aA	96.00±2.82aA	100.0±0.00aA	21.06	0.001
	0.7	68.00±3.35bC	78.00±1.15bB	97.00±1.91aA	99.0±1.00aA	100.0±0.00aA	100.0±0.00aA	19.98	0.001
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.6	22.67	22.36	21.67	21.8	22.83		
	P-value	<0.001	<0.001	<0.001	0.001	0.001	<0.001		

Appendix 8. Mortality after 24 h of *An. coluzzii* adults exposed to different concentrations of methanolic extracts and essential oils of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum* in the laboratory.

Plant products	Conc (mg/mL/bottle)	Plant species – mortality of adults after 24 h			*H-value*	*P-value*
		O. basilicum	*C. lusitanica*	*P. crispum*		
Methanol extracts	0	0.00±0.00e	0.00±0.00e	0.00±0.00e		
	0.1	48.00±2.82dA	40.00±1.63dB	47.00±1.00dA	6.56	0.037
	0.3	70.00±5.29cA	49.00±1.91cB	60.00±3.63cAB	7.81	0.02
	0.5	77.00±2.51cA	54.00±2.58cB	60.00±1.63cB	8.73	0.013
	0.7	87.00±1.91bA	69.00±3.41bB	79.00±1.91bA	8.9	0.012
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	21.83	22.04	22.02		
	P-value	0.001	0.001	0.001		
Essential oils	0	0.00±0.00e	0.00±0.00f	0.00±0.00d		
	0.01	48.00±2.82dAB	42.00±2.00eB	60.00±5.88cA	7.29	0.026
	0.03	67.00±1.91cB	56.00±1.63dC	84.00±2.82bA	9.95	0.007
	0.05	86.00±2.00bB	66.00±2.58cC	100.0±0.00aA	10.35	0.006
	0.07	100.0±0.00aA	84.00±2.82bB	100.0±0.00aA	10.50	0.005
	Deltamethrin	100.0±0.00a	100.0±0.00a	100.0±0.00a		
	H-value	22.74	22.58	22.78		
	P-value	<0.001	<0.001	<0.001		

Appendix 9

REPUBLIQUE DU CAMEROUN
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UNIVERSITY OF DOUALA

CEI - UD

INSTITUTIONAL ETHICS COMMITTEE FOR RESEARCH ON HUMAN HEALTH

N°2968/IEC-UD/03/2022/T

Douala, the 1st of March 2022

ETHICAL CLEARANCE

The Institutional Ethics Committee for Research on Human Health of the University of Douala (**IEC-UDo**) for the 1st of March 2022 evaluation session, has examined the research project entitled «**Insecticidal effects of *P. Crispum*, *O. Basilicum* and *C. Lusitanica* on the developmental stages of malaria vector species, *Anopheles coluzii***» submitted by **TAMUNJOH Stella Shinwin ATEYIM** for Thesis at the Faculty of Science of the University of Yaounde 1.

The present research project has a clear scientific interest and presents no risk for its participants. The objectives and methodology of this research project are clearly described. The principle of data confidentiality is respected. The required expertise for the supervision of the research is present.

From the above mentioned observations, the IEC-UDo approves this version of the project for a period of one year.

However, **TAMUNJOH Stella Shinwin ATEYIM** is responsible of the scrupulous respect of the methodology and ethical consideration, and should not amend it without approval of the IEC-UDo. Researchers are expected to collaborate with the IEC-UDo for a follow-up of the ethical aspects of the approved project. A copy of the final report of this research project should be submitted to IEC-UD for archival purposes.

The present ethical clearance is delivered to serve the purpose for which it is presented. It can be cancelled in case of non-respect of the above recommendations.

Copy

- MINPH

The PRESIDENT

CEI-UD

Pr Léopold Gustave LEHMAN

NB : Only one copy of an ethical clearance is delivered.

N° 0977/Minsante/SESP/SG/DROS of April 16, 2012
Campus de Logbessou, 3è étage du bloc pédagogique de la FMSP.
Tél. : (237) 680.35.98.35 / 695.39.35.50 / B.P. : 2701 Douala - Cameroun / e-mail : cei@univ-douala.com

Appendix 10

Mortality rate of adult female *Anopheles coluzzii* after 24h exposure in *Petroselinum crispum, Ocimum basilicum* and *Cupresuss lusitanica* methanolic extracts in the laboratory (laboratory and field strains).

The mortality rates of methanolic extracts against mosquito adults were reported in Table 18. It was observed that the mortality rate increases with concentrations for parsley, cypress and basil methanolic extracts ($p < 0.05$). There was 100% mortality for parsley methanolic extracts in 0.05mg/mL and 0.07mg/mL) which mean that all the concentrations used had the same effect ($p \geq 0.05$).

In the case of laboratory-reared mosquitoes (adult), the mortality rate varied from 48% to 92% and for field-reared mosquitoes (adults), it was from 40% to 80% for parsley, 36% to 76% and 20 to 56% for basil respectively ad 48% to 80 ad 32% to 72% for cypress respectively. Thus, methanolic extracts were statistically more active on laboratory-reared mosquitoes than on field-reared ones ($p < 0.05$). From the concentration 0.1mg/mL to 0.7mg/mL, parsley methanolic extracts was the most active ($p < 0.05$). It is followed by cypress and then by basil methanolic extracts.

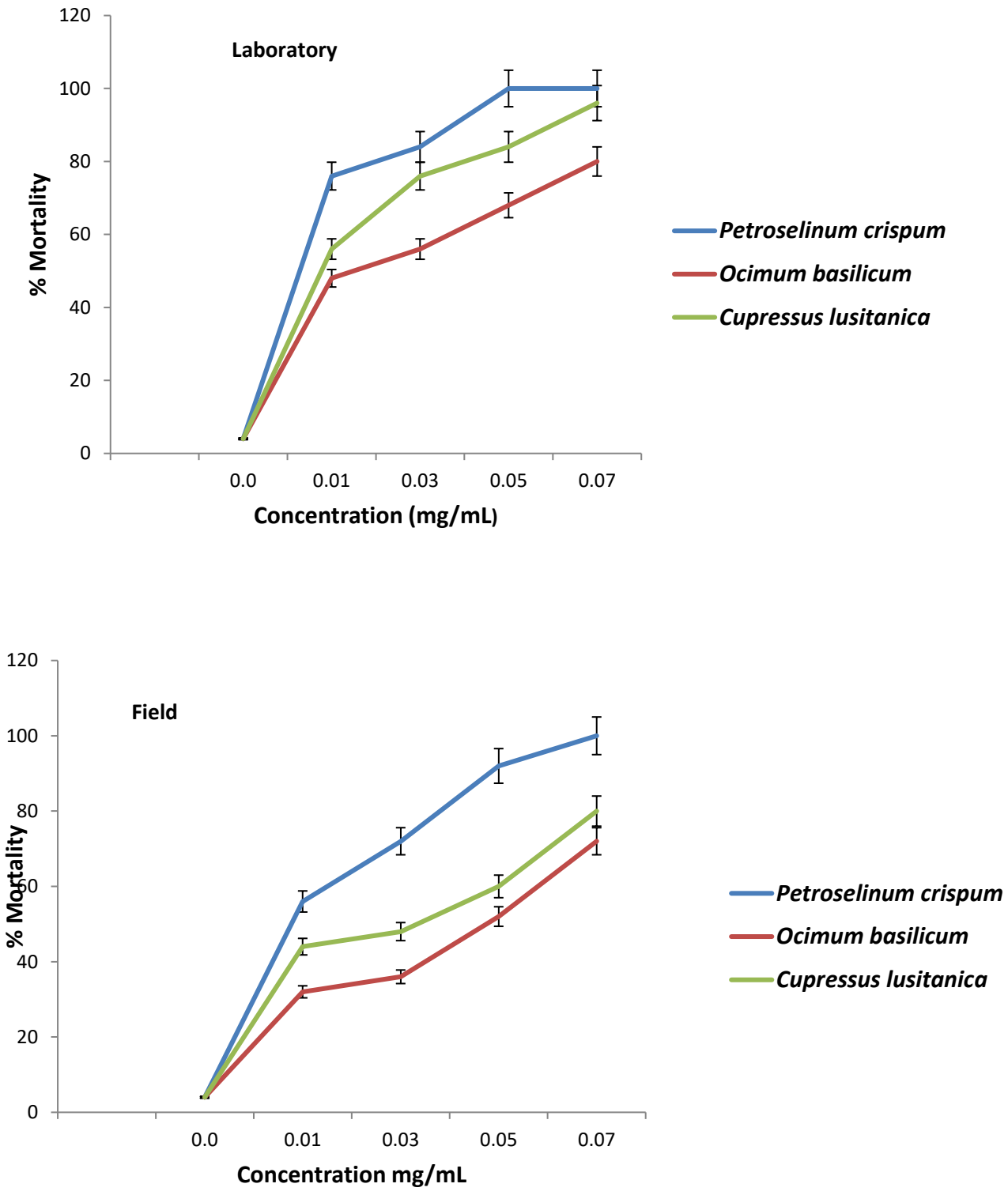

Figure 24: Mortality after 24 h of laboratory and fields strains of *An. coluzzii* adults exposed to different concentrations of essential oils of *Ocimum basilicum*, *Cupresuss lusitanica* and *Petroselinum crispum.*

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Research Article

Egg Hatching Reduction and Larval Mortality Induced by Essential Oil and Extracts of *Petroselinum crispum* (Parsley) Leaves in the *Anopheles coluzzii* Malaria Vector Species

Tamunjoh Stella Shinwin Ateyim[1,2], Foko Dadji Gisele Aurelie[2], Baudelaire Elie[3,4,5], Dicko Amadou[3], Djieukap Njieyap Laurelle[6,7], Akono Ntonga Patrick[8], Antonio-Nkondjio Christophe[6,9], Tamesse Joseph Lebel[2], Awono-Ambene Herman Parfait[6,10*]

[1]Faculty of Science, University of Yaounde1, Yaounde, Cameroon

[2]Department of Biological Sciences, Higher Teacher Training College, University of Yaounde I, Cameroon

[3]Laboratory of Physics and Chemistry, Jean Baritiot Institute, University of Lorraine, Metz, France

[4]National School of AgroIndustrial Sciences (ENSAI), University of Ngaoundere, Ngaoundere, Cameroon.University of Ngaoundere, Ngaoundere, Cameroon

[5]AGRITECH, France

[6]Institut de Recherche de Yaoundé (IRY), OCEAC, Yaoundé, Cameroon

[7]Faculty of Science, Laboratory of Parasitology and Ecology, University of Yaounde I, Cameroon

[8]Faculty of Science, University of Douala, Douala, Cameroon

[9]Vector Biology Liverpool School of Tropical Medicine Pembroke Place, Liverpool, UK

[10]School of Health Sciences, Catholic University of Central Africa, Yaoundé, Cameroon

[*]Corresponding Author: Awono-Ambene Herman Parfait, Institut de Recherche de Yaoundé (IRY), OCEAC, Yaoundé, Cameroon

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Abstract

The interest of plant-based products is increasing as alternative solutions to current synthetic insecticides associated with detrimental effects on the environment. Here we assessed the potential deterrent effect of parsley (*Petroselinum crispum*) formulations on immature stages of the African malaria vector, *Anopheles gambiae* s.l. In vitro bioassays were performed to evaluate egg-hatching reduction and larval mortality induced 24 hours post exposure at various concentrations by crude powder, methanol extract and essential oil of parsley leaves. Plant powder and methanol extract were rich in alkaloids, saponins and phenolic compounds, while myristicin (67.1%) was the main compound in essential oil.

Parsley induced 19-75% egg hatching reduction, 43-88% overall larval reduction and 26-77% mortality on 3rd and 4th instars, with significant variations by formulations and concentrations. Essential oil (LC50=0.011-0.014 mg/mL, LC95=0.12-0.26 mg/mL) showed low effective concentrations against *An. coluzzii* larvae compared with the methanol extract (LC50=0.17-0.20 mg/mL, LC95=5.44-6.54 mg/mL). These findings provide evidences that *P. crispum* formulations, especially essential oil might be identified among new potential plant-based products to evaluate towards alternative tools for malaria vector control.

Keywords: Egg hatching; Larval mortality; *Petroselinum crispum*; *Anopheles coluzzii*; Malaria vector

Abbreviations

GC/MS: Gas Chromatography coupled with Mass Spectrometry (GC/MS); IRY: Institut de Recherche de Yaoundé; LC: Lethal Concentration; OCEAC : Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale

1. Background

Mosquito control techniques in use to combat adults or immatures stages depend principally on the application of synthetic insecticides such as pyrethroids, organochlorines, organophosphates, and carbamates [1]. However, these synthetic insecticides products widely in use are harmful to humans and other non-target living organisms and they pollute the environment, and their improperly application contributes to mosquito resistance problems [2]. Therefore, during these three last decades, research efforts were mostly focused on the development of eco-friendly alternative insecticides. Some constituents of plant extract and essential oil like tannins, flavonoids, alkaloids, glycosides, saponins, terpenoids, steroids, hydrogenate and dehydrogenate monoterpenes as well as sesquiterpenes were reported to possess toxic effect against developmental stages of mosquito species [3]. Intirach et al. [4] reported the insecticidal effect of *P.*

crispum essential oil against *Aedes aegypti* mosquitoes. Essential oil of *P. crispum* also significantly decreased weight, volume and energy reserves of *Culex pipiens* and *Culiseta longiareolata* larvae and pupae [5]. Botanicals are largely documented as potential effective insecticides which are target specific, ecofriendly safe, and their phyto-constituents may overcome the resistance problem developed by some insect pests [6]. Numerous mosquito species including the widespread malaria vector species, *Anopheles gambiae* s.l., have gradually developed resistance to common insecticide families i.e. organochlorides, organophosphates, carbamates and pyrethroids.

Therefore, WHO has recommended for actions to mitigate adverse effects of such multi-resistance towards the control of mosquito-borne diseases like malaria, dengue fever, lymphatic filariasis and some arboviruses which are transmitted through the bites of infected females of *Anopheles* species [7-10]. Among these diseases, malaria remains the most deadful parasitic infection, and the *Plasmodium falciparum* species remains the first cause of malaria cases and deaths. The parasite is transmitted essentially by sibling species of the *An. gambiae* complex, *An. gambiae* and *An. coluzzi* [11]. In 2019, Cameroon registered up to 6.2 million cases and 11,233 deaths [12]. The disease epidemiology remains stable due to, among others factors, the nationwide spread of mosquito resistance to pyrethroid-based formulations used for vector control.

The country has developed recently a national plan for resistance management, with special interests on alternative strategies or approach including environmental management and biocides. Several aromatic plants used locally for domestic or pharmaceutical purposes have been tested so far to check for their potential activity against both larval and adult mosquitoes [13]. Among them,

Petroselinum crispum (Apiaceae) which is native to the Mediterranean region (Greece, Spain, Italy, Malta, Tunisia, Algeria and Morocco) until its dissemination worldwide [14], is one of the most cultivated vegetables in Cameroon. Here, we assessed the potential deterrent effect of parsley (*Petroselinum crispum*) formulations on immature stages of the African malaria vector mosquitoes, *Anopheles gambiae* s.l., with as expected ambition in providing further evidences that parsley-based formulation might be among potential active biocides to control mosquitoes.

2. Materials and Methods

2.1 Plant collection

P. crispum leaves were collected early in the morning (around 7:00 am) from Santa (North West Region of Cameroon). The plant species was identified in the department of Botany (Higher Teacher Training College) and confirmed at National Herbarium of Cameroon at Yaounde under the registration number 403884/SFRcam. The plant leaves were dried at shade for 15 days and pulverized in the electric blender. The grinded leaves were passed through 0.5 mm mesh size sieve and the powder obtained was packaged in the sealed plastic container until its use for extraction, chemical screening and biological assays.

2.2 Plant methanolic extraction

A weight of 129 g of *C. lucitanica* powder was macerated in 3000 mL of methanol for 72 h and then filtered using Whatman No.1 filter paper. The filtrate was submitted to rotary evaporator to remove the solvent and then dried in the oven set at 60°C. The dry methanolic extract obtained was weighed and the extraction yield was calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of plant powder used (g)}} \times 100$$

2.3 Plant essential oil extraction

Essential oil was isolated from the leaves by hydro distillation process using Clevenger apparatus-type in the laboratory of Microbiology, Faculty of Science, University of Yaounde 1, Cameroon. Traces of water in the essential oil recovered were discarded using anhydrous sodium sulphate and kept in dark glass bottle in the refrigerator till its use for phytochemical analysis and larvicidal assay. Essential oil extraction yield was determined following the formula below:

$$\text{Essential oil yield (\%)} = \frac{\text{Weight of essential oil recovered (g)}}{\text{Weight of plant fresh leaves used (g)}} \times 100$$

2.4 Chemical and GC/MS analysis of plant extracts Methanolic extract and powder of *P. crispum* were submitted each to phytochemical screening to identified the presence of potential active plant constituents including alkaloids, saponins, tannins, flavonoids, terpenoids and phenolic. The main constituents of essential oils were determined by Gas Chromatography coupled with Mass Spectrometry (GC/MS), as described by Adams [15]. GC/MS analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer line temperatures were 220 °C and 250 °C, respectively. The oven program temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH2Cl2, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the m/z range 35300; electron multiplier 1460 eV; scan rate, 2.96 scan/s. The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of n-alkanes (C9–C22). Their mass spectra were compared with the standards (for main components) and values found in the literature including the NBS75K database and Wiley 7th NIST 2014 EPA/NIH Mass Spectral Library Upgrade, provided by the GC/MS control and data processing software guidelines. The percentage composition of the essential oils was computed by the normalization method from the GC/FID peak areas, assuming an identical mass response factor for all compounds.

2.5 Anopheles mosquito strain

Anopheles coluzzii mosquito progenies from the IRYOCEAC insectary were used for bioassays. This susceptible *An. coluzzii* Ngousso strain were adapted to artificial rearing conditions of ambient temperature

(28–30°C) and relative humidity (70–80%) since 2006 [16].

2.6 Egg hatching and growth reduction assays with parsley powder

Freshly laid eggs of *A. coluzzii* collected from ovipositors and matured at 26-28°C and 70-80% HR under photoperiod 12L: 12D, were then transferred to individual petri dishes at various concentrations of parsley powder (0.1, 0.3 and 0.5 g/mL) to check for hatching rate. Each replicate of 25 eggs (four replicates per concentration) was monitored until egg hatching and the counting of active first instar larvae (L1). After 48 h post-treatment, each batch of the tested mosquito eggs were recovered retaining them on a muslin cloth, then cleaned with water, and observed under microscope at 10 X magnification for hatching assessment after counting non-hatched eggs. The percentage of non-hatched eggs was calculated based on the number of eggs with unopened opercula at the end of the test. For larval growth reduction assays, larvae (L1) were then pooled per concentration into individual containers and fed with Tetramin fish food (0.625 mg/25 larvae/day). The larval growth reduction was calculated at the end of complete larval development to fourth instars (L4), adjusted with the number of emerged pupae. Control arms (without parsley powder) were used for both egg hatching and larval development follows up.

The following formula described for aquatic toxicity testing [17] was adapted for the calculation of the percentage of egg hatching and larval growth reduction rate for each concentration: $Rr\ (\%) = \left[\left(\frac{\mu C - \mu T}{\mu C}\right) \times 100 \right.$, where *Rr* (%) was the reduction rate in percentage; μC, the average mean hatched eggs/ emerged pupae in the control group, and μT, the average mean hatched eggs/emerged pupae for a given concentration.

2.7 Egg hatching and bioassays with methanol extract and essential oil

Four replicates of 25 mature eggs of *A. coluzzii* each were exposed in plastic cups containing various concentrations of methanolic extract (0.1, 0.3 and 0.5 mg/mL) and essential oil (0.01, 0.03 and 0.05 mg/mL), to record the hatching rate by checking unhatched eggs under microscope at 10X magnification. In parallel, four batches of 25 *An. coluzzii* larvae (3rd and 4th instars) was separately transferred to plastic containers and reared at such methanolic extract and essential oil concentrations, respectively. The number of dead larvae induced by parsley formulations was recorded 24h post-treatment by concentration ranges, and Abbott's formula [18] was used for correction when larval mortality in the control arm ranged from 5 to 20%. A set of 100 larvae (50 third instars and 50 forth instars) were distributed into four plastic cups containing 99 mL of spring water and 1 ml of ethanol each were monitored as control batches.

2.8 Statistical analyses

The Pearson's Chi-square and Kruskall-Wallis nonparametric tests were used to compare egg hatching and mortality rates by concentrations and formulations. Probit analysis [19] was employed to calculate LC50 and LC95 values of the plant methanol extract and essential oil causing 50% and 95% mortality of

mosquito stages. Differences were considered significant at a rate of probability (P) less than 0.05 ($P<0.05$).

3. Results

3.1 Phytochemical composition of powder and methanol extract

Results of the phytochemical screening shown in table 1 revealed the presence of alkaloids, saponins and phenolic compounds in plant powder and in methanol extract. However, methanolic extract showed additional group of compounds, terpenoids, whereas both extracts were negative for other phytochemical groups of components such as tannins and flavonoids.

Phytochemical groups evaluated	Plant powder	Methanolic extract
Flavonoids	-	-
Alkaloids	+	+
Tannins	-	-
Terpenoids	-	+
Saponins	+	+
Phenolic compounds	+	+

Abbreviations: + = present; - = absent

Table 1: Phytochemical compounds isolated from powder and methanol extracts of *Petroselinum crispum* leaves.

No.	RT (min)	Compounds	Phytochemical category	Proportion %
1	14.97	Myristicine	Phenylpropanoids (phenolic compounds)	67.1
2	16.77	Apiol		3.6
3	20.37	3,4α,7,7,10α-Pentamethyl-3-vinyldodecahydro-1Hbenzo[f]chromene		1.0
4	10.49	Estragole		0.8
5	15.96	Bisabolene < (E)- iso-ϒ>	Sesquiterpenes (terpenoids)	8.6
6	15.74	β -Sesquiphelandrene		5.4
7	16.48	Sesquisabene hydrate		2.6
8	10.36	4-(1-Methylethyl)-2-cyclohexen-1-one	Monoterpenes (terpenoids)	2.9
9	10.27	4-Terpineol		2.1
10	8.89	Linalool		1.1
11	7.81	1-Isopropyl-4-methylenebicyclo[3.1.0]hexane		0.9

12	10.07	3-Thujen-2-one		0.8
13	12.65	α-Terpineol acetate		0.7
14	7.68	p-Isopropyltoluene		0.5
15	8.79	α,p-Dimethylstyrene		0.4
16	7.01	β-Myrcene		0.3
17	7.75	Limonene		0.3
18	10.32	m-Methylacetophenone	other aromatic compound	0.9
		Total		**100.0**

Abbreviations: No: number; RT: retention time; %: percentage

Table 2: Name and retention time (RT) of chemical constituents isolated from essential oil of Petroselinum crispum leaves.

3.2 Chemical composition of essential oil The MC/GC profile of *P. crispum* essential oil was composed by 18 different active compounds. These belong to at least three main phytochemical groups including four phenylpronanoids (72.5%), thirteen terpenoids (26.6%, i.e. three sesquiterpenes: 16.5%, 10 monoterpenes: 10.1%), one benzenoid (1.9%) and another not classified aromatic compound (Table 2). Among these identified constituents, myristicine (phenylpronoid) was the main compound (67.1%), following by sesquiterpene compounds such as basibolen (8.6%) and β-sesquiphelandrene (5.3%).

3.3 Egg hatching inhibition and larval reduction induced by crude powder

The number of eggs hatched into larvae is concentration dependent. The trend of hatching rate was inversely proportional to increase concentration ranges (P=0.003). The crude powder induced 19.45%, 45.83% and 58.33% egg inhibition at 0.1 g/mL, 0.3 g/mL and 0.5 g/mL, respectively (Table 3). The similar trend was also recorded for larval development inhibition, with rates of 43.82%, 77.53% and 87.64% at 0.1 g/mL, 0.3 g/mL and 0.5 g/mL of crude powder, respectively. Bioassay results showed a significant reduction on larval development from 1st instar to pupae induced by parsley powder regardless the concentration range (P<0.01).

This toxic effect against *An. coluzzi* mosquitoes was concentration-dependent, with high reduction rates recorded at highest concentrations. However, larval reduction rates did not vary significantly between the concentrations tested (X^2=3.623, df=2, P=0.163).

Variables	**0 g/mL (control)**	**0.1 g/mL**	**0.3 g/mL**	**0.5 g/mL**
Number of eggs tested	100	100	100	100
Hatched eggs (% ± 95% CI)	96.00 ± 3.84	77.00 ± 8.25	52.00 ± 9.79	40.00 ± 9.60
Egg hatching reduction (% ± 95% CI)	-	19.79 ± 7.81	45.83 ± 9.77	58.33 ± 9.66
Number of 1st instar larvae that have reached the pupae	89	50	20	11
Larval growth reduction (% ± 95%CI)	-	43.82 ± 11.08	77.53 ± 11.34	87.64 ± 10.20

Abbreviations: g/L: gramme per milliLiter; CI: Confidence Interval; %: percentage; T°C temperature; RH: relative Humidity

Table 3: Hatching inhibition and mortality induced by *P. crispum* powder based formulations on the immature stages (eggs and all instar larvae) of *A. coluzzii* in the labotatory conditions (T°C: 25 ± 2°C; 75 ± 4% RH).

3.4 Egg hatching inhibition and larval mortality induced by methanol extract and essential oil These parameters were monitored against *An. coluzzii* mosquitoes (Table 4). The methanol extract and essential oil induced the increasing egg hatching inhibition rates, varying accordingly by concentrations from 25% to 75% with methanol extract (X^2=48.240, df=2, $P<10^{-4}$) and from 17% to 46% for essential oil (X^2=16.310, df=2, $P<10^{-3}$). The inconsistency observed for egg hatching inhibition between the both formulations was not statistically significant (X^2=0.453, df=2, P=0.797).

Concerning the larval mortality, the overall mortality rate reached 40% at 0.1 mg/mL, 53% at 0.3 mg/mL and 72% at 0.5 mg/L of methanol extract, with significant variations between the three concentrations (X^2=20.929, df=2, $P<10^{-4}$). The trend of larval mortality recorded with essential oil was similar with that of methanol extract, varying according to increased concentration ranges from 47% to 77% (X^2=19.887, df=2, $P<10^{-4}$). Mortality rates did not differ between 3rd and 4th instars regardless the type of formulation (X^2-values < 2.000, df=2, P>0.450).

Globally, LC_{50} and CL_{95} (mg/mL) values of *P. crispum* methanolic extract and essential oil varied with the larval stages. The LC_{50} and LC_{95} ranges were estimated for 3rd instar (LC_{50}=0.17 mg/mL; LC_{95}=5.44 mg/mL) and 4th instar (LC_{50}=0.20 mg/mL; LC_{95}=6.54 mg/mL) for methanolic extract. Lethal concentrations were also calculated with essential oil against 3rd (LC_{50}=0.011 mg/mL; LC_{95}=0.257 mg/mL) and 4th (LC_{50}=0.014 mg/mL; LC_{95}=0.123 mg/mL) instar larvae of *An. coluzzii*.

Plant formulations	**Concentration (mg/mL)**	**Egg hatching Rr** (%± 95% CI)	**Mortality ranges**		
			Overall (%± 95% CI)	3rd instar	4th instar
Methanolic extract	0.1	25.00	40.00 ± 9.60	41.00	38.00
	0.3	41.67	53.00 ± 9.78	54.00	52.00
	0.5	75.00	72.00 ± 8.80	73.00	70.00
Essential oil	0.01	16.67	37.00 ± 8.52	47.00	26.00
	0.03	29.17	57.00 ± 9.70	72.00	41.00
	0.05	45.83	68.00 ± 9.14	77.00	58.00

Abbreviations: mg/mL: milligramme per milliliter; CI: Confidence Interval; %: percentage; T°C temperature; RH: Relative Humidity, Rr: Reduction rate

Table 4: *Anopheles coluzzii* egg hatching reduction and larval mortality rates induced by Petroselinum crispum methanolic extract and essential oil in the laboratory conditions (Temperature=25 ± 2°C; 75 ± 4% Relative Humility).

4. Discussion

Petroselinum crispum (parsley) is a perennial plant in the family of Apiaceae, in use in Cameroon and elsewhere by local communities as spice herb for culinary purpose and/or as medicinal plant. Apart from such consumption, parsley as other aromatic plants, is increasingly under investigation to assess their potential activity in controlling pests and disease borne vectors such as mosquitoes [20-22]. Mosquitoes belonging to the *Anopheles gambiae* complex of species transmit the most infectious parasite species, *Plasmodium falciparum*, involved in the majority of deadly malaria infections in Cameroon [11]. Because of adaptative responses developed by natural malaria vector populations to escape current vector control strategies (i.e. behavioural changes, resistance to insecticides, etc.), the development of new biocides including eco-friendly plant-based insecticides might be potential alternatives for an improved management of vectors and associated environments [23, 24].

It is therefore in this context we proposed this paper to describe potential effects of parsley-based formulations against immature stages of *Anopheles coluzzii*, one of the major malaria vector species of the *Anopheles gambiae* complex. The first information gathered from this study is that local parsley leave extracts are composed predominantly by phenolic compounds, especially myristicin. This phenolic constituent was in high concentration in essential oils obtained from leaves as well as from other parts of parsley including roots, fruits and herbs [25, 26]. Other chemical compounds usually found in various aromatic plants were terpenoids, saponins and alkaloids [27]. The chemical composition of parsley showed variations by seasons, locations and extracts [4, 6, 26-28]. Recent works reported from 17 to 25 different compounds in parsley extracts, among which pulegone, D-Limonene, thymol, *p*-cymene and γterpinene were the main active compounds. The most frequent secondary metabolites of plants found toxic for insects belong to terpenoids, steroids, phenols, flavonoids, tannins, alkaloids and cyanogenic glycosides compounds [29-31]. The present study suggests that parsley plants collected locally were rich of phenylpropanoid compounds, especially myristicin which was the main secondary metabolite isolated from essential oil. This compound (myristicin), known for its potential hallucinogenic effects on human [32] had been so far identified as potential natural insecticide and synergist [33].

The growth reduction (16-77%) and mortality rates (36-68%) induced by parsley-based formulations (powder, methanol extract and essential oil) on the *Anopheles coluzzi* immature stages are evidences for its toxicity, probably increased by the presence of other active compounds i.e. terpenoids and alkaloids. These findings are consistent with that reported previously by Foko Dadji et al. on *Capsicum annuum* powder [21]. Other studies have also indicated that extracts of *Atlantia monophylla*, *Pseudocalymma alliaceum*, *Cardiospermum halicacabum*, *Hyptis suaveolens* and others caused a high disturbance in the growth regulation and larval survival among various mosquito species including *Anopheles*, *Aedes* and *Culex* mosquitoes [14, 34-39]. Some authors attributed this development disturbance to the presence of growth regulator enzymes in plant extracts that causing

morphological and physiological disorders which interfere with total development of insect [24, 40]. The level of parsley toxicity against Anopheles developmental stages varied by concentrations and by extracts. The essential oil displayed 68% mortality rates after 24 h postexposure at the lowest concentrations (0.05 mg/mL), whereas mortality induced by 0.1% methanol extract was 40%. As observed previously with synthetic myristicin [33] and essential oil extracts [41, 42], the above observation confirms that parsley essential oil might be a potential phyto-chemical formulation for mosquito control. However, the not negligible deterrent effect induced by crude powder could be also addressed at the level of community, because of its limited access to essential oil based products.

5. Conclusion

This paper aimed at assessing potential growth inhibition induced by parsley-based formulations against immature stages of *Anopheles coluzzii*, one of the major African malaria vector species. Here, we provided supplementary data presenting the ovocidal and larvicidal activity of parsley formulations on the developmental mosquito stages. This toxicity was concentration-dependent and showed variations by extracts. Globally, the low concentrations of essential oil revealed significantly effective for egg hatching inhibition and larvicidal effect against tested mosquito stages. Thus, this formulation of parsley might be identified among new potential plant-based products to evaluate towards alternative tools for malaria vector control.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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