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Phytochemical constituents and larvicidal activity of medicinal plant hydroethanolic extract against mosquito species

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Dedication

No expression, however elaborate, could express my deep gratitude and appreciation for all these years.

I dedicate this modest work ;

to my dear father , who was always there for me.

To the dearest in the world,pieace of my heart my mother ,God rest her soul.

my second mother Sabrina who has always encouraged me throughout my years of study.

To all my dear brothers

To my dear sister Amira and Oumaima

To all my family

My best friend Sonia

To all the teachers who have contributed to my education

Dedication

In the name of allah,the most gracious the most merciful,all praise to him without him this work would never been done

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To the soul of my grand mothers and those who shared with me all the emotional moments during the complexion of this work. They warmlysupported me throughout my jorney.

To all my friends ,relatives and colleagues.

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To my besties : Omaima , Sonia , Imene

To my family , my beloved ones and those who give me love and vivacity.

الملخص

كان الهدف من هذه الدراسة هو تقييم تأثير المستخلص المائي الأنتانولي المائي من ريتاما سفيركاربا على نوع من البعوض Cs longiareolata، الأكثر انتشارًا في منطقة

منطقة تبسة. تمت دراسة عدة جوانب:

الجانب السمي المتمثل في تحديد التركيزات المميتة. أظهر المستخلص المائي الحولي المائي من ريتاما سفيركاربا سمية ليرقات3 Culiseta longiareolata

و4 يرقات Culiseta longiareolata مع وجود علاقة بين الجرعة والاستجابة.

الجانب المورفومتري المتمثل في وزن وحجم جسم يرقات Culiseta longiareolata حديثة الطور الثالث والرابع L3 و L4.يُظهر تحليل البيانات أن المستخلص المائي الأنتانولي المائي من ريتاما

spherocarpaيؤثر على هذه البارامترات.

تم تحديد جانب كيميائي حيوي عن طريق قياس الاحتياطيات الأيضية في يرقات الطور الثالث والرابع التي تم إخراجها حديثاً.

أظهرت أن هذه الاحتياطيات مضطربة نسبيًا بسبب المستخلص المائي الأنتانولي من ريتاما سفيركاربا وخاصة البروتينات.

الكلمات المفتاحية : Culiseta longiareolata ، Retama spherocarpa، السمية، قياس التشكل، التركيب الكيميائي الحيوي.

Abstract

The aim of this study was to evaluate the effect of the hydro-etnanolic extract of Retama spherocarpa on a species of mosquito Cs longiareolata, the most widespread in the region of

Tébessa region. Several aspects were studied:

A toxicological aspect represented by the determination of lethal concentrations. The hydro-etnanolic extract of Retama spherocarpa showed toxicity to Culiseta longiareolata larvae 3

and 4 larvae of Culiseta longiareolata with a dose-response relationship.

A morphometric aspect that is represented by the weight and body volume of newly exuviated Culiseta longiareolata third and fourth instar L3 and L4 larvae. Analysis of the data shows that the hydro-etnanolic extract of Retama

spherocarpa affects these parameters.

A biochemical aspect was determined by measuring metabolic reserves in newly exuviated third and fourth instar larvae show that these are relatively disturbed by the hydro-etnanolic extract of *Retama spherocarpa*, and more particularly proteins.

Key words: Culiseta longiareolata, *Retama Spherocarpa*, toxicity, morphometry, biochemical composition.

Résumé

Cette étude vise à evaluer l'effet de l'extrait hydro-etnanolique de Retama spherocarpa à l'égard d'une espèce de moustique Cs longiareolata, la plus répandue dans la région de Tébessa. Plusieurs aspects ont été étudiés:

Un aspect toxicologique qui représentés par la détermination des concentrations létales. L'extrait hydro-etnanolique de Retama spherocarpa montre une toxicité à l'égard des larves 3 et 4 de Culiseta longiareolata avec une relation dose-réponse.

Un aspect morphométrique qui est représentés, par le poids et le volume corporel des larves du troisième et quatrième stade L3 et L4 nouvellement exuvies de Culiseta longiareolata. L'analyse des données montre que l'extrait hydro-etnanolique de Retama spherocarpa affect ces paramètres.

Un aspect biochimique qui a été déterminé par le dosage des réserves métaboliques chez des larves du troisième et quatrième stade nouvellement exuviées, les résultats obtenus montrent que ces sont relativement perturbée par l'extrait hydro-etnanolique de *Retama spherocarpa*, et plus particulièrement les protéines.

Mots clés: Culiseta longiareolata, *Retama Spherocarpa*, toxicite, Morphométrie, Composition biochimique.

List of figure

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List of abbreviation

1. **µg** - Microgram
2. **m ± SD** - Mean ± Standard Deviation
3. **n** - Number of samples
4. **mm^3** - Cubic millimeter
5. **ppm** - Parts per million
6. **FL** - Field Laboratory **µg** - Microgram
7. **Cs** – *Culiseta*
8. **R**.S- *Retama Sphearocarpa*
9. **m ± SD** - Mean ± Standard Deviation
10. **n** - Number of samples
11. **mm^3** - Cubic millimeter
12. **ppm** - Parts per million
13. **FL** - Field Laboratory
14. **TCA** - Trichloroacetic Acid
15. **rpm** - Revolutions per minute
16. **°C** - Degrees Celsius
17. **BSA** - Bovine Serum Albumin
18. **BBC** - Brilliant Coomassie Blue

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Introduction

1. Introduction

Phytochemical constituents, a vast array of natural compounds derived from plants, havegarnered significant interest due to their diverse biological activities and potential applicationsin various fields, including medicine, agriculture, and pest control. Theseconstituents encompass a wide range of chemical classes, such as alkaloids, flavonoids,terpenoids, phenolic compounds, and others, each with unique properties and therapeutic potential **(Kumar *et al*., 2012).**

Among the myriad of plant species harboring phytochemical diversity, *Retama sphaerocarpa*,commonly known as Spanish broom, emerges as a noteworthy candidate. This Mediterraneanshrub has long been recognized for its medicinal properties in traditional folk medicine, withdocumented uses ranging from wound healing to gastrointestinal disorders. The rich phytochemical profile of *Retama sphaerocarpa* encompasses a plethora of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and terpenoids,which contribute to its therapeutic effects **(Mouhaddach *et al*., 2018)**.

Mosquitoes transmit serious human diseases, causing millions of deaths every year **(Ghosh *et al*., 2011)**. Major genera of mosquitoes that act as vector for various disease including dengue, chikungunya, malaria, yellow fever, filariasis, japanese encephalitis, lyme, disease, andepidemic polyarthritis **(Kalita *et al*., 2013)**. Only females are hematophagous unlike males and vector **(Dahchar *et al*., 2016)**. Mosquitoes are generally controlled by synthetic insecticides derived from those developed for agriculture and are very often based on organophosphates, pyrethroids, organochlorines, carbamates or cyclodienes **(McAllister and Adams. 2010)**.

However, these chemicals have caused environmental contamination as well as side effects to non-target organisms. Moreover, mosquito control techniques present serious threats because of the emergence of resistance to synthetic insecticides widely used **(Ouma and Russell. 2013 ; Chirchir *et al*., 2013)**. Therefore, there is an urgent need to develop new insecticides which are biodegradable and environmentally safe **(Pavela *et al*., 2014)**.

An alternative and recent approach for mosquito control is the use of natural products of plant origin, known as botanical derivatives. Many botanical natural products are effective, environment-friendly, easily biodegradable and inexpensive. They have no negative effects on non-target organisms and have varied novel modes of action **(George *et al.* 2014)**.

In this context and in the present study, *Retama spherocarpa* hydro-ethanolic extract was evaluated for their larvicidal activity against *Cs. longiareolata*, the most interesting mosquito

species in Algeria, particularly in Tebessa area **(Bouabida. 2014)**. In addition, its effects on morphometric measurements and on main biochemical components (carbohydrates, proteins and lipids) in whole body of the larval stage were investigated.

Matriels and methods

2. Materials and methods

2.1. *Culiseta longiareolata*

Culiseta longiareolata is a species of the family Culicidae belonging to the subfamily *Culicinae* **(Khaligh *et al.*, 2020)**. It is an insect that is resistant to full metamorphosis and thrives in warmer climates. The size of this insect varies between 3 and 5 mm. It has a small body with long, slender legs and long, thin, membranous wings. **(Villeneuve and Desire, 1965)**. The identification of this species is not difficult. The species is considered to be a vector of avian plasmodia **(Bruhnes *et al.*, 1999)**. The larvae of this species are characterised by a short, conical siphon with irregularly arranged siphuncle teeth. Adults have at least one dark scale patch on the wing, a thorax with three longitudinal white stripes and the absence of long, strong bristles on the basal lobe of the gonocoxite. This species may have a winter diapause in female imagos (cold regions) and larvae (temperate regions). Adults are present throughout the year, with a maximum density in spring and another in autumn **(Bruhnes *et al.*, 1999)**.

2.1.1. Systematic position

Table 01: The systematic position of *Cs longiareolata* (Dris, 2019).

Kingdom	Animalia
Sub-kingdom	Metazoa
Phylum	Arthropoda
Sub-phylum	Hexapoda
Super-class	Protostomia
Class	Insecta
Sub-class	Ptrerygota
Infra-class	Neoptera
Super-order	Endopterygota
order	Diptera
sub-order	Nematocera
Infra-family	Culicomorpha
Family	Culicidae
Sub-family	Culicinae
Genus	*Culiseta*

2.1.2. Life cycle

Mosquitoes are holometabolous insects whose life cycle includes aquatic and aerial stages **(figure 11) (Dris, 2019).**

A. Eggs

Females lay their eggs on the surface of various breeding sites (aquariums, abandoned wells, rock holes, seas, ponds, canals, cisterns, rainwater, etc.). where the water is always stagnant and rich in organic matter. These sites may be permanent or temporary, shaded or sunny, filled with fresh or brackish water, clean or polluted **(Paul, 2009)**. The eggs are spindle-shaped and 0.5 to 1 mm in size. They are whitish at the time of oviposition and quickly turn black due to the oxidation of certain chemical components of the theca **(Peterson, 1980)**.

B. Larvae

Mosquito larvae can be found in almost all areas of stagnant water. The larval habitats are varied, whether on the surface or in underground shelters, in permanent or temporary water, with natural or artificial facies, and with oligogenic water natural or artificial facies, with oligotrophic water (water that is particularly poor in nutrients) or eutrophic water (water that is particularly rich in nutrients). Mosquito esuse a wide range of habitats as long as they can stand water for at least 5 days (for many species) **(Laëtitia, 2017).** Larval development at this stage is exclusively aquatic. They have characteristic wriggling movements and their development comprises four stages, ranging in size from 2mm to 3mm. stages, ranging from 2 mm to 12 mm **(Boulkenafet, 2006)**.

The larvae live for about 10 days. The rate at which the larvae develop depends on the amount of contained in the water of the breeding site **(Peterson, 1980).**

C. Nymph

They floaton the surface of the water and is vulnerable to predators. However, the nymphs of some species are relatively resistant to desiccation **(Becker *et al.*, 2010)**. The nymph or pupa, which is also aquatic, is comma-shaped and mobile, but does not feed during this stage, which lasts between 2 and 5 days, the moth is not active. It sucks air from the atmosphere by means of two trumpets on the cephalothorax. Its body is made up of 2 parts: a (antennae, trunk, legs and wings) and an abdomen in the form of a tail. This distinguishes the sexes; The tail is shorter in females **(Guitsevitch *et al.*, 1974 ; Perez, 1985).**

D. Adult

has an elongate body, 5 to 20 millimetres long **(Rodhain & Perez, 1985)**. The body has three parts ; the adult has at least one patch of dark scales on the wing, the thorax with three longitudinal white stripes **(Bouabida, 2014)**.

The adult mosquito or (imago) has an elongated body, 5 to 20 millimetres long. It is generally light brown, with pale anterior stripes on the abdominal tergites. The exoskeleton is made up of rigid plates (sclerites) joined together by thin chitinous membranes. Each body segment (metamer) is a ring formed by: The sclerite tergite (dorsal), the sternite (ventral) and the pleurites (lateral). The body is composed of 3 parts: the head, the thorax and the abdomen **(Brahmi and Snoussi, 2021).**

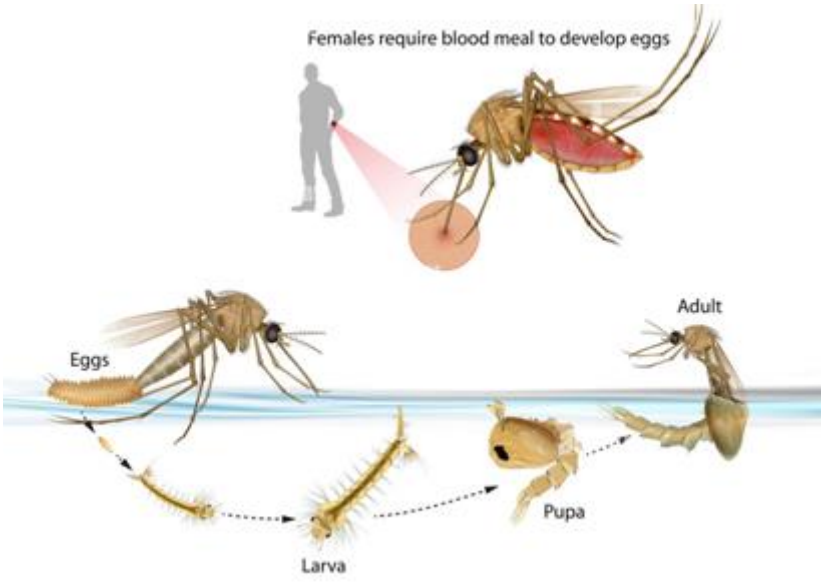


Figure 1 : Life cycle of *Culisita langiareolata*.

2.2. Collecting and breeding

The mosquitoes larvea are collected from different locations in Tebessa city (1er November, Boulhef-edir). Where we find a dirty water (aquarium , dirty pool).

The larvea are raised in plastic glasses containing 150 ml of dechlorinated water and feed them with a mixture of 75% biscuits and 25% baking powder .The water is renewed every two days **(Dris, 2019)**. The diet plays a major role in fertility because proteins enable the fimale to lay more eggs then females feeds on sugar alone **(Wigglesworth , 1972)**.

Figure 02: The collect locations

Figure 03: Collection of mosquitoes in the laboratory

2.3. Presentation of *Retama sphearocarpa*

These shrubs are 1 to 2 meters tall with more or less erect pubescent branches, characterized by small yellow flowers (5-6 mm) clustered on lateral branches of mature stems. The leaves are very small, and the pods are globular, yellow-brown, measuring 7-13.5 mm. They are typically found in rocky pastures **(Quezel & Santa, 1962).**

Figure 4 : *Retama sphearpcarpa*. (Gagui et Guelil, 2021).

2.3.1. Classification

According to **Quezel and santa (1962)**, retams are classified in the following taxon:

- **Kingdom:** Plantae
- **Phylum:** Spermatophyta
- **Subphylum:** Angiospermae
- **Class:** Magnoliopsida
- **Order:** Fabales
- **Family:** Fabaceae
- **Genus:** Retama
- **Species:** *Retama sphaerocarpa*

2.3.2. Botanical description of *Retama sphaerocarpa*

Retama sphaerocarpa (L.) Boiss is a perennial shrubby legume, reaching a height of 1 to 2 m, with more or less erect pubescent branches. It bears a large number of green branches of varying lengths, giving the species an open structure and representing the majority of its aboveground biomass. The deciduous leaves are very small, and the flowers are also small and yellow (5-6 mm), arranged in lateral clusters of 8 to 15 flowers on older branches. The fruits are covered by a hard layer and germinate in winter (January–February). They are globose pods, yellow-brown**. (figure 04).**

Figure 5 : The parts of *Retama sphearocarpa* (Gagui and Guelil, 2021).

Transpiration and photosynthesis are primarily carried out by cylindrical green cladodes, arranged on randomly oriented stems, with cladodes being produced annually at the level of axillary buds **(Brenner *et al.*, 1995 ; Pugnaire *et al.*, 2006)**. In spring, the plant produces new shoots in each node, with sessile lanceolate leaves that persist for only 3 to 4 weeks until their axillary cladodes complete growth. The shrub thus becomes devoid of leaves, with leaves contributing to a negligible part (<1%) of photosynthesis during a short period in spring **(Haase *et al.*, 1996).**

2.3.3. Geographical distrubtion

It is a plant that grows in rocky mountain grasslands, clay soils, sparse forests, and along streams in the steppe. This species is found in Spain, Portugal and North Africa and is rare in the Sahara. In Algeria, *R. sphaerocarpa* was reported from Ain Sefra, Oued M'zab, Constantine,Maillot, Bouira **(Maire, 1987).**

Figure 6 : The distrubtion of *Retma shearocarpa* (Bensaid, 2021)

2.3.4.. Uses of the plant

In Algeria, *Retama sphaerocarpa* is utilized as a medicinal plant known as "chemma," prepared from the branches of the species. According to **Bellakhdar (1997)**, the species is traditionally used in various forms:

- The stems and leaves, crushed with honey and taken orally, are used as an emetic. As rectal enemas, a decoction of the leaves is administered as a purgative and vermifuge.
- Powder made from the dried leaves and flowers of the species is used as a healing agent in circumcisions, as a vulnerary, antiseptic, and sedative in local wound care for skin abrasions, wounds, ulcers, and purulent pimples.
- The plant, ground with milk or butter, is used for the same indications, and the decoction is used in friction against itching and human and animal scabies.
- The roots of this species are widely used, as an abortifacient, in fumigations, or as vaginal enemas.
- The infusion of leaves and flowers can be consumed, but due to the known risks of intoxication, this form of use is rather rare.
- The plant has been used to poison wells during tribal warfare in Morocco.
- In the Sahara, Retama stems are used to make fire points against various pains in sciatica.
- Flagellation using Retama stems is used in the treatment of swelling and madness to expel evil spirits, which, according to traditional beliefs, are responsible for the loss of reason.
- In Algeria, bread is prepared to be eaten with the infusion of *Retama sphaerocarpa* branches to cure rabies in humans. These branches are given to animals suffering from rabies to eat as they are**.**

2.3.5. Chemical Composition of *Retama sphaerocarpa*

2.3.5.1. Coumarins

Coumarins are secondary phenolic metabolites characterized by the presence of a benzopyrone (coumarin) nucleus resulting from the lactonization of ortho-hydroxy-cis cinnamic acid.**(Gagui and Guelil, 2021).**

Figure7 : Coumarins.(Gagui Asma et Guelil, 2021).

2.3.5.2. Tannins

Tannins are substances from the polyphenol family, with a molecular weight of between 500 and 3000 Da **(Delcambre, 2010)**. They are found in the bark, leaves and fruit of many plants, and are divided into two types plants, and are divided into two types, hydrolysable tannins and condensed tannins.**(Hemingway, 1992).**

Tannins have a number of therapeutic properties, and are used for tanning animal skinrepairing tissue damage due to eczema and anti-constipation.**(Isrein *et al,* 2001).**

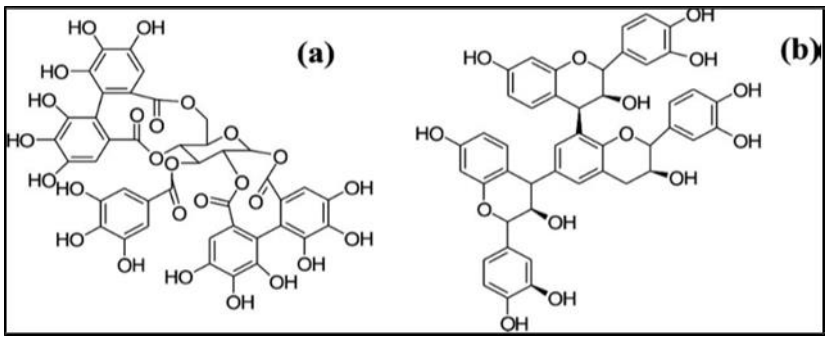


Figure 8 : Structure of (a) hydrolysable and (b) condensed tannin (Naczk and shehidi, 2006).

2.3.5.3. Saponins

The term saponing and derived from the word soap, are heterosides, found in the following forms triterpene and steroid form **(Robinet, 1951)**. They have surface-active properties (**William and Hopkins, 2003).**

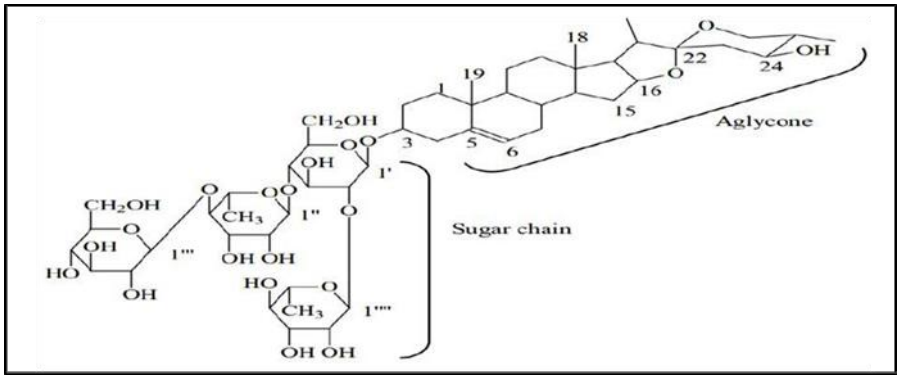


Figure 9 : Structure of saponins**.** (Moghimipour and Handali, 2015)

2.3.5.6. Alkaloids

Are cyclic compounds containing one or more nitrogen atoms in their chemical structure. They are stored in plants as products of various biosynthetic biosynthetic pathways. Some structures are relatively simple, while others are quitecomplex. Alkaloids can be found in all parts of the plant and the part in which the alkaloids accumulate is not always the same. alkaloids accumulate is not necessarily the part where they are synthesized **(Harborne,1995)**. They can be found infamilies of plants, and most alkaloids are soluble in water and alcoholwater and alcohol. **(Doncheva, 2014).**

They are bitter and aperitive, and form the basis of many therapeutic molecules (Marouf. 2007).

2.3.5.7. Flavonoids

Are natural polyphenolic compounds. They are considered to be almost universal plant pigments. pigments in plants. Structurally, they are generally in free form or in the form of aglycoside. They are found in various plant organs such as roots, stems, wood, leaves, flowers and fruit **(Marfak. 2003).**

2.4. Preparation of the extract

In a 1000 mL capacity beaker, a mixture comprising 500 mL ethanol and 500 mL distilled water was prepared, followed by thorough agitation. Subsequently, plant powder was introduced into the solution, and the amalgamation was subjected to agitator for 20 - 40 minutes. Following complete homogenization, the solution was refrigerated for 24 hours. It was then subjected to filtration using filter paper into another receptacle to isolate the liquid fraction. Post-filtration, the solution was transferred to a rotary evaporator set at 45°C for

further processing. The resultant extract was stored in an electric evaporator apparatus to obtain the final extract (Merghem ***et al.***, 1995).

The yield of plant extract is the ratio between the weight of the extract and the weight of the dry matter of the plant, evaluated from 4 extractions, it is expressed as a percentage and calculated by the following formula: Y = (We/Wp) x 100

Y = yield of the extract in%

We = Weight of the extract in g

Wp = Weight of the dry matter of the plant in g

2.5. Toxicity bioassays

Larval bioassays were conducted as previously described **(Boudjelida *et al.* 2005)**. Different concentrations of *Retama spherocarpa* hydro-ethanolic extract were added for each stage (newly ecdysed third-instar larvae, newly ecdysed fourth-instar larvae of *Cs. longiareolata* in order to detect mortalities. Newly ecdysed third-instar larvae and newly ecdysed fourth-instar larvae of *Cs. longiareolata* were exposed to different concentrations (100, 200, 400, 600, 1200, 1600 ppm) and (200, 400, 800, 1000, 1200, 2000ppm) respectively for 24 h according to the World Health Organization standard procedure **(Anon 1983, WHO 2005)**. *Retama spherocarpa* hydro-ethanolic extract were dissolved in 1 ml ethanol solvent, and then diluted in 150 ml of filtered tap water to obtain the desired concentrations. The controls were prepared using 1 ml of ethanol in 150 ml of water for positive controls and no additive with negative ones. After the exposure time of 24 h, larvae were removed, washed with untreated water and placed in clean water. The test was carried out with four replicates each containing 15 larvae per concentration. Mortality was registered at 24, 48 and 72 hours following treatment. The mortality percentage obtained was corrected (Abbott 1925), Sub-lethal and lethal concentrations (LC25, LC50 and LC90) and 95% confidence limits (95% CL) were calculated.

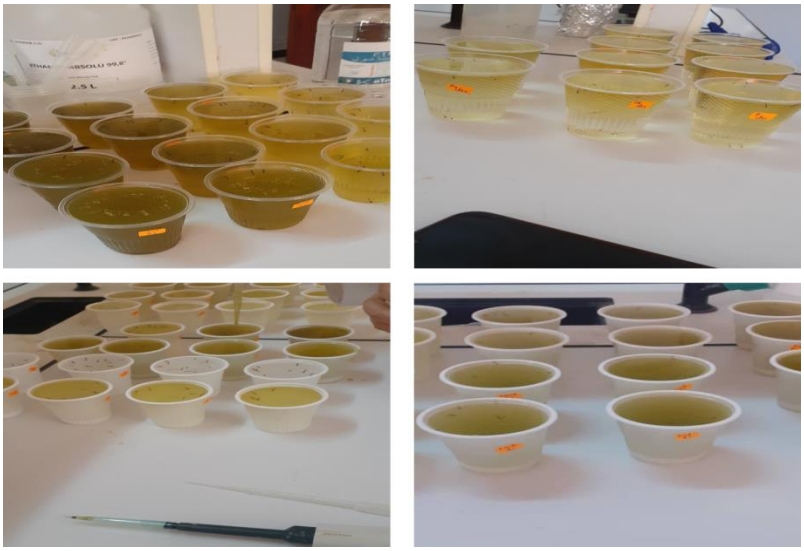


Figure 10 : Concentrations applied to mosquitoes

2.6. Morphometric study

The morphometric study was based on two parameters: the weight and body volume of individuals, calculated from the cubic value of the thoracic width of larvae **(Timmermann & Briegel, 1999).** Measurements were made under a calibrated binocular loupe.

2.7. Determination of biochemical constituents

Control and treated (LC50 and LC50) individuals were sampled at different stages (larva 3 and larva 4) and preserved in 1 ml of 20% TCA (trichloroacetic acid). The major biochemical components (proteins, carbohydrates and lipids) were extracted according to the method of **Shibko *et al*. (1966)**. After ultrasonic homogenisation followed by centrifugation (5000 rpm at 4°C for 10 min), the supernatant I obtained was used for the determination of total carbohydrates according to the method of **(Duchateau & Florkin 1959)**. To pellet I, 1 ml of ether/chloroform mixture (1V/1V) is added and after a second centrifugation (5000 rpm, 10 min), supernatant II and pellet II are obtained. Supernatant II is used for the determination of lipids **(Goldsworthy *et al*., 1972)** and pellet II, dissolved in sodium hydroxide (0,1 N), is used for the determination of proteins according to **Bradford, (1976).**

2.7.1. Determination of total protein

The protein determination is carried out according to the method of **Bradford (1976)** in an aliquot of 100 µl to which 4 ml of Brilliant Commassie Blue (BBC) G 250 reagent (Merck) is

added. This reveals the presence of proteins by blue colouration. The absorbance is read on a spectrophotometer at a wavelength of 595 nm. The calibration range is based on a solution of bovine serum albumin (BSA) at 1 mg/ml.

2.7.2. Determination of total lipids

Total lipids were determined by the method of **Goldsworthy *et al* (1972)** using the sulphophosphovanillin reagent. The lipid assay was performed on 100 µl aliquots of lipid extract or standard series, to which the solvent was completely evaporated and then 1 ml of concentrated sulphuric acid was added. The tubes were shaken and placed in a sand bath at 100°C for 10 minutes. After cooling, 200 µl of this mixture was taken and 2.5 ml of sulphophosphovanillin reagent was added. After 30 min in the dark, the optical density was read in a spectrophotometer at a wavelength of 530 nm. On heating, the lipids form pink complexes with sulphuric acid in the presence of vanillin and orthophosphoric acid. The lipid stock solution was prepared as follows 2.5 mg of edible oil (sunflower, 99% triglycerides) was placed in an Eppendorf tube and 1 ml of ether-chloroform (1V/1V) was added.

2.7.3. Determination of total carbohydrates

Total carbohydrates were determined by the method of **Duchateau & Florkin (1959)**. In this method, 100 µl of the supernatant contained in a test tube is mixed with 4 ml of Anthrone's reagent and the mixture is heated at 80°C for 10 minutes. A green colour develops, the intensity of which is proportional to the amount of carbohydrates present in the sample. The absorbance is read at a wavelength of 620 nm. The calibration range is based on a glucose stock solution (1 mg/ml).

2.8. Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean ± standard deviation (SD). The significance between different series was tested using one-way analysis of variance (ANOVA) at 5% level followed by Tukey's multiple comparaison test. All statistical analyses were performed using Prism 8.0 for Windows (GraphPad Software Inc., www.graphpad with a significant level $p<0.05$).

Results and Discussion

3. Results and discussion

3.1. Results

3.1.1. Yield of *Retama spherocarpa* hydro-ethanolic extract

The extraction of the aerial part of the plant was prepared following the protocol described by Merghem ***et al*** (1995). The yield of the aerial part of *Retama spherocarpa* hydro-ethanolic extract is 28.12 %

3.1.2. Larvicidal efficacy of *Retama spherocarpa* hydro-ethanolic extract against Cs. longiareolata

Toxicological tests were used to determine the efficacy of *Retama spherocarpa* hydro-ethanolic extract on the basis of the mortality recorded in treated individuals after different periods of 24, 48 and 72 hours.

*** Larval stage (L3)**

Different concentrations: 100, 200, 400, 600, 1200, 1600 ppm were applied to newly exuviated third instar larvae. Negative controls (water only) and positive controls (water + 1ml ethanol) were applied in parallel. No mortality was observed in either control series. The corrected mortalities given in the table show a significant increase according to the concentrations applied and the periods tested.

The data were subjected to an analysis of variance with one classification criterion, revealing a very highly significant concentration effect ($p < 0.0001$) at 24, 48 and 72 hours. The dose-response curve expressing the mortality rate corrected as a function of the logarithm of the concentrations applied (Fig. 11) was used to estimate the values of the various sublethal (LC25 and LC50) and lethal (LC90) concentrations, along with their confidence intervals and the Hill Slope (Table 02).

Table 02 :. Efficacy of *Retama spherocarpa* hydro-ethanolic extract applied on third instar larvae of *Cs. longiareolata*: corrected mortality (m ± SD, n = 4 replicates each containing 15 larvae)

Time (hours)	100 ppm	200 ppm	400 ppm	600 ppm	1200 ppm	1600 ppm	P
24	4.44 ± 7.69	11.11 ± 10.18	19.99 ± 6.66	55.55 ±	84.44 ±	100.00 ±	<
48	13.33 ±	20.00 ± 6.66	28.89 ±	3.85	7.69	0.00	0.0001
72	6.67	28.89 ± 10.18	10.18	57.77 ±	84.44 ±	100.00 ±	<
	13.33 ±		37.78 ± 3.85	7.69	7.69	0.00	0.0001
	6.67			57.77 ±	86.66 ±	100.00 ±	<
				7.69	6.66	0.00	0.0001

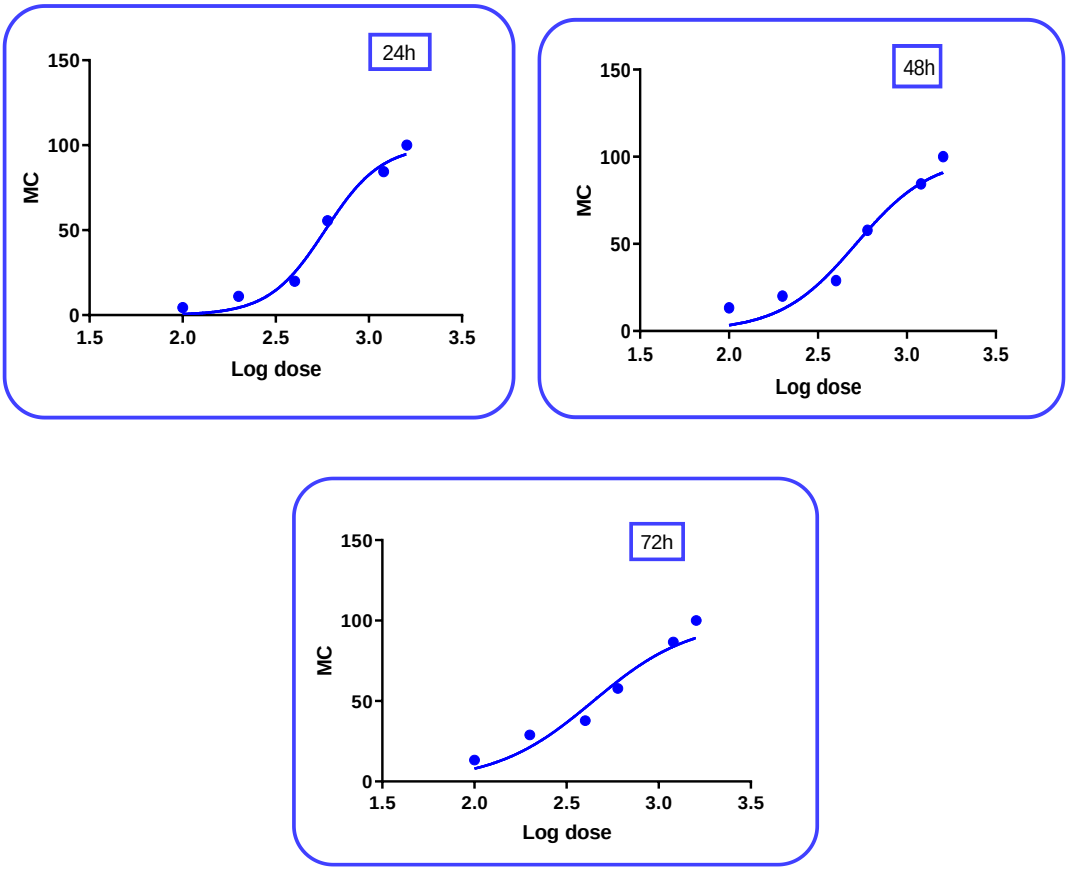


Figure 11: . Efficacy of *Retama spherocarp* ***a***hydro-ethanolic extract (ppm) applied on third instar larvae of *Cs. longiareolata*: Dose-response curve expressing the percentage of mortality corrected according to the logarithm of doses

Table 03 : . Sublethal and lethal concentrations (ppm, FL) of *Retama spherocarpa* hydro-ethanolic extract against third instar larvae *Cs. longiareolata*

Time (hours)	***Cs. Longiareolata***			
	LC25, ppm (95 % FL)	LC50, ppm (95 % FL)	LC90, ppm (95 % FL)	r^2
24	350.36	560.36	1554.0	0.99
	293.5 to 494.2	489.3 to 698.4	804.7 to 2016	
48	312.5	518.56	1500.2	0.99
	156.5 to 467.2	363.0 to 700.3	793.5 to 3768	
72	300.6	450.36	1460.8	0.99
	110.4 to 381.6	297.7 to 621.4	907.1 to 4453	

*** Larval stage (L4)**

Retama spherocarpa hydro-ethanolic extract was tested on *Cs. longiareolata* larvae 4 at different doses 200, 400, 800, 1000, 1200, 2000ppm. The natural mortality recorded in the positive and negative control series was zero. The treatment revealed a larvicidal effect with a dose-response relationship.

Analysis of variance using a classification criterion revealed very highly significant differences ($p < 0.001$) (Table 3). The dose-response curve expressing the percentage of mortality corrected as a function of the logarithm of the dose applied (Fig. 12) was used to determine the values of the various sublethal and lethal concentrations, together with their confidence intervals and the HillSlope (Table 04).

In addition, the L4 larval stage was more resistant to *Retama spherocarpa* hydro-ethanolic extract than the L3 larval stage.

Table 04 : . Efficacy of *Retama spherocarpa*hydro-ethanolic extract applied on fourth instar larvae of *Cs. longiareolata*: corrected mortality (m ± SD, n = 4 replicates each containing 15 larvae)

Time (hours)	200 ppm	400 ppm	800 ppm	1000 ppm	1200 ppm	2000 ppm	P
24	8.88 ± 3.85	15.55 ± 3.85	22.22 ± 3.85	46.66 ±	75.55 ±	100.00 ±	<
48	11.11 ±	15.55 ± 3.85	35.55 ± 7.20	6.66	13.8	0.00	0.0001
72	3.85	22.22 ± 3.85	40.00 ± 0.00	57.78 ±	75.55 ±	100.00 ±	<
	15.55 ±			7.69	13.8	0.00	0.0001
	3.85			57.78 ±	82.22 ±	100.00 ±	<
				3.85	7.69	0.00	0.0001

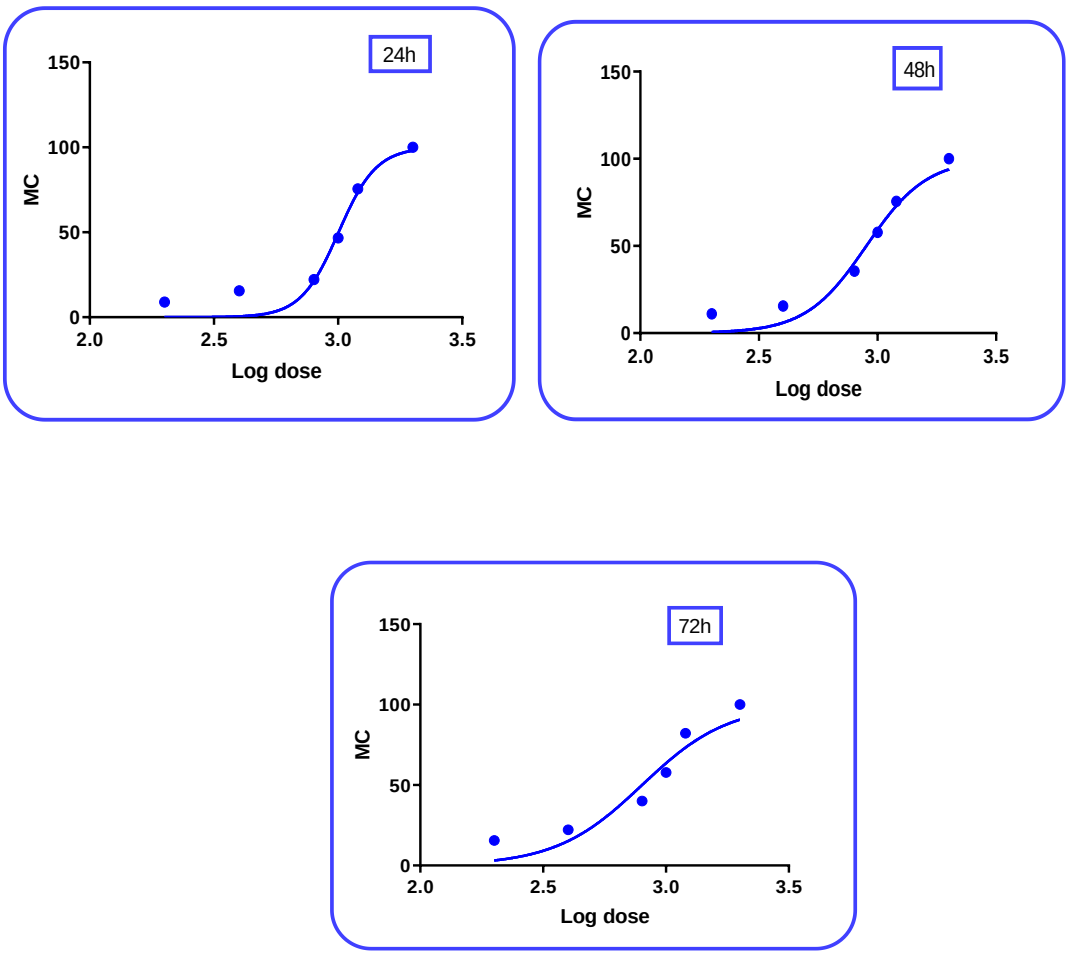


Figure 12 : Efficacy of *Retama spherocarpa* hydro-ethanolic extract (ppm) applied on fourth instar larvae of *Cs. longiareolata*: Dose-response curve expressing the percentage of mortality corrected according to the logarithm of doses

Table 05 : Sublethal and lethal concentrations (ppm, FL) of *Retama spherocarpa* hydro-ethanolic extract against fourth instar larvae *Cs. Longiareolata*

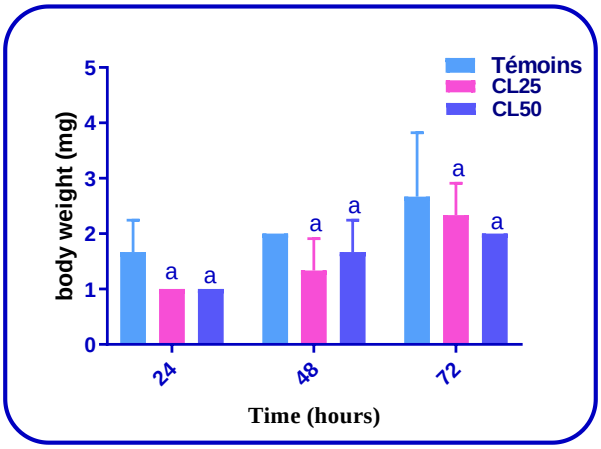
Time (hours)	*Cs. Longiareolata*			
	LC25, ppm (95 % FL)	LC50, ppm (95 % FL)	LC90, ppm (95 % FL)	r^2
24	825.36	1050.4	1476.0	0.99
	529.7 to 977.0	845.9 to 1149	1166 to 2865	
48	650.9	899.6	1700.2	0.99
	371.8 to 859.5	689.4 to 1056	1184 to 3461	
72	521.6	798.36	1960.8	0.99
	197.6 to 874.5	504.3 to 1063	1084 to 6652	

3.1.3. Impact of *Retama spherocarpa*hydro-ethanolic extract on mosquito growth

3.1.3.1. Effect of *Retama spherocarpa*hydro-ethanolic extract on the weight growth of Culiseta longiareolata

*** Larval stage (L3)**

The results of changes in the body weight of *Culiseta longiareolata* third instar larvae are shown in Table 04 and Figure 13. For the control and LC25 and LC50 treated series, there were no significant differences in larval body weight over the test period (24, 48 and 72 h).



Figur13: Effects of of *Retama spherocarpa* hydro-ethanolic extract on weight (mg) against against third instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

***Larval stage (L4)**

The results of changes in the body weight of *Culiseta longiareolata* fourth instar larvae are shown in Figure 14. These results show a highly significant difference between the control and treated series (p=0.0001).

Comparison of the means between the control and treated series using the Dunett test revealed a significant decrease in the body weight of the 4 larvae at 24 and 72 h for the series treated with LC50, and a significant decrease only at 24 h for the series treated with LC25.

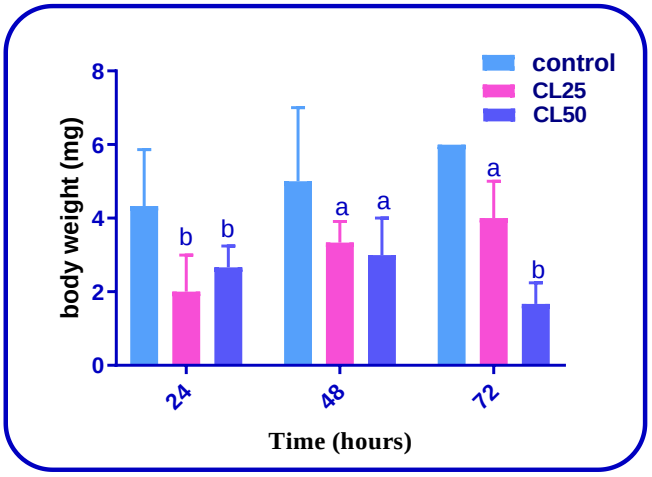


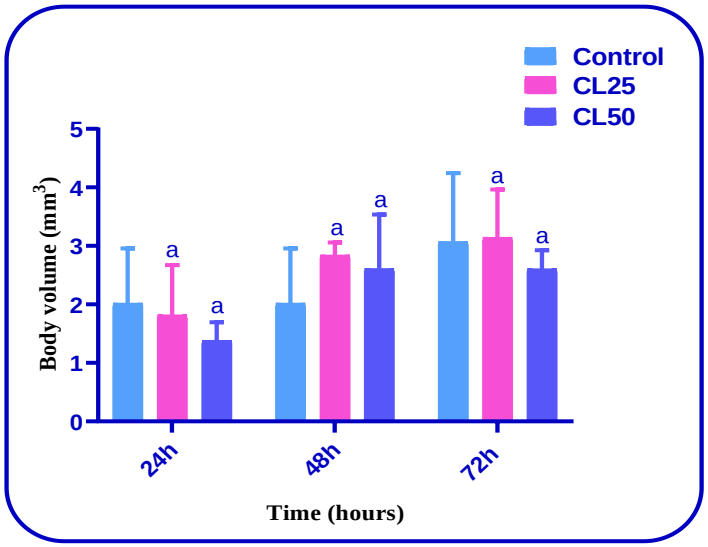
Figure 14 : Effects of of *Retama spherocarpa* hydro-ethanolic extract on weight (mg) against against fourth instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

3.1.3.2. Effect of *Retama spherocarpa* hydro-ethanolic extract on the linear growth of Culiseta longiareolata

*** Larval stage (L3)**

The results obtained show that the body volume of control and treated individuals at LC25 and LC50 present non-significant differences with (p = 0.5706) during the three stages.

The comparison of the means by the Dunnett test shows that the treatment with the two concentrations has no effect during the three times.



1. **Figure 15 :** Effects of of *Retama spherocarpa* hydro-ethanolic extract on Body volume (mm3) against third instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

***Larval stage (L4)**

For the stage (L4), the results obtained show that the body volume decreases in a very highly significant manner ($p<0.0001$) when comparing the three series during 24, 48 and 72 hours after treatment (Fig. 16)

Comparison of the means by the Dunnett test shows that the treatment with the two concentrations causes a significant reduction during the three times compared to the control series.

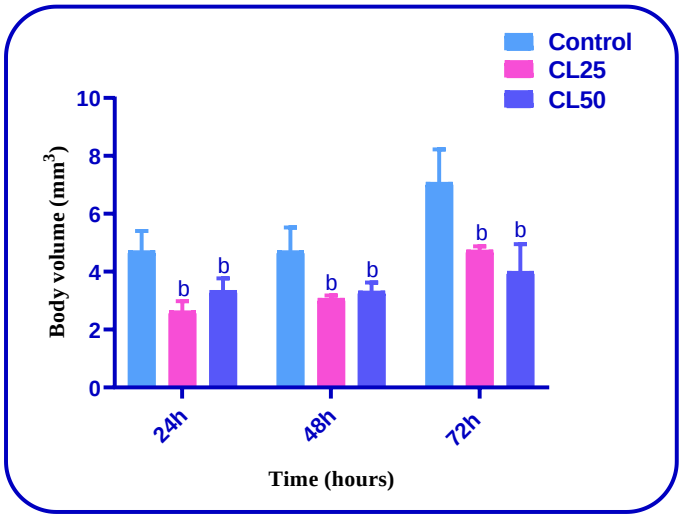


Figure 16 : Effects of *Retama spherocarpa* hydro-ethanolic extract on Body volume (mm3) against fourth instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

3.1.4. Impact of *Retama spherocarpa* hydro-ethanolic extract on the biochemical composition of *Culiseta longiareolata*

The LC25 and LC50 of the hydroethanolic extract of *Retama spherocarpa* were applied to newly exuviated third and fourth instar larvae of *Culiseta longiareolata*. The effect of this extract was assessed on the carbohydrate, lipid and protein content.

The effect of this extract was evaluated on the carbohydrate, lipid and protein content during different periods after treatment (24, 48 and 72 h).

3.1.4.1. Effect on total protein content

*** Larval stage (L 3)**

The results of the assay are shown in Figure 19. The values show a non-significant non-significant variation (p=0.2466) in protein content in both control and treated treated series from 24h to 72h.

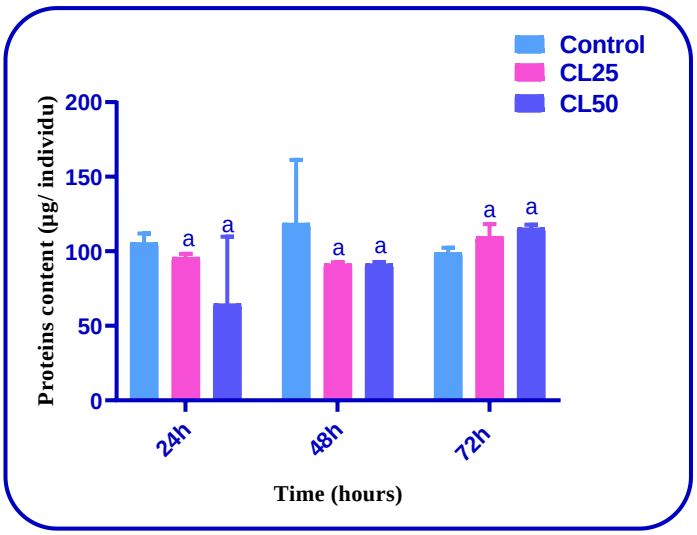


Figure 17: Effects of *Retama spherocarpa* hydro-ethanolic extract on the proteins content (µg/ individu) against third instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

*** Larval stage (L4)**

The assay results are shown in Figure 14. These values show a highly significant variation (P<0.0001) in protein content between the control and treated series and a highly significant variation (p= 0.0064) when compared between the three times (24h, 48h and 72h).

The multiple comparison test revealed a highly significant decrease in protein content under the effect of treatment at 24h, 48h and 72h.

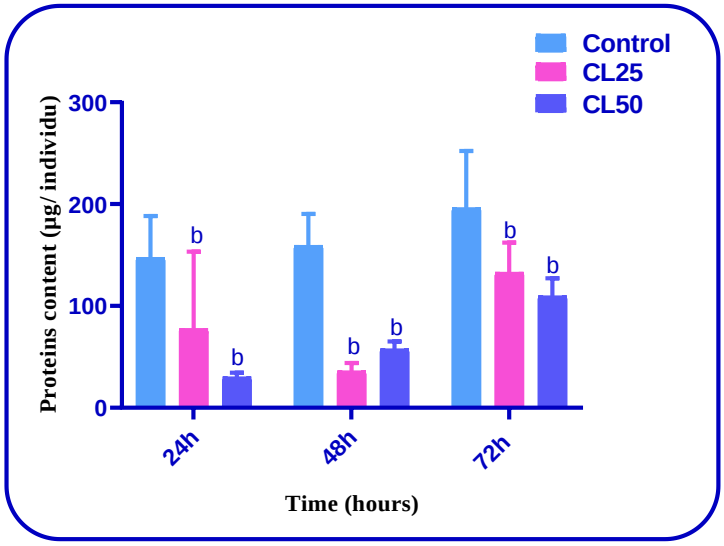


Figure 18 : Effects of *Retama spherocarpa* hydro-ethanolic extract on the proteins content (µg/ individu) against fourth instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

3.1.4.2. Effect on total carbohydrate content

*** Larval stage (L3)**

The results of the assay are shown in figure 19. These values show a highly significant variation (p= 0.0009) in carbohydrate content between the control and treated series and a highly significant variation (p= 0.0163) when compared between the three times (24h, 48h and 72h).

The multiple comparison test revealed a highly significant decrease in protein content under the effect of the treatment at 24h and 48h.

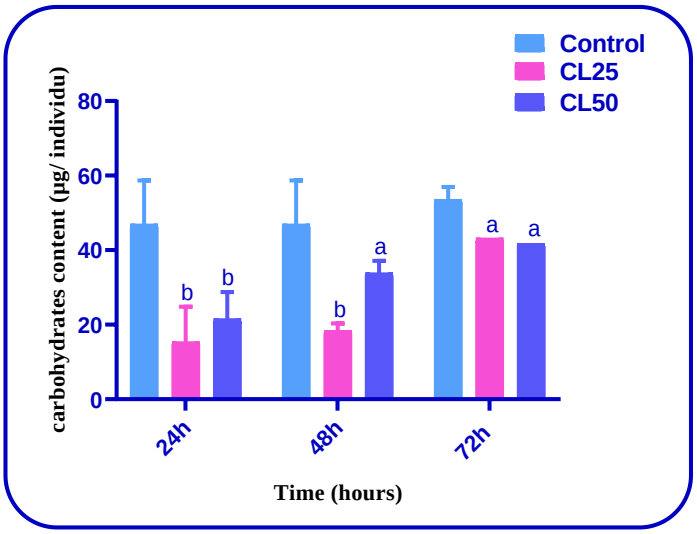


Figure 19 : Effects of *Retama spherocarpa* hydro-ethanolic extract on the carbohydrates content (µg/ individu) against third instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

*** Larval stage (L4)**

The comparative study between the control and treated series shows that the hydroethanol extract of *Retama spherocarpa* leads to a very highly significant variation ($P<0.0001$). And a highly significant variation ($P<0.0001$) over the three times.

The multiple comparison shows a highly significant decrease for the series treated with the LC50 at 24h and 48h.

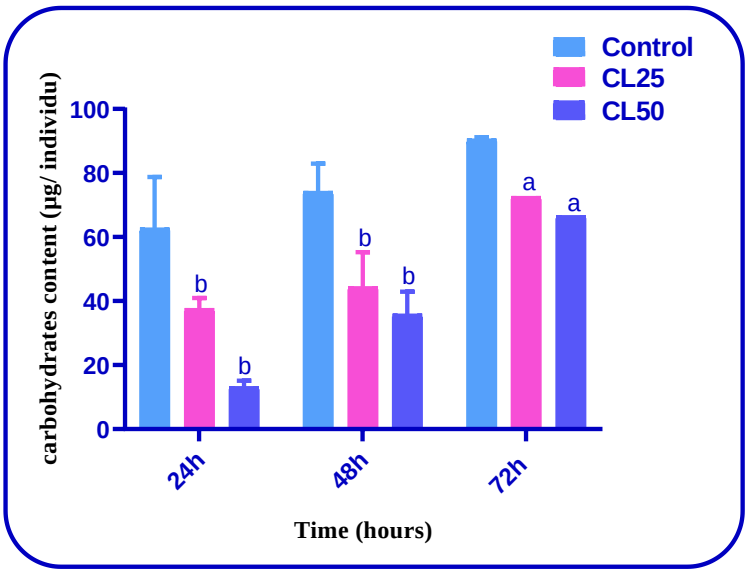


Figure 20 : Effects of *Retama spherocarpa* hydro-ethanolic extract on the carbohydrates content (µg/ individu) against fourth instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

3.1.4.3. Effect on total lipid content

*** Larval stage (L3)**

The results of the assay are shown in Figure 21. These values show a significant variation (p= 0.0101) in lipid content between the control and treated series and a non-significant variation (p= 0.0727) when compared between the three times (24h, 48h and 72h).

The multiple comparison test revealed a highly significant decrease in protein content under the effect of the 24h treatment.

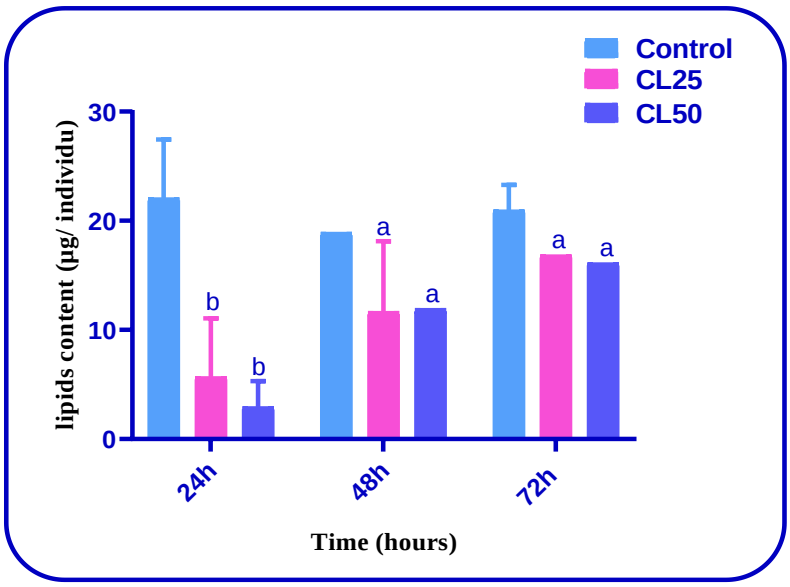


Figure 21 : Effects of *Retama spherocarpa* hydro-ethanolic extract on the lipids content (µg/ individu) against third instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

*** Larval stage (L4)**

In the treated series, the lipid content decreased very significantly ($p<0.0001$) and there was a non-significant variation between the three stages ($p= 0.0711$).

Multiple comparison showed a decrease at 24 and 72 hours after treatment.

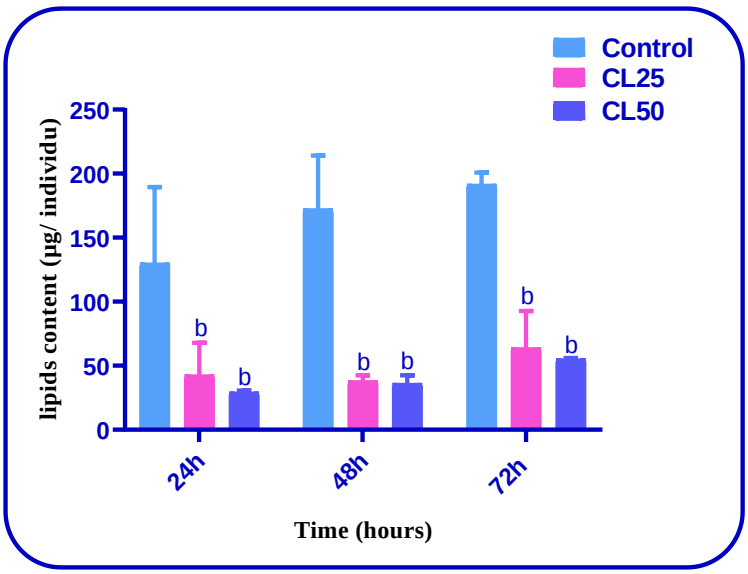


Figure 22: Effects of *Retama spherocarpa* hydro-ethanolic extract on the lipids content

(µg/ individu) against fourth instar larvae *Cs. longiareolata*: (m ± SD, n = 5 pools each containing 10 individuals).

3.2. Discussion

3.2.1. Yield of *Retama spherocarpa* hydro-ethanolic extract

According to the results obtained in the last calculation for *R. sphearocarpa* extract yield we found the yield was 28.12% inferieur than that obtained by **(Hamdi Cherif and S.2022)**.This difference could be due to a difference in climatic conditions between the sites and the harvest period **(Ndomo *et al.*, 2009)**. This shows that there are several factors influencing yield, including geoclimatic factors (soil type, temperature), the harvesting period (temperature), the harvesting period, the drying time, the extraction method and the parts of the plant used **(Fadil *et al.*, 2015).**

3.2.2. Toxicity test

Toxicological tests were introduced to test the sensitivity of larvae to insecticides used in control campaigns **(OMS, 1963**). The use of chemical insecticides leads to an eco-toxicological mess, accompanied by a spectacular increase in the number of resistant species. The use of natural products remains the most beneficial method for the health of living beings and their environment **(Benayad, 2008).**

The research into plant-insect interactions has led to the potential use of plant extracts for pest control. Biopesticides have multiple targets: they can affect the insect nervous system, causing paralysis and subsequent death, alter the conductance of several ion channels (calcium, potassium, sodium), disrupt the arrangement of lipid bilayers, and also act at the level of presynaptic terminals **(Windley *et al.*, 2012).** These multiple targets of biopesticides work against the development of resistance in insects...

Toxicity, as assessed by the mortality rate recorded after treatment, depends on the dosesadministered.

Our study test the toxicity of hydro-ethanol extracts *Retama spherocarpa* on newly exuviated third- and fourth-stage larvae of *Cs longiareolata*, the results of which showed larvicidal activity with a dose-response relationship. However, lethal and sublethal concentrations of the hydroethanolique extract of *Retama spherocarpa* showed variable values as a function of time: LC25 (350.36 ; 312.5 and 300.6 ppm,FL) LC50 (560.36 ;518.56 ;450.36 ppm,FL) and LC90 (1554.0 ;1500.2 ;1460.8ppmFL) at 24, 48 and 72 hours after

treatment, respectively on newly exuviated third-stage larvae of *Cs longiareolata*. And LC25 (825.36 ;650.9 ;521.6ppm FL) , LC50 (1050.4 ;899.6 ;798.36ppm, FL) and LC90 (1476.0 ;1700.2 ;1960.8ppm, FL) at 24, 48 and 72 hours after treatment on newly exuviated fourth-stage larvae of *Cs longiareolata*.

Previous experiments have shown that the aqueous extract of *T. vulgaris* has a higher larvicidal activity than the aqueous extracts of *Ricinus communis* and *Tetraclinisarticulata*.

The aqueous extracts *of Ricinus communis* and *Tetraclinisarticulata* applied to the larvae of four mosquito species showed a marked sensitivity of *Cx. pipiens* and Cs. longiareolata larvae 2 **(Bouguerra, 2019).** The insecticidal activity of the crude ethanolic extract of the aerial part of the plant A. judaica (Asteracea), known for its insecticidal activity, was evaluated. The results of toxicity tests on the crude extract of the plant showed good ovicidal and larvicidal activity, with a dose-response relationship.

Aqueous extracts of castor oil leaves (*Ricinus communis* L.) and thuja wood (*Tetraclinis articulata)* ,were applied to the second and fourth instar larvae of four mosquito species: *Culex pipiens, Aedescaspius, Culiseta longiareolata* and Anopheles maculipennis, show comparable results between the species tested, except that *Culiseta longiareolata* is the most sensitive species compared to the others, with LD 50 s of 110 mg/L for castor oil extract and 250 mg/L for *thuja wood*, in contrast to *Anopheles maculipennis* for which these extracts are less toxic **(Aouinty *et al*., 2006)**.

Effect of *Retma Sphearocarpa* hydroethanol extract applied to *Cs.longiareolata*: higher mortality percentage than *Retama spherocarpa* effect on the same mosquito species.

3.2.3. Morphometriec study

The body weight of insects generally depends on the availability of food in their habitats, environmental conditions and, most importantly, the hereditary characteristics of each species).

Plant-based biopesticides have been shown to have adverse effects on the growth and development of insects, reducing the weight of larvae, pupae and adults and lengthening developmental stages **(Talukder, 2006)**.

The results obtained in our experiment show that the extract (LC25 and LC50) applied to culiseta longiareolata L3 instar larvae did not cause a significant decrease in body weight. On the other hand, the results obtained LC25 when applied to fourth-stage Culiseta longiareolata larvae caused a significant reduction in body weight only in 24h ;LC50 caused

significant reduction in 24h and 72h **(Bouabida, 2017)** showed that spiromesifene applied to third and fourth instar larvae of Cs. *longiareolata* disrupted the morphometric parameters of individuals. In another mosquito species, *Culex pipiens*, treatment with *Eucalyptus globulus* **(Kheled &Dib, 2015)** and *Lavandula dentata* **(Sahbi and Aouni, 2015)** showed the same results.

Ethanolic extract of *Melia azedarach* reduced food consumption and digestibility of larvae and induced a decrease in growth rate of *Spodoptera littoralis* larvae, blockage of pupationand reduction in pupa weight **(Akacha *et al*., 2017).**

Body volume was estimated from the cubic value of larval thoracic width, pupal cephalo thoracic width and adult wing length. Mosquito body volume can influence a number of key parameters such as the volume of blood meal consumed, the degree of its utilisation in metabolic pathways and the number of eggs that reach maturity **(Hosoi, 1954 ; Van Den Heuvel, 1963).**

The results obtained in our experiment show that *Retama spherocarpa* extract (LC25 LC50) applied to *Cs.longeriolata* third instar larvae does not significantly affect body volume.

On the other hand, *Retama spherocarpa* extract (LC25 LC50) applied to fourth-stage larvae ofthe same species had a highly significant effect on body volume. Results and in the same species (*Culex pipiens*), application of E.H. extracted from *Eucalyptus globulus* **(Kheled & Dib, 2015)** and *Lavandula dentata* **(Sahbi & Aouni, 2015)** caused adecrease in thoracic width, weight and body volume of larvae 4. Furthermore, in anothermosquito species, Culiseta longiareolata, treatment with *Ocimum basilicum* (LC50) showedthe same results **(Bouzidi, 2015).**

3.2.4.3. Assay of chemical compositions

Whole body assays of major components in control and treated *Cs. longiareolata* L3 L4 larvae show changes in biochemical components such as proteins, carbohydrates and lipids after treatment with *R spherocarpa* extracts at 24, 48 and 72 hours. When the insect comes into contact with the insecticide, it penetrates the organism and reaches more or less rapidly, at the cellular level, the target proteins and enzymes whosenormal functioning it interferes with

Proteins are important biochemical components required for the development and growthof organisms and for the performance of vital activities **(Yazdani *et al.*, 2014)**.

Proteins play a fundamental role in the organism of all known living biological species**(Mahler *et al.*, 1968).** The results obtained in our experiment show that the application of hydroethanol extract (LC25; LC50) has no significant effect on the amount of proteins in the third instar larvae ofthe mosquito *Cs longeriolata*. On the other hand, there was a significant decrease in protein levels after application of the extract to 4th instar larvae CL25 CL50 of the same species. As a result, an increase was recorded in *Cx. pipiens* after treatment with *O. Basilicum* **(Dris *et al.* 2017)** and in *Cx. pipiens and Cs. longiareolata* treated with lavender and mint **(Dris,2018).**

Carbohydrates are a very important group of compounds. Some are a source of energy forliving organisms, either immediately usable (trehalose) or in the form of reserves (glycogen);others have a structural role (cellulose, chitin, hyaluronic acid) **(Nation, 2008).** Our results show that treatment with hydroethanol extract, with LC25 and LC50 in L3 and L4,had a significant effect over the period tested. a large reduction in carbohydrate levels. Previous work has shown the negative effects of essential oils on carbohydrate reserves **(Sak *et al*, 2006 ; Abdul Razak & Sivasubramanian, 2007).** An increase in this biochemical compound has been reported in *Cx pipiens* after treatmentwith *Ocimum basilicum* **(Khamene, 2014).**

Lipids are the main source of energy in insects **(Beenakers *et al.*, 1985).** Our results show that treatment with hydroethanolic extract CL25 and CL50 in L3 L4 of *Cs longeriolata* causes alarge decrease in lipids over the period tested. **Sneha & Preet (2016)** study the effect of neem EO on *A. aegypti* larvae and report a 90% and82% decrease in carbohydrate and lipid content, respectively, in treated compared to controls.

Conclusion

The investigation into the phytochemical constituents and larvicidal activity of the hydroethanolic extract of *Retama sphaerocarpa* on Culiseta longiareolata larvae provides compelling evidence of its potent insecticidal properties. The extract exhibited significant larvicidal activity, effectively reducing larval populations in experimental settings. This observed effect underscores the potential of *Retama sphaerocarpa* as a natural larvicide for controlling mosquito vectors.

The larvicidal activity of the extract can be attributed to its complex phytochemical composition, including alkaloids, flavonoids, tannins, saponins, and terpenoids. These bioactive compounds likely act synergistically or through specific mechanisms to disrupt vital physiological processes in mosquito larvae, leading to their mortality. Such mechanisms may include interference with growth and development, disruption of metabolic pathways, or damage to essential tissues.

Furthermore, the effectiveness of the extract against *Culiseta longiareolata* larvae suggests its broad-spectrum activity, which could potentially extend to other mosquito species of public health significance. This versatility enhances its value as a natural larvicide for integrated mosquito management strategies.

The demonstrated larvicidal activity of the *Retama sphaerocarpa* extract holds promise for the development of environmentally sustainable mosquito control methods. By harnessing the natural insecticidal properties of plant-derived compounds, such as those found in *Retama sphaerocarpa*, it may be possible to reduce reliance on synthetic insecticides that pose risks to human health and the environment.

However, further research is needed to optimize extraction methods, determine the optimal concentration and application protocols, and assess the long-term efficacy and environmental impact of the extract. Additionally, studies on the potential development of resistance in mosquito populations and the safety of the extract for non-target organisms are essential for ensuring its sustainability and effectiveness in mosquito control programs.

In conclusion, the hydroethanolic extract of *Retama sphaerocarpa* demonstrates significant larvicidal activity against Culiseta longiareolata larvae, highlighting its potential as a natural and eco-friendly alternative for mosquito control. Continued research and development efforts are warranted to unlock the full potential of this botanical resource for combating mosquito-borne diseases and promoting public healt

References

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- Abu-Dahab, R., ***et al***. "Antioxidant activity of three plants in Jordan." Pharmaceutical Biology 45.3 (2007): 197-201.
- Al-Qura'n, S., ***et al***. "Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan." Journal of Ethnopharmacology 123.1 (2009): 45-50.
- A.E. Tchoundjeu ***et al***., "Potential of indigenous agroforestry trees for domestication and adoption in the West African Sahel", in Agroforestry Systems, 1999.
- Abdel-Malek, A. (1960). The culicine mosquitoes of the northern region of the United Arab Republic, Bulletin de la Société Entomlogique d'Egypt., 44: 11-128.
- Akacha, M., Chaieb, I., Laarif, A., Haouala, R. & Boughanmi, N. (2017). Effects of Melia azedarach Leaf Extracts on Nutritional Behavior and Growth of Spodoptera littoralis. Tunisian Journal of Plant Protection 12: 61-70.
- Aouinty, B., Oufara, S., Mellouki, F. & Mahari, S. (2006). Évaluation préliminaire de l'activité larvicidedes extraits aqueux des feuilles du ricin (Ricinus communis L.) etdu bois de thuya (Tetraclinisarticulata (Vahl) Mast.) sur les larves de quatre moustiques culicidés : Culex pipiens (Linné), Aedescaspius (Pallas), Culiseta longiareolata (Aitken) et Anopheles maculipennis (Meigen). Biotechnol.Agron. Soc.Environ., 10 (2): 67 – 71.
- Abdul Razak, T. & Sivasubramanian, P. (2007) Effect of three botanical oils oncarbohydrate content in Cheilomenes sexmaculata Fabricius and Chrysoperla carneaStephens. Asian Journal of Biochemistry 2: 124-129.

-B-

- Bellakhdar, J. (1997). La pharmacopée marocaine traditionnelle: Médecine arabe ancienne et savoirs populaires. Ibis Press.
- Bruneton J. (1999). Pharmacognosie, Phytochimie, Plantes Médicinales. Editions Tec & Doc Lavoisier.

- Bruhnes, J., Rhaim, A., Geoffroy, B., Angel, G. &Hervy, J.P. (1999).Les Culicidae de l'Afrique méditerranéenne. Logiciel de l'institut de recherche et de développement de Montpellier (France).
- Bensaid Sara Ouissem. Étude phytochimique et biologique des plantes médicinales algériennes *Retama sphaerocarpa* L., Lepidium draba L. et Cedrus atlantica . 2021.
- Bouzid, S., Alayat, M. S., & Rharrabe, K. (2016). Larvicidal activity of some Moroccan plants against Culex pipiens (Diptera: Culicidae). Journal of Asia-Pacific entomology, 19(1), 223-227.
- Bouabida, H., Tine-Djebbar, F., Tine, S. & Soltani, N. (2017a). Activity of spiromesifen on Growth and development of Culex pipiens (Diptera: Culicidae): Toxicological, Biometrical and biochemical aspects. Journal of Entomology and Zoology Studies 5(1): 572-577.

- Bouzidi O. Ziani R., 2015. Etude de l'impact des huiles essentielles d'une plante larvicide, l'Ocimum basilicum sur une espèce de moustique Culiseta longiareolata : aspect morphométrique et biochimique. Mémoire du diplôme de Master. Université des sciences exactes et sciences de lanature et de la vie-Tébessa. 28p.

- Benayad, N. (2008). les huiles essentielles extraites des plantes medicinales marocaines : moyen efficace de lutte contre les ravageurs des denrees alimentaires stockees .63 p

- Bradford, M.M. (1976). A rapid and sensitive method of the quantitation microgram quantities of Protein utilising the principale dye binding. Analytical Biochemistry 72: 248 - 254.
- Beenakers A.M.T.H., Vander Host D.G. & Van Marrewijk W.J.A., 1985. Insect lipids and lipoproteins and their role in physiological process. Prog. Lipid. Res., 24 : 19-67.

-D-

- Delcambre A. (2010). Une apprche moléculaire de l'astringence des vins : utilisation de sondes pour l'étude dss intéraction entre protéines de la salive et polyphénols.Thèse de doctora de Chimie analytique etenvironnement Uiversité de Bordeaux I. Ecole doctora des sciences chimique, p. 176.

- DRIS, D. (2019). Etude de l'activité larvicide des extraits de trois plantes : Mentha piperita, Lavandula dentata et Ocimum basilicum sur les larves de deux espèces de moustiques Culex pipiens (Linné) et Culiseta longiareolata (Aitken). Thèse de doctorat. Biologie animale. Université Badji Mokhtar Ŕ Annaba. P 181.
- Duchateau, G. & Florkin, M. (1959). Sur la tréhalosémie des insectes et sa signification.Archives of Insect Biochemistry and Physiology 67: 306-314.

-E-

- Evaluation de l'effet antimicrobienne d'extrait brute et l'étude phytochimieque e de la plante Retama Reatam.2018.

-F-

- Fernández, María, ***et al***. "Antimicrobial activity of retama sphaerocarpa extracts." Journal of Ethnopharmacology 41.1-2 (1994): 39-42.

-G-

- González-Trujano, M. E., Peña, E. I., Martínez, A. L., Moreno, J., Guevara-Fefer, P.,Deciga-Campos, M., & López-Muñoz, F. J. (2007). Evaluation of the antinociceptive effect of Rosmarinus officinalis L. using three different experimental models in rodents.Journal of ethnopharmacology, 111(3), 476-482.
- Gagui asma , Guelil Sassia.Etude phytochimique de la plante Retama sphaerocarpa (L) Boiss et évaluation de son activité antibactérienne ;2021
- Goldsworthy, A. C., Mordue, W. & Guthkelch, J. (1972). Studies on insect adipokinetic hormone. General and Comparative Endocrinolog. 18: 306-314.

-H-

- Haase, P., Pugnaire, F.I., Fernández, E.M., Puigdefábregas, J., Clark, S.C., Incoll, L.D., 1996. Investigation of rooting depth in the semi-arid shrub *Retama sphaerocarpa* (L.)Boiss. By labeling of ground water with a chemical tracer. J. Hydrol 170, 23-31.

- Haase, P., Pugnaire, F.I., Clark, S.C., Incoll, L.D., 1999. Diurnal and seasonal changes in cladode photosynthetic rate in relation to canopy age structure in the leguminous shrub *Retama sphaerocarpa*. Functional Ecology 13, 640-649.
- Hemingway, R.W. (1992). Structural variation in proanthocyanidins and their derivatives.In: Lapant polyphénols: synthesis, proprieties, sinificande. Laks P.E, Hemingway R.W Newyork.
- Hassan, A., Khalid, R., & Abdelgaleil, S. A. M. (2019). Insecticidal and biochemical effects of Melia azedarach L. and *Retama sphaerocarpa* (L.) Boiss extracts on spodoptera littoralis larvae. Journal of Pest Science, 92(2), 763-774.
- Hosoi, T. (1954). Egg production in Culex pipiens pallens coquillett. I V. Influence of breeding conditions on wing length, body weight and follicule production. Japanese Journal of Medical Science & Biology 7: 129 - 134.
- Haubruge, E. & Amichot, M. (1998). Les mécanismes responsables de la résistance aux insecticideschez les insectes et les acariens. France. Biotechnologie, Agronomie, Société et Environnement 2 (3):161–174.

-I-

- Iserin P., Masson M., Restellini J. P., Ybert E., DE LAAGE DE MEUX A., Moulard F.,ZHA E., DE LA ROQUE R., DE LA ROQUE O., VICAN P., DEELESALLE - FEAT T.,Biaujeaud M., Ringuet J., Bloth J., Botrel A., 2001. Larousse des plantes médicinales :identification, préparation, soins. 2éme édition de VUEF, Hong Kong.

-J-

- Jean, B., 2009. Pharmacognosie, phytochimie, plantes médicinales (4e éd.). Lavoisier.

-K-

- Khaligh, F. G., Naghian, A., Soltanbeiglou, S. & Gholizadeh, S. (2020).Autogeny in Culiseta longiareolata (Culicidae: Diptera) mosquitoes in laboratory conditions in Iran. BMC Research Notes [En ligne]. 5(1).
- Kumar, A., Shukla, R., Singh, P., & Dubey, N. K. (2012). Chemical composition antifungal and antiaflatoxigenic activities of Ocimum sanctum L. essential oil and its safety assessment as plant based antimicrobial. Food and Chemical Toxicology, 50(5),1675-1681.

- Khaled I. & Dib D., 2015. Evaluation de l'activité des huiles essentielles de l'Eucalyptus globulus à l'égard d'une espèce de moustiques Culex pipiens : toxicologie, développement,morphométrie et biochimie. Mémoire de Master. Université de Tébessa. p : 39-44
- Khamene I. 2014. Etude de l'activité insecticide d'extrait de l'Ocimum basilicum à l'égard d'une espèce de moustiques Culex pipiens. Mémoire du diplôme de Master. Université de Tébessa 43p.

-M-

- M. B. Qaddoury, H. E. Moussadek, "Plant diversity and vegetation in the Gharb area, northwest of Morocco", in Acta Botanica Gallica, 2001.
- Maire, R. (1552-1987). Flore de l'Afrique du Nord (Maroc, Algérie, Tunisie, Tripolitaine,Cyrénaique et sahara). Volumes I à XVI. Lechevalier, Paris in Encyclopédie biologique.
- Martin, S., Andriantsitohaina, R., 2002. Mécanismes de la protec tion cardiaque et vasculaire des polyphénols au niveau de l'endothélium, Annales de Cardiologie et d'Angéiologie. Elsevier, pp. 304-315.
- Mouhaddach, A., Cáceres, A., Schinini, A., Matabosch, X., & Cañigueral, S. (2018) .Chemical characterization and biological activity of *Retama sphaerocarpa* (L.) Boiss extracts and their main components. Industrial Crops and Products, 117 22-29.
- Mahler H. & Cordes E., 1968. Biological chemistry, Harper and Row.

-N-

- Navarro, Teresa, ***et al***. "In vitro antioxidant and antifungal activity of the contents of Retama raetam twigs." Journal of Agricultural and Food Chemistry 52.4 (2004): 1141-1144.
- Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence,extraction and analysis. Journal of pharmaceutical and biomedical analysis, 41(5), 1523-1542.
- Ndomo, A.F., Tapondjou, A.L., Tendonkeng, F., Tchouanguep, F.M., 2009.Evaluation des Propri_et_es insecticides des feuilles de Callistemonviminalis (Myrtaceae) contre

les Adultes d'Acanthoscelidesobtectus (Say) (Coleoptera; Bruchidae). Tropicultura 27, 137–143.

-P-

- P. N. Ravindran, K. Nirmal Babu, K. Sivaraman, "Fabaceae: Taxonomy and Ecology", in Genetic Resources, Chromosome Engineering, and Crop Improvement: Medicinal Plants, CRC Press, 2008.

-Q-

- Quezel, P., & Santa, S. (1962). Nouvelle flore de l'Algérie et des régions désertiques méridionales. Paris: Centre National de la Recherche Scientifique.
- Quezel et Santa ; (1962). Nouvelle flore de l'Algérie. Tome I.p 156-162.

-R-

- ROBINET, F.G. (1951).Saponosides stéroïdes et triterpéniques de synthèse.Ecole Polytechnique Fédérale, Zurich.

-S-

- Santos-Buelga, C., Gonzalez-Manzano, S., Dueñas, M., Gonzalez-Paramas, A.M., 2012.Extraction and isolation of phenolic compounds. Natural products isolation, 427-464.
- Sahbi F., Aouni M., 2015. Impact des huiles essentielles de Lavandula dentata sur la biochimie, la morphométrie chez une espèce de moustique Culex pipiens. Mémoire de Master. Université de Tébessa. p : 28-32.
- Sharma, P., Mohan, L. & Srivastava, C.N. (2006). Phytoextract-induced developmental deformities in malaria vector. Bioresource Technology 97 (14): 1599-1604.
- Sharma, P., Mohan, L. & Srivastava, C.N. (2009). Anti-juvenile activity of Azadirachta indica extract on the development and morphometry of filaria vector, Culex quinque fasciatus (Diptera: Culicidae) Say. Parasitology Research 105 (5): 1193–1203.

- Sahbi F., Aouni M., 2015. Impact des huiles essentielles de Lavandula dentata sur la biochimie, la morphométrie chez une espèce de moustique Culex pipiens. Mémoire de Master. Université de Tébessa. p : 28-32.
- Sak, O., Uckan, F. &; Ergin, E. (2006) Effects of cypermethrin on total body weight, glycogen, proteinand lipid contents of Pimpla turionellae L. (Hymenoptera: Ichneumonidae). Belgian Journal of Zoology 136: 53-58.

-T-

- Tine-Djebbar, F., Bouabida, H. &Soltani, N. (2016).Répartition spatio-temporelle des Culicidés dans la région de Tébessa. Editions Universitaires Européennes.
- Talukder, F.A. (2006). Plant products as potential stored product insect management agentsA mini review. Emirates Journal of Food and Agriculture 18(1): 17–32.
- Timmermann, S.E. & Briegel, H., (1999). Larval growth and biosynthesis of reserves in mosquitoes. Journal of Insect Physiology 45: 461–470.

-V-

- Villeneuve F et Desire CH. 1965. Zoologie. 1ére Édition : P : 323.
- Van Den Heuvel. (1963). The effect of rearing temperature on the wing length, thorax length, leg length and ovariol number of the adult mosquito, Aedes aegypti (L.). Transactions royal entomological society london 115: 197 – 216.

-W-

- WILLIAM G., HOPKINS M (2003).Physiologie végétale. Traduction de la 2ème édition américaine par serge Rambour, Bibliothèque Nationale, Paris, 268-273.
- Windley, M.J., Herzig, V., Dziemborowicz, S.A., Hardy, M.C., King, G.F. & Nicholson,G.M. (2012).Spider-venom peptides as bioinsecticides. Toxins 4: 191-227.

-Y-

- Yazdani, E., Sendi, J.J., Aliakbar, A.R. & Senthil Nathan, S. (2014). Effect of Lavandula angustifolia essential oil against lesser mulberry pyralid Glyphodes

pyloalis Walker (Lep: Pyralidae) and identification of its major derivatives. Pesticide Biochemistry Physiology 107: 250-257.

-Z-

- Zemouli Nadjett. L'extrait de la plante *Retama sphaerocarpa* .L comme inhibiteur de la corrosion de l'acier API 5L Gr-B dans l'acide chloridrique.2017.