Algerian Democratic and Poaire Republic

Ministry of Higher Education and Scientific Research

EcheikhLarbi Tébessi University -Tebessa-

Faculty of Natural Sciences and Life Sciences

Department: Applied Biology

Laboratory: Bioactive molecules and applications

Thesis

In view of obtaining the PhD degreeL.M.D

Speciality: Biological Sciences
Option: Pharmacotoxicology

Title

The toxicity Evaluation of a medicinal plant *Ruta montana* in a biological model *Drosophila melanogaster* under laboratory conditions

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Academic year: 2025/2024

Acknowledgements:

In the first, I would like to thank of all almighty Allah for giving the health, the patience, the power and the will for carry this thesis and complete my research.

First and foremost, the acknowledgment must be made to my supervisor Pr. Bouabida Hayette (Professor in the Department of Biology of Living Beings, University of Tebessa). I would like sincerely to thank her for all the time and effort he spent in assisting me in my work. Thank you for being an inspirational mentor and for motivating me to pursue relevant research.

Great thanks are also to Dr. Dris Djemaa (Doctor in the Department of Biology of Living Beings, University of Tebessa) for her valuable help, encouragement, plentiful advice and their co-operation during this work.

I' m deeply indebted to PrZorgui Lazher (Professor in the Higher Institute of Applied Biology Medenine, Tunisia) for his warm welcome, kind support, sincere guidance, and invaluable advice during my thesis.

I would express my profound gratitude to Dr. Cheriak Linda for accepting to preside my thesis defense committee. Many thanks go to Dr.Fengour Hind, Dr. Bouguessa Slim and Dr. Leelmi Nassima for accepting to review my thesis manuscript and for their presence in defense committee.

Acknowledgements:

I am so grateful to my family and my husband for their support and for encouraging me to furither my studies. To my parents for their unconditional confidence which always sustains me.

To my sisters and my brother,Thank you for always taking an interest in my research and for providing endless support while i pursue my goals.

For my son, thank you for giving me love for encouraging me to furither my studies.

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ملخص:

تهدف هذه الدراسة إلى تحليل التركيب الكيميائي للزيوت الأساسية المستخلصة من نبات **فيجل الجبل** (*Ruta montana*) وتقييم فعاليتها البيولوجية ضد يرقات وبالغات نوع الحشرات .*Drosophila melanogaster*

تمت تجربة الزيوت الأساسية باستخدام تركيزات مختلفة على يرقات وذباب الخل البالغ *D. melanogaster*، في ظروف مختبرية وفقًا لإرشادات منظمة الصحة العالمية. تم تقييم تأثير هذه الزيوت من خلال قياس معدلات الوفاة بين اليرقات والبالغين، بالإضافة إلى تحليل المؤشرات الحيوية الأنزيمية مثل **GSTs**و**catalase**، وكذلك دراسة المعايير الفسيولوجية والبيوكيميائية للبالغين.

مردود الزيوت الأساسيةتم استخلاص الزيوت الأساسية منالأجزاء الهوائية لنبات*Ruta montana*المأخوذ من منطقتين مختلفتين:المنطقة الأولى :جبل *ونزة* والمنطقة الثانية:جبل *بولحاف الدير*فكانت نسبة مردود الزيت من المادة الجافة 1.49 ± 0.27٪ و0.75 ± 0.083٪ على التوالي.

تم تحليل التركيب الكيميائي باستخدام تقنية .**CPG/SM**أظهر التحليل أنالزيوت المستخلصة من نباتات المنطقة الأولى تحتوي على 21 مركبًا، تمثل 87.43٪ من إجمالي التركيب، حيث يتضمن68.47٪ أحاديات التربين30.54و٪ هيدروكربونات و بالنسبة للمنطقة الثانية فالزيوت المستخلصة من نباتاتها تحتوي على 17 مركبًا، يمثل التربينات 91.38٪ من مكوناتها، بينما تشكل المكونات الكيميائية الأخرى مثل الألكانات، السيمين، والمركبات العضوية العطرية 8.62٪ فقط.

و أظهرت اختبارات السمية، وفقًا لإرشادات منظمة الصحة العالمية، أن الزيوت الأساسية تمتلك خصائص مبيدة للحشرات. أظهرت نتائج الاختبارات على البالغات التي عُولجت بتركيز **LC50**انخفاضًا في نشاط نظام إزالة السموم، مما انعكس على انخفاض في مستويات **GSTs**و **catalase**.

و اظهرت الزيوت الاساسية تأثيرات إضافية حيث سببتفي انخفاضًا كبيرًا في أعداد الذكور والإناث في أجيال *D. melanogaster*المختلفةبالاضافة إلى تقليل مستويات البروتينات، الكربوهيدرات، والدهون لدى الحشرات البالغة من كلا الجنسين.

تم توصيف التربة في المنطقتين من حيث:حجم الجسيمات.المحتوى العضوي.الأس الهيدروجيني.(pH) لتوصيل الكهربائي.

كشفت التحليلات عن وجود تركيزات عالية من بعض المعادن الثقيلة، خاصة الحديد والزنك، في العينات المدروسة.

الكلمات المفتاحية: الزيوت الأساسية، *R.montana*، السمية،*D.melanogaster*، المؤشرات الحيوية، المكافحة البيولوجية، التركيب الكيميائي الحيوي، التوصيف الإيكولوجي.

Résumé :

Cette présente étude a pour but de déterminer la composition chimique d'huile essentielle extraite de *Ruta montana* (Rue) et d'évaluer ses activités insecticides à l'égard d'une espèce biologique medicalement le plus important, *Drosophila melanogaster*. L'HE extraite de *R.montana*a été testé à différentes concentrations sur les pupes, larves et adultes de *Drosophila melanogaster*dans les conditions du laboratoire selon les recommendations de l'organisation mondiale de la santé.

Les effets ont été examinés sur la mortalité, les biomarqueurs enzymatiques (GSTs et catalase)et la composition biochimique des adultes.

Les HEsobtenue par hydrodistillation de type clevenger des parties aériennes de la plante médicinale *Ruta montana* (P1) appartenant à la famille des Rutaceae prélevées dans la zone 1 (montagne Ouenza) et de la zone 2 (Boulhaf –Edirmontain) a respectivement un rendement de 1.49±0.27% et 0.75 ± 0.083% de la matière sèche. Leur composition chimique a été déteminées par CPG/SM.L'analyse chimique a montré que les HEs de *R.montana*Les composés terpéniques identifiés dans la plante de zone 1(P1) représentent 21 composés, 87,43% de la composition totale dont 68,47% sont des monoterpènes tandis que 30,54% sont des hydrocarbures terpéniques sesquiterpènes de composés terpéniques totaux.L'HE de *R. montana* de la zone 2 (P2) se compose de 17 composés, 91,38% de dérivés terpéniques, correspondant aux monoterpènes tandis que le reste représente 8,62% d'autres composants chimiques (alcane, cymène, composés organiques et aromatiques).

Les tests toxicologiques effectués selon le protocole standard de l'OMS, ont révélé l'activité pupicide, larvicide et adulticide d'HE de *R.montana* sur l'espèce *D.melanogaster.*

L'activité enzymatique déterminée chez les adultes traitées (CL_{50})aux huiles essentielles a révélé une diminution de l'activité desGSTs et de la catalase, respectivement. L'HE de *R.montana* peut être efficace dans lalutte biologique contre la mouche de vinaigre *D.melanogaster*.

Par ailleurs, l'étude de cycle de developpement montre que les HEs provoquent une diminutionde nombre des males et des femelles de differnetesgenerations. De plus, elles réduisent significativement le contenu en protéines, glucides et lipides chez les adultes mâles et femelles. Cela est probablement dû aux conditions de stress imposées par le traitement à ces insectes qui ont besoin d'un apport supplémentaired'énergieprovenant du métabolisme glucidique et lipidique.

Aussi, La caractérisation écologique des deux sols de zone (1 et 2) est effectuée : granulométrie, matière organique, pH et conductivité électrique. Les résultats d'analyse

montrent que les échantillons analysés sont caractérisés par une abondance de certains métaux lourds Spécialement pour le fer et le zinc.

Mots clés:,Huiles essentielles,*R.montana*, Toxicité,*D.melanogaster*, Biomarqueurs, lutte biologique, Composition biochimique, caractérisation écologique.

Abstract:

The purpose of this study is to determine the chemical composition of essential oil extracted from *Rutamontana* (Rue) and to evaluate its insecticidal activities with respect to a medically important biological species, *Drosophila melanogaster*. The EO extracted from *R.montana* was tested at different concentrations on pupae, larvae and adults of *D.melanogaster* under laboratory conditions according to the recommendations of the World Health Organization (WHO).

Effects were examined on mortality, enzyme biomarkers (GSTs and catalase) and biochemical composition of adults.

EOs obtained by clevenger-type hydrodistillation of aerial parts of the medicinal plant *Rutamontana* (P1) belonging to the family Rutaceae collected in zone 1 (mountain Ouenza) and zone 2 (Boulhaf –Edir montain) has respectively a yield of 1.49 0.27% and 0.75 0.083% of the dry matter. Their chemical composition was determined by CPG/SM. The chemical analysis showed that the EOs of *R.montana* The terpenic compounds identified in the zone 1(P1) plant represent 21 compounds, 87.43% of the total composition of which 68.47% are monoterpenes while 30,54% are terpene hydrocarbons sesquiterpenes of total terpene compounds. *R.montana* EO of zone 2 (P2) consists of 17 compounds, 91.38% terpene derivatives, corresponding to monoterpenes while the remainder represents 8.62% other chemical components (alkane, cymene, organic and aromatic compounds).

Toxicological tests carried out according to the WHO standard protocol, revealed the pupicide, larvicide and adulticide activity of *R.montana* EO on *D.melanogaster*.

Enzyme activity determined in adults treated with EO (LC50) revealed a decrease in GSTs and catalase activity, respectively. *R.montana* EO can be effective in biological control of vinegar fly *D.melanogaster*.

Moreover, the development cycle study shows that EOcauses a decrease in the number of males and females of different generations. In addition, they significantly reduce protein, carbohydrate and fat content in both male and female adults. This is probably due to the stress conditions imposed by the treatment of these insects that need an additional supply of energy from carbohydrate and lipid metabolism.

The ecological characterization of the two zone soils (1 and 2) is carried out: particle size, organic matter, pH and electrical conductivity. The analysis results show that the samples analyzed are characterized by an abundance of certain heavy metals especially for iron and zinc.

Keywords:, Essential oils, *R.montana*, Toxicity, *D.melanogaster*, Biomarkers, biological control, biochemical composition, ecological characterization.

Introduction

1. INTRODUCTION

In the recent past, the use of medicinal herbs in politics has increased. Their application and preservation is a cross-sectoral issue that includes, in addition to medical treatment, preserving biodiversity, economic growth, trade, and numerous legal issues (Khursheed *et al.,* 2022).

New preventative techniques and products are continuously investigated for to ensure a better intervention while protecting the environment as much as possible. Therefore, the use of new pest control solutions is further promoted in order to support the sustainable management of the environment. Alternatives to insecticides include natural compounds with a wide range of pharmacological effects, such as bactericides, fungicides, acaricides, nematicides (Sparks & Bryant, 2022).

While biological control may consider many different forms, the use of natural insecticides derived from plants is one that's capturing researchers' attention at the moment. Due to their positive effects, traditional herbal treatments have been utilized for a long time without anyone understanding what they were. Although certain confirmed pharmacological effects on animals have been assigned to substances such alkaloids and derivatives, terpenes, steroids, and poly compounds phenolic, it is still difficult to identify the molecules that are in charge of the activity. Such chemical compounds are abundant in aromatic and medicinal plants (Jamshidi-Kia *et al.,* 2017; Nagulapalli Venkata *et al.,* 2017). Plant extracts have been used as insecticides for a very long time; pyrethrum, nicotine, and rotenone are just a few examples. In even more recent research, insects have been evaluated for some plants' insecticidal capabilities (Mpumi *et al*., 2016; Pavela, 2016; Jouzani*et al.,* 2017). Plants acquire expensive defense mechanisms as a result of insect and plant co-evolution to protect themselves against herbivores. The induction of defense proteins is one of these strategies. The discharge of volatile chemicals to draw predators or parasitoids of phytophagous insects or the production of secondary compounds (Pickett & Khan, 2016; Merillon&Ramawat, 2020; Ramawat& Goyal, 2020).

The insects have developed defense systems to deal with these secondary chemicals and avoid ingesting excessive quantities of foods that may be toxic. Initially of all, phytophage insects have taste cells that can detect harmful and/or unpalatable plant components. Additionally, each species has a distinct visual window that can distinguish between plants that are a host and those that are not (Raglan*det al.,* 2015). Although insects may not be able to discern between various bitter compounds based on their chemical identities, they can do so based on their intensity or starvation. However, different substances can be processed by various signaling pathways, which may result in the recognition of different molecules

(Ragland *et al.,* 2015; Asparch*et al.,* 2016). Insects have created postestive procedures to decrease the impact of ingesting substances secondary, to sensory detection of harmful molecules, They have efficient detoxification processes that enable them to remove the majority of toxic compounds from the plants they consume by utilising mechanisms including oxidation, reduction, hydrolysis, and then conjugation of molecules (Hammer& Bowers, 2015; Heidel-Fischer & Vogel, 2015; War *et al.,* 2020).

Some insects can hide toxins (De Maagd*et al.,* 2001; Pigott & Ellar, 2007), Chemicals can be employed to ward off prospective predators while also lessening the requirement for their purification (Montemezzani*et al.,* 2015). Insects can finally develop a relationship with plants with the negative effects they have on poststatives in order to avoid them (Facknath& Lalljee, 2005).

Fruit flies sometimes more than rats respond to the taste of these chemicals in a way that is quite similar to man (Gordesky-Gold *et al.,* 2008). They are drawn to sweets, avoid bitter and harmful chemicals, and adjust how much acid and salt they eat based on their internal needs (Wright, 2016). Fruit flies and mammals have anatomically different taste systems, although they function quite similarly functionally (Ebbs & Amrein, 2007.). *Drosophila melanogaster* is a suitable model to research the sense of taste because of the molecular technologies that are currently available and the relative simplicity of the insect taste system (Amrein & Thorne, 2005;Ebbs & Amrein, 2007). The 3000 species of the Algerian flora, 15% of which are endemic (Dalila *et al*., 2015), and which are spread over numerous botanical families (Sebaa*et al.,* 2018). Still needs a lot of pharmacological and photochemical exploration.

For our part, we have chosen to study species such as the *Rutamontana* focusing first on their toxicity and insecticidal effects, particularly on *Drosophila melanogaster*, a laboratory animal model well-known in the scientific community.

To confirm and carry out this particular purpose, a laboratory experiment is required. This research contributes to the understanding of the effects of *R.montana* essential oils on *D. melanogaster* behavior and control tests.

The overall goals of this brief are to improve and conserve the environment while simultaneously protecting our natural resources. It is necessary to discuss each of the three chapters that feature this topic individually in order to shed further light on it:

- The first chapter evokes the bibliographical reviews concerning the plant medicinal *Rutamontana* and the biological species studied *Drosophila melanogaster* in general
- The second chapter describes the materials and methods used in the work Experimental

- The third chapter presents all the results obtained with their discussion and finally our brief concludes with a general conclusion with perspectives.

Materials and methods

II-Materials and methods:

2.1. Presentation of the *Drosophila melanogaster* species

Drosophila melanogaster belong to the Drosophilidae family and are often referred as fruit flies, vinegar flies, wine flies, or pomace flies (Farzana khan, 2018), it is a pest of agriculture (Khan *et al.,* 2017; Dong *et al.,* 2021) which is able to colonize live, ripening fruits by using of an ovipositor, resulting in significant agricultural damage (Silva-Soares *et al.,* 2017; Deutscher *et al.,* 2018). The vital model organism *D.melanogaster* is frequently used in biological and medical research projects and incredibly effective in resolving basic biological issues (Morris *et al.,* 2012 ; Malloy *et al.,* 2016 ; Staats *et al.,* 2018 ; Mestetskiy*et al.,* 2023). The *D.melanogaster* is a member of the order Diptera, or the higher flies. The full metamorphosis of the holometabolous insect Drosophila, which has four phases in total, includes the change from larval to pupal form (McLaughlin and Bratu, 2015).

This species is commonly used in research because of its rapid life cycle, its relatively simple genetics with only four pairs of chromosomes and its large number of offspring per generation (Cook *et al.,* 2012; Kaun *et al.,* 2012; Frankham, 2023).

Like all insects, fruit fly is covered with a chitinous exoskeleton; has three main body segments; and has three pairs of segmented legs (Moussian, 2019; Goldsmith *et al.,* 2022; Majumdar& Dey, 2022). The typical adult fruit fly is just around 3 mm length and 2 mm wide, and it tends to be yellow-brown (tan) (Nath, 2017; Rajawat *et al,* 2021). It has a rounded head with large compound redeyes; three small single eyes and short antennae (Volonté*et al.,* 2022; Hou *et al.,* 2016). The rear end of the abdomen of males has more dense black coloring (Akiyama *et al.,* 2022; Schember and Halfon, 2022; Weber *et al.,* 2022). *D.melanogaster* has a single pair of wings that form from the middle segment of its chest (Mestetskiy*et al.,* 2023). On the last segment of its gorgese develops a set of rudimentary wings that act as gnarly balancing organs (Rajawat *et al,* 2021), These balancing organs are called dumbbells (Giraud *et al.,* 2021). Their eyes are sensitive to slight differences in light intensity (Senthilan*et al.,* 2019; Palavalli-Nettimi& Theobald, 2020). Fruit flies, as their name implies, are mostly plant-based organisms and feed on decaying plants and fruit (Liu et *al., 2017*; Knittelfelder*et al.,* 2020). Because of this, Drosophila is regarded as a serious pest in several regions of the world (Asplen et *al.,* 2015; Kenis *et al.,* 2016). According toMeigen, 1830, the systematic position of *D.melanogaster* is as follows:

Table 1: The systematic position of *D.melanogaster*according toMeigen, 1830 is as follows:

Kingdom	**Animalia**
Class	**Insect**
Order	**Dipteral**
Family	**Drosophilidae**
Subfamily	**drosophilinae**
Tribe	**Drosophilini**
Genus	**Drosophila**
Species	**Melanogaster**
Descriptor	**Meigen, 1830**
Scientific name	***Drosophila melanogaster***
Common name	**Fruit fly, vinegar fly**

2.2. Life cycle of *D. melanogaster*

Drosophila is characterized by sexual dimorphism (David *et al.,* 2011; Setoguchi*et al.,* 2014), the use of a sex comb makes sexual dimorphism less noticeable (Ng & Kopp, 2008;David *et al.,* 2011), drosophila male's reproductive capacity also varies greatly (Carey *et al.* 2022). Females lay 400 eggs (embryos) into rotting fruits or other suitable materials such as decaying mushrooms and sap fluxes, about 1 to 5 at a time (Farzana khan, 2018 ; Patra *et al.*, 2020).

D.melanogaster eggs are around 0.5 mm long, silvery, oblong, ovoid, and almost compacted when seen horizontally. (Moharana, 2015; Cetera *et al.,* 2016; Duhart *et al.,* 2017; Dye, 2022). Under normal environmental circumstances, eggs hatch after 20-22 hours at 25°C into little, white first instar maggots (larvae) (Wipfler *et al.,* 2013),The resultant maggots then consume food, and their growth continues for approximately 4 days (at 25°C)(Farzana khan, 2018), They then molt twice, becoming second and third instar maggots 24 and 48 hours after hatching, respectively (Sévigny, 2018). The larvae eventually encapsulate (pupate) in the puparium and go through a 4-day metamorphosis (at 25°C), following which the adults emerge (Ahmad, 2016; Reji, 2019).As with many ectothermic species, the developmental time of *D. melanogaster* varies with temperature (Mikkelsen *et al.,* 2010; An *et al.,* 2017; Petino Zappala *et al.,* 2018; Kim *et al.,* 2020).

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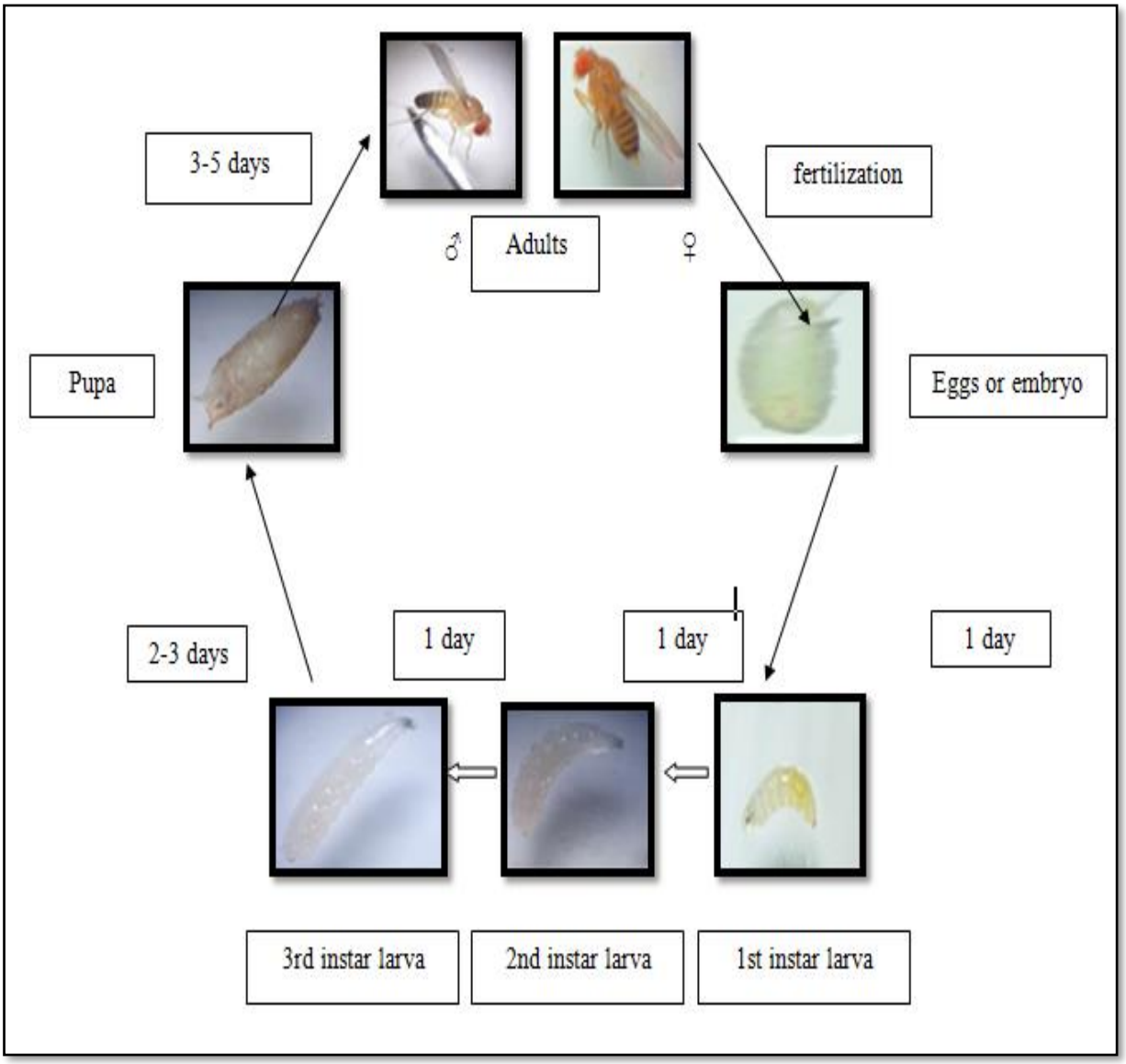


Fig 1: Life cycle of *D.melanogaster*

2.3.Collection and breeding:

Breeding is carried out in glass bottles using artificial media. The rate of development depends on temperature. In addition to providing food for insects, it also serves as the environment where larvae are going to develop. It ought to be sturdy but not too compact so that larvae are able to grow. One litre of culture solution (10 vials) includes:

- 750 mLof H2O, 100 grams of corn flour
- 20 g of agar-agar
- 80 g of yeast
- 50 mL alcohol solution of hydroxybenzoate methyl 10 %

All these products will be mixed and Heat gently stirring until boiling and cooked 25 minutes in the pressure cooker, 100 mL approximately in each bottle. The resulting mixture must be allowed to cool, and stored in the refrigerator if necessary.

Each vial is limited to 10 pairs of *D.melanogaster*adult'swhitch were identified by Pr. BOUABIDA Hayette at the laboratory level of living beings of EchikhlaarbiTebessi University.

If the pairs of the fruit fly have been asleep, care should be taken to place them on the rough paper support to avoid the engluement. The vials must be at a temperature between 24° and 28°, slight variations in temperature are not very important (between 18° and 28°), but they affect the speed of development. Eggs are observed laid on the surface of the nutrient medium and on the inner surface of the vial. After a few days, larvae will move in this medium. Around the 6th, 8th day, there larvae goes up along the walls of the bottle. They undergo pupation.

In order not to injure insects during handling, care should be taken to only handle and move them with a soft brush.



Fig2 : Breeding bottles (Original photo)

2.4.Presentationof the plant studied*Rutamontana*:

R.montana, also known as Fidjel, is part of the Rutaceae family (Drioiche*et al*., 2020; El-Ouady&Eddouks, 2021; Nadra *et al*., 2022). Rutaceae are frequently secretory, woody plants (Kubitzki*et al*., 2010; Machado *et al*., 2017; Bennaoum&Benhassaini, 2019). With pockets secretory of a type that is not encountered in any other family called schizolysigens (Maggi &Benelli, 2018; Bennaoum&Benhassaini, 2019; Butnariu, 2021). These epidermal-derived pockets are always superficial and release their EO content with the least amount of pressure

(Das, 2013; Sadgrove& Jones, 2015;Bennaoum&Benhassaini, 2019). This family has more than 1500-2096 species (Groppo*et al*., 2012; Cheek *et al*., 2018).

Three subfamilies of the genus Rutaceae are distinguished by differences in the ovary and fruit (Kubitzki*et al.,* 2010; El Ottra*et al.*, 2022).

Numerous rutaceae plants are employed in both the food and medicinal industries (Govindarajan, 2010; Zhang *et al.,* 2017).Rutaceae are rich in alkaloids, triterpenes, coumarins and essential oils (Sadgrove*et al.,* 2014 ; Bissim*et al.,* 2019),The genus Ruta belongs to the family Rutaceae (Daoudi *et al.,* 2016), Ruta comes from the Greek 'rhyté' means saved, prevent (Bourgaud*et al.,* 2008), in Algeria, there are 04 species: *R.montana* , *R.chalepensis*, *R. tuberculata* and *R. latifolia* (Atoui, 2021), Species differ in appearance leaves, fruiting bunch, bracts and sepals (Kennaz*et al.,* 2022).

2.5. Position in the systematic

Table 2: The systemic position of *Rutamontana* according to Bonnier, 1999.

Kingdom	**Plantae**
Class	**Magnoliopsida (dicotylédons)**
Order	**Sapindales**
Family	**Rutaceae**
Subfamily	**Rosidae**
Tribe	**montana**
Genus	**Ruta**
Species	***Rutamontana***
Descriptor	**Medicinal plant**
Scientific name	***Ruta montana***
Common name	Fidjel (Bonnier, 1999; Wiart, 2006 ;Takhtajan, 2009)

2.6. Botanical descriptionof the plant studied

R.montana commonly known as Mountain Street is a shrub of the family of Rutaceae, belonging to the genus Ruta (Drioiche*et al.,* 2020; Rhafouri*et al.,* 2020). It is a Mediterranean plant (Pollio *et al.,* 2008; Khadhri*et al.,* 2014)and is abundant in tropical and temperate regions. Numerous reaserch have documented the antioxidant and antibacterial properties of EO of the Ruta species (Drioiche*et al*., 2020; Jianu *et al*., 2021; Nahar *et al*., 2021).

In traditional medicine, *R.montana*, an herb from the Rutaceae family, is frequently used to treat diabetes mellitus, infections, as a convulsive for children, and to treat psychic illnesses (Benkhnigue*et al*., 2014; Coimbra *et al*., 2020; Benkhaira*et al*., 2022).

The presence of 2-Undecanone, a ketone that has been shown to be a promising antifungal and antibacterial molecule, reveals the volatile diversity of the essential oils of the Ruta species (Reddy, & Al-Rajab, 2016; BarbouchiBenzidia, 2021).

2.7. Presentation of Essential Oil

Essential oils are complicated entities naturally occurring, volatile molecules.Essential oils are naturally occurring chemicals that are odorized by a strong aroma and produced by aromatic plants as secondary metabolites (Shaaban, 2020; Bilia *et al*., 2014;Solgi Ghorbanpour, 2014).

They are often obtained using steam or hydro-distillation, which Arabs invented in the Middle Ages (Bakkali *et al*., 2008 ;Sarma *et al*., 2021), They are used in embalming, food preservation, and as antibacterial, analgesic, sedative, anti-inflammatory, spasmolytic, and locally anesthetic treatments. They are also known for their antiseptic, bactericidal, virucidal, and fungicidal, as well as therapeutic characteristics and their scent (Akthar *et al*., 2014; Brochot*et al*., 2017; Tariq *et al*., 2019). EO are used in nature to protect plants from herbivores by decreasing their desire for certain plants and acting as pesticides, insecticides, antibacterials, and antivirals (Dubey *et al*., 2011 ; Chen *et al*., 2013 ; Singh & Pandey, 2021).Additionally, they might attract certain pests to help pollen and seed dispersal or deter undesired ones (Kalita *et al*., 2013; Laborda *et al*., 2013; Trivedi *et al*., 2018).

EO are made from a variety of fragrant plants that are typically found in temperate to warm regions like the Mediterranean and tropical areas, where they play a significant role in the ancient pharmacopeia (Manaila*et al*., 2016; Božović & Ragno, 2017;Elshafie&Camele, 2017; Asdadi*et al*., 2020).They have a density typically lower than that of water and are liquid, volatile, limpid, infrequently colored, lipid soluble, and soluble in organic solvents (Nieto, 2017; Bilia *et al*., 2018). All plant organs, including buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, and bark, are capable of producing them. They are then stored in secretory cells, cavities, canals, epidermic cells, or secretory trichomes (Miguel, 2010; Carvalho*et al*., 2016).

For the extraction of essential oils, a variety of extraction methods, including solvent extraction and steam distillation, are frequently used (Bhattacharya, 2016; Ozdemir *et al*., 2018).

While only making up a small portion of a plant, EOs provide aromatic plants their distinctive qualities, which are employed in the food, fragrance, and pharmaceutical industries (Costa *et*

al., 2015; Armijos *et al*., 2020). The plant's essential oil content is primarily responsible for the insecticidal capabilities (Hanif *et al*., 2019; Dassanayake *et al*., 2021).

Different techniques can be used to extract EOs:

2.7.1. Cold Expressionextraction technique

EO is virtually solely produced using the earliest extraction technique, cold pressing (Hanif *et al*., 2019; Teigiserova*et al*., 2021). Any physical procedure that causes EO glands in the cuticles and peel to rupture in order to release the oil is referred to as this approach (Chakravarty *et al*., 2021; Valussi*et al*., 2021). A watery emulsion is created as a result of this procedure, and the essential oil is then separated from it using centrifugation (Bento *et al*., 2020; Padilla-de la Rosa *et al*., 2021).

2.7.2. Solvent Extractiontechnique

The extraction of thermally labile EO can be accomplished using solvent extraction (Morsy, 2015; Giacometti *et al*., 2018; Pandey & Shrivastava, 2018). This process involves putting the plant material into a solvent bath to dissolve it (Vinatoru*et al*., 2017 ;Maquirriain*et al*., 2022), Following extraction, the liquid mixture containing the essential oil and other chemicals undergoes filtration followed by distillation (Ali & Chow, 2019 ; Manousi*et al*., 2019). Alcohol, hexane, ethanol, petroleum ether, and methanol are among the solvents that are frequently employed for extraction (Al-Alwani *et al*., 2015; Fornasari*et al*., 2017). Since the diffusion rates are influenced by temperature, it is possible to speed up the process by employing hot solvents. Solvent extraction is cheap and generally quick (Flórez*et al*., 2015; Ameer *et al*., 2017). The created essential oil will have a trace amount of solvent left over, making it unable to use it in food applications (El Asbahani*et al*., 2015; Reyes-Jurado *et al*., 2015).

2.7.3. DistillationExtractiontechnique

The steam distillation or water distillation method is the most popular extraction technique (Filly *et al*., 2016; Parliment, 2020). The duration of this extraction procedure ranges from 1 to 10 hours (David *et al*., 2019). The amount of oil produced is influenced by the temperature, pressure, and type of plant material used during distillation (Božović *et al*., 2017; Golmohammadi*et al*., 2018). Plant materials are subjected to heated steam or boiling water during distillation in order to evaporatively liberate EO contained therein (Aziz *et al*., 2018; Basavegowda& Baek, 2021). In a container commonly referred to as the "Florentine flask," the condensed steam and essential oil vapors are gathered and separated (Bhardwaj *et al*., 2020 ; Dhar *et al*., 2023). Esters can hydrolyze during distillation into alcohols and acids,

which could have catastrophic consequences in the event of oils with high ester content (Mahanta *et al*., 2021; Umaru *et al*., 2023).

2.7.4. Extraction with Supercritical Gases

Typically, standard extraction techniques like steam distillation and solvent extraction have been employed (Prado *et al*., 2021; Xiao *et al*., 2021). Fortunately these techniques have drawbacks such limited yield, volatile component loss, lengthy extraction durations, and hazardous solvent residues (Marić *et al*., 2018; Chouhan *et al*., 2019). Supercritical fluid extraction (SFE), which has been established and extensively researched, can be used as an alternative to these traditional procedures (Ding *et al*., 2017; Chai *et al*., 2020). Batch, semi-batch, and continuous SFE modes are all available (Moraes *et al*., 2015; Djas&Henczka, 2018). In order to achieve the proper extraction conditions, the solid material is often placed in a vessel into which the supercritical fluid is introduced at a certain flow rate (Sánchez-Camargo *et al*., 2019; Yousefi *et al*., 2019).

2.8. Collect of plant materiel *R.montana:*

The samples (aerial parts) of *R.montana*(the first sample S1) were collected in October 2021 from the Ouenza forest located in the region of Tebassa (North-eastern Algeria), (GPS: 35°9.4′77.91″ N, 8°0.7′76.22″ E and 1206 m altitude) as it is shown in figure 1. The second sample S2 were collected in October 2023 from Boulhef-Edir mountain located in the region of Tebessa(North-eastern Algeria), as it is shown in figure. The identification of this species was carried out by Dr. Seghir Hanene (Department of Biology of living beings Tebessa University).

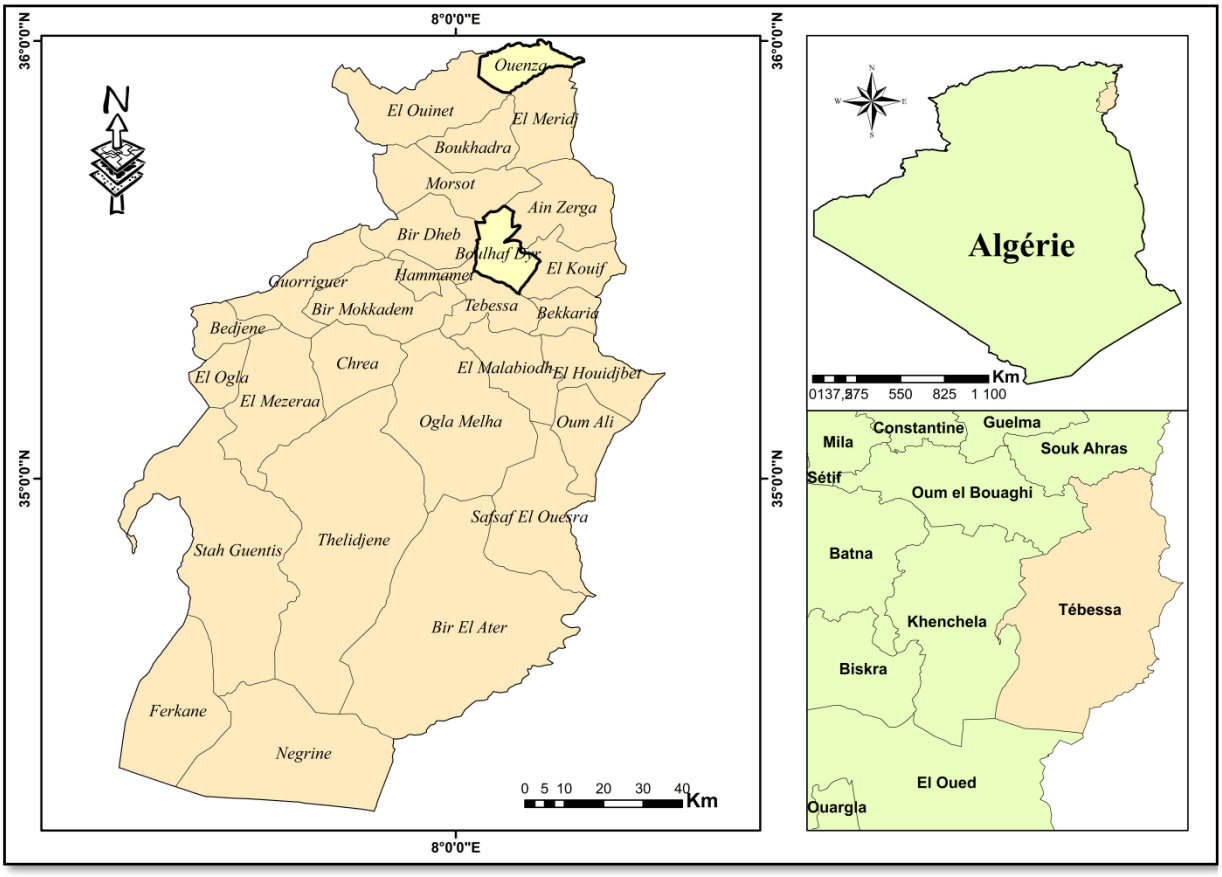


Fig 3: Geographical location of *R.montana* plant collection areas Tebessa(North-eastern Algeria)

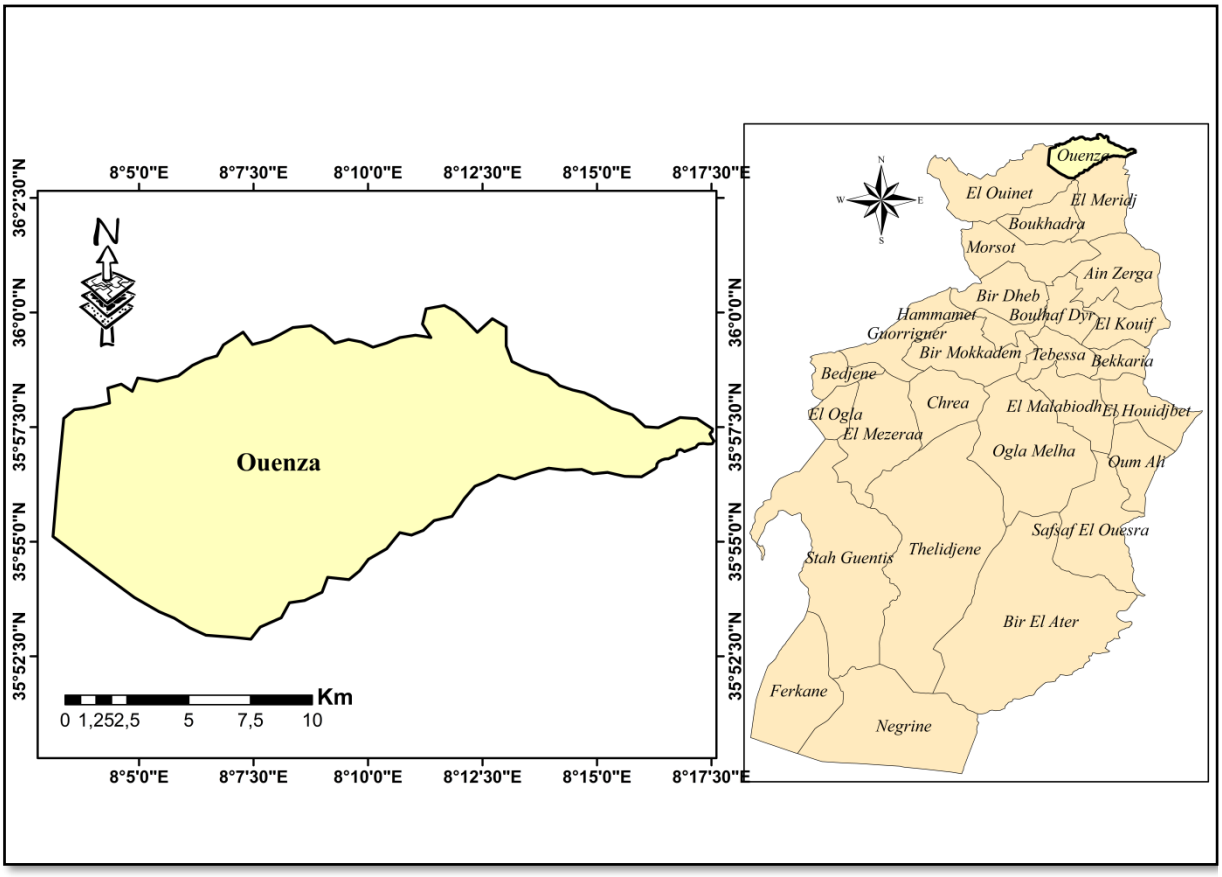


Fig4: Geographical location of *R.montana* plant collection areas Ouenza forest Tebessa(North-eastern Algeria) (collection of first sample S1)

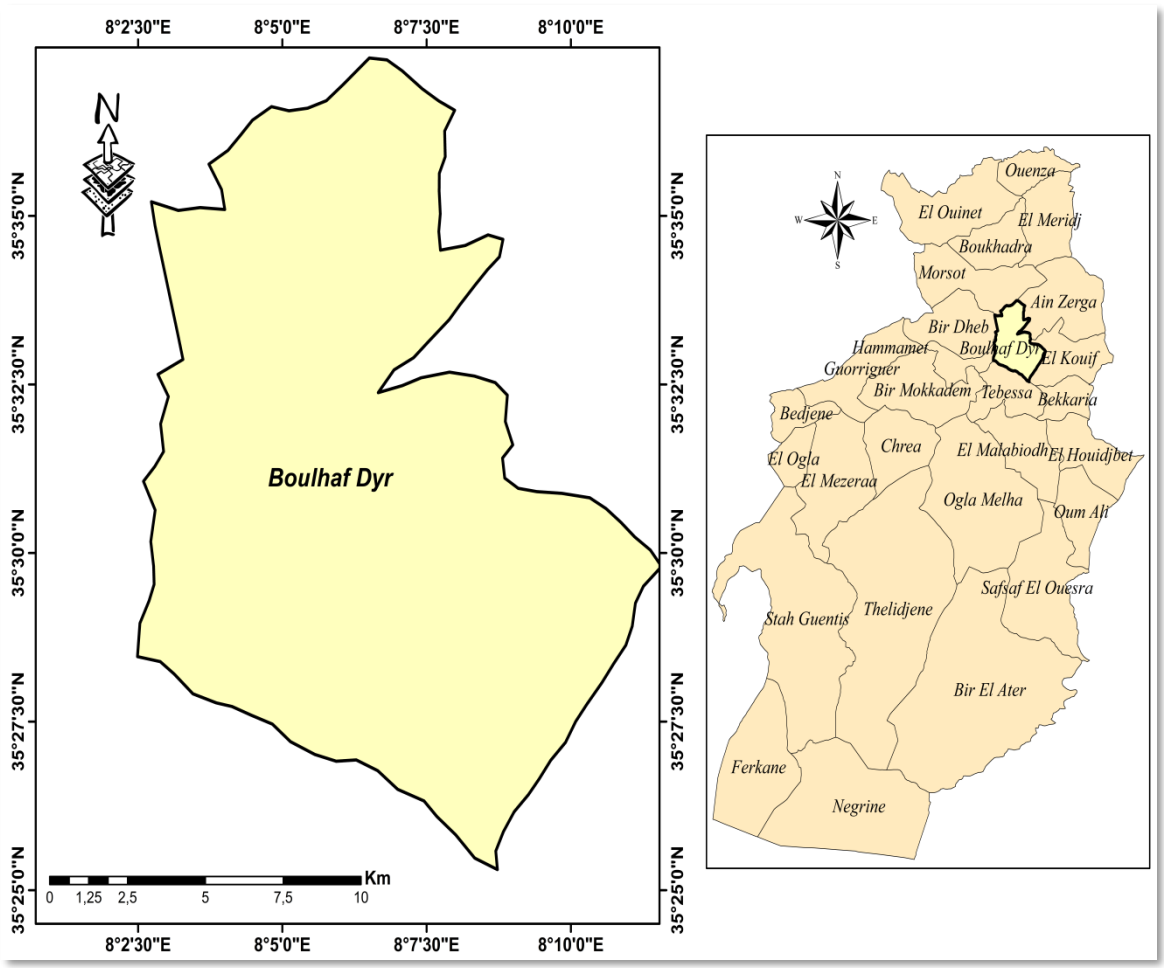


Fig 5: Geographical location of *R.montana* plant collection areas Boulhaf- Edir Tebessa(North-eastern Algeria) (collection of the second sample S2)



Fig 6:*R.montana*collected from the two stations (Ouenza forest and Boulhaf-Edir mountain)



Fig 7: Ouenza mountain / forest (station 1)



Fig 8: Boulhaf-Edir mountain (station 2)

2.9. Extraction and yield of Essential Oil by hydrodistillation

For the best yields, hydrodistillation is still the most frequent and efficient extraction method (Giacometti *et al*., 2018; Katekar*et al*., 2023).The extraction was made at the level of the Animal Biology laboratory at the University of Tebessa by a hydrodistiller of type clevenger. The aerial parts of the plant were harvested fresh and dried out of direct sunlight for 10 days.



Fig 9: The steps to collect and dry the medicinal plant obtained from the two stations

Using Clevenger-typehydrodistillation for 3 hours 50 g of dryand crushed plant matter are introduced with 700 mL of distilled waterinto a round-bottomed, one-litre balloon with 3 collars or flasks, topped by a 60 cm long column, The entire will be linked to the remaining extraction equipment and placed on a balloon heater at a temperature near 100°C.



Fig 11: Operating mode of EO extraction by a Clevenger

The mixture is brought to a boil for 3 hours, during which time the steam moves to the gooseneck and then into the refrigerator where it quickly condenses and falls into the settling bulb in the form of oil.After the essential oils were gathered, any remaining water was filtered out with the use of sodium sulfate (Na2SO4). The EO collected in glass bottlessealed tightly and shielded from light and air with aluminum foil. Weighing the obtained oil allows us to calculate its yield. Then refrigerated at 4°C. The EO yield is the ratio between the weight of the oil extracted and the weight of the dry matter of the plant, evaluated from 5 extractions (Dikbaset al., 2008; Bouabida and Dris, 2020)

It is expressed as a percentage and calculated by the following formula:

$$\mathbf{Y = [\Sigma W_B / \Sigma W_A] \times 100}$$

Y: Oil yield in %

$\mathbf{W}_B$: Oil weight in g

$\mathbf{W}_A$: Plant dry matter weight in g

2.10. Analysis of Essential Oil by CG-MS

Chemical analysis of EO was performed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). This technique was done at the Valorisation of Active Biomolecules (VBA) research unit in Medenine (Gabes, Tunisia).

GC/MS, or gas chromatographic analysis combined with mass spectrometry, is carried out using a Hewlett Packard 6800 plus chromatograph, connected to a Hewlett Packard 5973 mass spectrometer with an impact electron operating in the EI 70 eV mode.The capillary column used is type VB-5 (30 m x 0.25 mm; film thickness 0.25 µm).Helium is the carrier gas that makes up the mobile phase, and its flow rate is 1 mL/min. The injector temperature is 250ºC, and the injection is done in split mode. The oven temperature is progressively increased to 250ºC over the course of eight minutes, starting at 60ºC and going up by 2ºC per minute. For the mass spectrometer, the detection temperature is 250ºC. Fragmentation is performed by electronic impact under a 70eV field, and a pressure of 6.75Psi. The injected volume is 0.2µl. The apparatus is connected to a computer system managing a library of mass spectra. By comparing each essential oil's mass spectrum to that of reference goods from Willey databases, the components of each essential oil are identified (Mclafferty and stauffer, 1994), NIST (NIST, 1999) as well as by their retention indices calculated using C5-C18 alkanes compared to the reference product indices and literature data (Kondjoyan&Berdagué, 1996; Adams, 2001).



Fig 12: The CG-MS machine used to identify chemical compositions of *R.montana* EO (personal photo)

2.11. Study of the antioxidant activity of *R.montana* EO:

2.11.1. Antioxidant activity of R.montana Essential Oil by DPPH:

a. Principle:

Anti radical test is measured by the degradation of DPPH which is a relatively stable free radical: This test is performed according to the method ofSanchez et *al* (1998). This discoloration explains the power of the plant extract to trap this radical.

DPPH + AH ⟹ DPPH + A

(Violet) (Colorless)

This discoloration highlights the antioxidant power of a sample by its ability to trap the free radical.

b. Operating mode:

Different concentrations of EO (from 0.0125 to 5 mg/mL) are added to 1.9 ml of the DPPH ethanolic solution (0.025g/L = 2.5mg/100 mL ethanol).In parallel, a negative control is prepared by mixing 100µL of ethanol with 1.9 mL of DPPH ethanolic solution.

The absorbance reading is made against a blank prepared for each concentration at 515 nm after 30 min incubation at dark and room temperature.The positive control is represented by a solution of a standard antioxidant; the ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration the test is repeated 3 times. The results were expressed as percentage of inhibition (I%).

I%= [(control Abs – test Abs)/ control Abs] x 100

c. IC50 calculation

IC50 represents the amount of antioxidant substance needed to reduce 50% of the amount of DPPH initially present. The IC50 is obtained by the equation of the mean line of the percentage of residual DPPH as a function of the antioxidant concentration.The highest antiradicalar activity corresponds to the fraction that has the lowest IC50.

The curve % OD sample = f (Sample) is drawn and the projection of the point corresponding to 50% of the sample OD on the x-axis allows to calculate the IC50 in µg/ml extract (Wollinger et *al.*, 2016).

2.11.2. Antioxidant activity of *R.montana*Essential Oil by Reducing power of iron (FRAP):

a. Principle:

The FRAP method is a simple, fast and reproducible test. It is universal can be applied both in plants in organic and aqueous extracts(Li et *al*., 2007).

The presence of reducers in the extracts causes the reduction of iron Fe^{3}+ ferricyanide complex to the ferrous form. Therefore, Fe^{2}+ can be evaluated by measuring and monitoring the increase in density of the blue-green color in the reaction medium at 700 nm.

b. Operating mode:

1mL of extract was taken at the desired concentrations (2, 4, 10, 50, 100) were mixed with 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe(CN) 6] (1%). The resulting mixture is incubated for 20 min at 50°C in a double boiler. After

incubation, aliquots of 2.5 mL of trichloroacetic acid TCA (10%) were added to the mixtures which were then centrifuged for 10 minutes at 4500 rpm.

2.5 mL of distilled water and 0.5 mL of FeCl3 are added to the recovered supernatant. Absorbance is measured at 700 nm after 10min. Better absorbance indicates better iron reduction. The addition of Ferricyanide to the extracts allows its reduction in Ferrocynide if there is antioxidant activity. The latter is detected by the addition of FeCl 3. Trichloroacetic acid (TCA) is added to stop the reaction. A high OD indicates a significant amount of Ferrocynide.

2.11.3. Determination of antioxidant activity of R.montana Essential oil by reduction of ABTS radical-cation:

a. Principle:

The study of antioxidant activity was carried out by the TEAC method according to Reet et *al*., 1999. In the TEAC (Troloxequivalentantioxidantcapacity) method, the total antioxidant activity of a molecule is deduced from its ability to inhibit the radical cation ABTS•+ figure obtained from ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)), compared to a reference antioxidant: Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Its cyclic molecular structure is similar to that of vitamin E. The radical cation ABTS•+ is blue-green in color, resulting from the contact of ABTS with potassium persulfate ($K_2S_2O_8$).

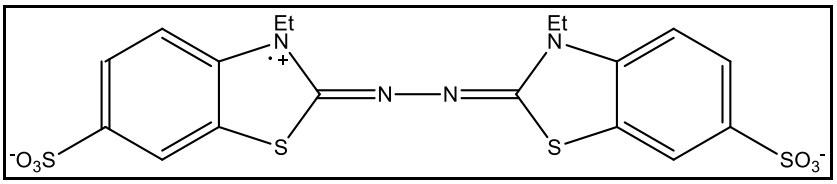


Fig16: Chemical structure of radical cation ABTS•+

The addition of antioxidants reduces this radical and causes discoloration of the mixture. This radical discoloration measured by spectrophotometry at 734 nm is proportional to the concentration of antioxidants. The radical cation ABTS•+ (absorbing at 734 nm) is formed by tearing an electron (e-) from an ABTS nitrogen atom. In the presence of Trolox (or H•-donor antioxidant), the nitrogen atom involved traps an H•, leading to ABTS+, resulting in discoloration of the solution.

b. Operating mode:

A 7 mM ABTS solution was prepared by dissolving 36 mg ABTS in 10 mL of distilled water. The radical cation (ABTS•+) was prepared by adding 88 µl of a $K_2S_2O_8$ solution (2.456 mM) to 5 mL of the ABTS solution. The ABTS•+ solution was stored in the dark for 12-16 h. This ABTS•+ solution was diluted, before use, with ethanol to obtain an absorbance of 0.70 0.02 at

734 nm. The Trolox standard curve was achieved by adding 20 µL of different concentrations of Trolox ethanolic solutions (0.2; 0.4; 0.6; 0.8 mM) to 2 ml of ABTS•+ Diluted solution. Absorbance at 734 nm was measured 6 min after the start of the reaction. The same procedure was followed for a concentration of ethanolic solutions of the extracts to be tested. Ethanol was used as blank. Fading extinction was calculated as a percentage of absorbance reduction. This percentage was determined based on the concentration and then calculated relative to the Trolox equivalent concentration. Activity was therefore expressed in mg trolox equivalent per g sample.

ABTS radical scavenging activity (%) = [A control - A sample / A control] x 100

2.12. Treatment and bioassays

2.12.1.Larvicidal activity of *R.montana* EO on *D.melanogaster* larvae:

EO dissolved in methanol is applied at different concentrations (1,2, 3, 5 and 7 µL/ mL) on larvae of the first, second and third instar larva of *D.melanogaster*. Three repetitions, each with 20 larvae, were performed for each concentration. A negative control series (individuals do not undergo any treatment) conducted in parallel. The treatment was applied in bottles containing food for 24 hours, according to the recommendations of the World Health Organization (OMS, 1963) with some modifications. Mortalities were recorded after 24 hours of exposure to EO and corrected according to Abbott formula (1925), to eliminate natural mortality. The corrected mortality percentages undergo an angular transformation according to the tables of Bliss (1938) in Fisher & Yates (1957). The standardized data are analyzed for variance to a classification criterion and dose classification by the Tukey HSD test. Finally, the nonlinear regression expressing the percentage of mortality corrected for the logarithm of the dose allowed to estimate the lethal concentrations (LC25, LC50 and LC90) and their confidence intervals (95% CI).

2.12.2. Pupicide activity of *R.montana* EO on *D.melanogaster*pupae:

EO of *R.montana* dissolved in methanol is applied at different concentrations (2, 3, 4 and 5 µL/ mL de methanol) on pupae of *D.melanogaster*. Three repetitions, each with 20 pupae, were performed for each concentration. A negative control series (individuals do not undergo any treatment) conducted in parallel. The treatment was administered for 24 hours in bottles with food, according World Health Organisation standardswith few modifications(Anonym, 1983). Mortalities were noted after 24 hours of exposure to EO and corrected according to Abbott formula (1925), to eliminate natural mortality.

2.12.3. Fumigant toxicity test of *R.montana* EO on *D.melanogaster* adults

The fumigation approach as outlined by Soonwera and Sittichok (2020) was used to test the toxicity of *R.montana* EO. Various doses of EO dissolved in 1 mL of ethanol (0.2, 0.4, 0.8, 1.6, 2.4, 4.2, and 5 µL/Lair) were applied to Whatman filter paper, with a diameter of 2 cm. after releasing 20 adults of *D. melanogaster* for one hour and transferring them to a tube without treatment, the screw caps of the glass bottles were sealed under the surface. The mortality rate was noted 24 hours following EO exposure. One milliliter of ethanol was applied as the positive control and the vials without EO were used as the negative control in the tests. There were five iterations of each treatment. When *D. melanogaster* flies did not move in reaction to the shaking, it was observed that they were dead inside the vial. Levels of adult vulnerability were categorized according to Soonwera and Sittichok's prior descriptions (2020).

Table 03: Classification of adult sensitivity levels

Susceptibilitylevels	**Status**
98 - 100%	Sensitive (S)
80-97%	Possiblyresistant (PR)
>80%	Resistant (R)

2.13. Impact of *R.montana* EO on *D.melanogaster*growth

EO of the medicinal plant is applied with LC50 on adults during developmentof*D.melanogaster*, affects the growth of individuals and causes morphological abnormalities (affects body weight). The individual weight test using a digital scale after treatment shows various morphological aberrations. (Bouabida and Dris, 2022)

2.14. Impact of *R.montana* EO on *D.melanogaster* development time

According to Bouabida and Dris, (2022b) (with some changes), *R.montana* EO (LC25, LC50) was applied to *D.melanogaster* adults to assess the development time of two generations after mating for five treated males and five treated females, compared to a control series.

The evolution was followed daily until the emergence of first, second and third generations.

2.15. Response time (LT50) bioassay against *D. melanogaster*

This experiment aims to find out the duration of the vegetable oil's insecticidal effect. For this purpose we applied the method of Aljedani, 2021 (with some modifications) on adults of *D. melanogaster*. Crude *R.montana* EO was tested at the highest concentration (5 µL/Lair) and mortality was recorded as a function of time (1, 3, 6, 12, 16, 20 and 24 hours) after exposure.

Each experiment was repeated three times. Adults are considered dead when there are no movements when examined with a brush. The time required to reach 50% mortality of *D. melanogaster* was calculated by the concentration (LT50) (Aljedani, 2021).

After calculating and applying Abbott's equation to each treatment, the percentages for mortality were evaluated.For each group, the lethal time 50 (LT50) was determined at p=0.000

2.16. Sex ratio test against *D.melanogaster*

Given that it has the power to determine reproductive capacity, the sex ratio is an essential part of population structure (Hardy, 2002; Hultine*et al*., 2016).

The effect of *R. montana* EO on the sex ratio of fruit flies has been studied according to the method of (Wang *et al*., 2020) with some modifications., we placed 10 females and 10 males treated with LC25 and LC50 of *R.montana* EO for 24 hours and poured into bottles containing normal food for mating, then we counted the number of male and female flies in the tubes for three generations and calculated their male-female ratio. A negative control series (the flies do not undergo any treatment) and a positive control series (the flies receive 1 mL of ethanol) are carried out in parallel. There are 5 repetitions per treatment.

2.17. Negative geotaxis behavior assay

Locomotor ability was assessed following negative geotaxis behavior as described by Boutkhil*et al*., (2009) with some changes. Twenty adult flies (1-2 days old, male and female) were exposed to EO with both concentrations (LC25 and LC50) in graduated tubes up to 40cm, after the hourly count of fly mortality, a negative geotaxis test was carried out simultaneously with the surviving flies, where with the surviving flies, where this consisted of counting the number of flies that rise above 40 cM in the tube of the experiment itself, during a time interval of 5 s. The tests were repeated five times at 1 min intervals. The results are presented as number of flies that reached the SD peak obtained from three independent experiments (De Lacerda Neto *et al.*, 2021).

2.18.Residual efficacy test of *R.montana* EO:

The residual efficacy of *R.montana* EO was evaluated by fumigation against Drosophila adults, after the application of the highest concentration ofEO (5µL) diluted in 1 mL of ethanol on Whatman paper in glass vials. The lid was closed after the release of 10 *D. melanogaster* flies. Every 24 hours the individuals are replaced by new ones in the same vials. This is to assess the duration of activity of the insecticide. (Kim*et* al., 2016 ;Gowton*et al*., 2020).

2.19. Extraction and determination of the main biochemical constituents

The various metabolites were extracted according to Shibko*et al*., (1966) and the main steps are summarized in Figure 21. The samples are placed ineppendorf tubes containing 1 mL of 20% trichloroacetic acid (TCA) and ground using an ultrasonic homogenizer. After a first centrifugation (5000 trs/ min at 4°C, 10 mn), the supernatant I obtained is used for the determination of total carbohydrates according to the method of Duchateau &Florkin (1959). At base I, 1 ml of ether/chloroform mixture is added (1V/1V) and after a second centrifugation (5000 trs/min, 10 mn), the supernatant II is obtained and the base II is obtained, the superngent II will be used for the determination of lipids (Goldworthy*et al*., 1972) and the base II, dissolved in soda (0.1 N), will be used for the determination of proteins according to Bradford, (1976).

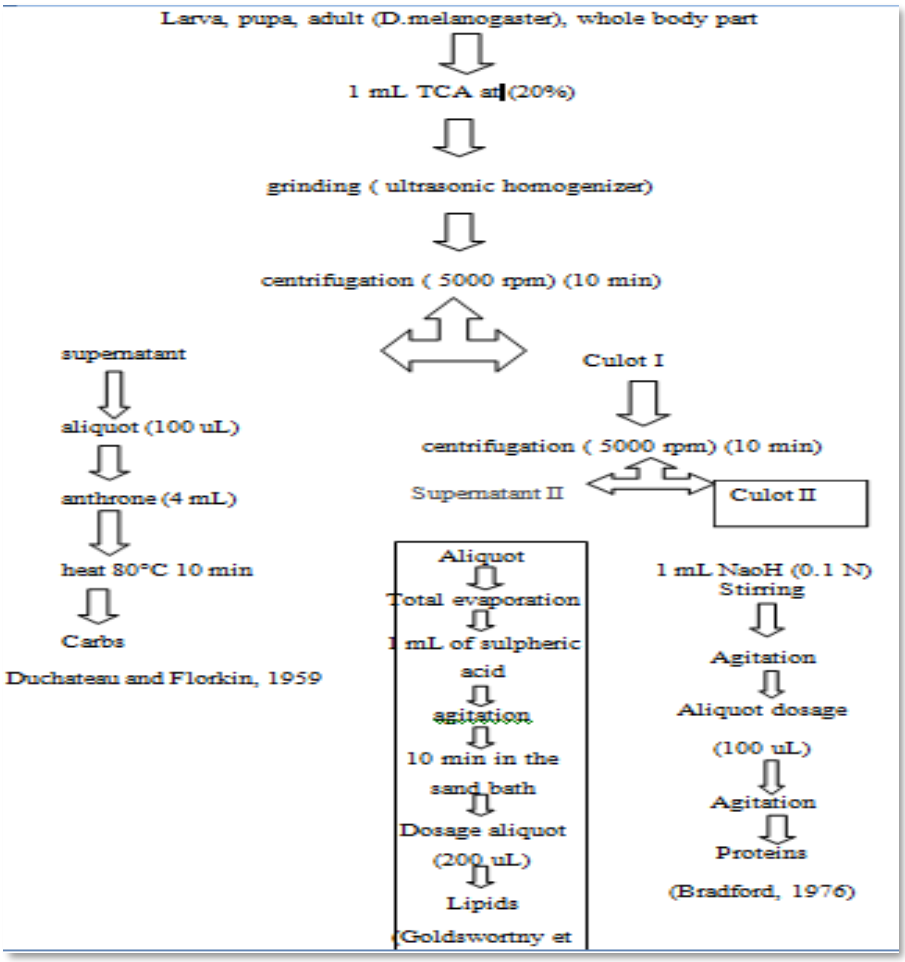


Fig 27: Extraction of carbohydrates, proteins and total lipids according to Shibko*et al.*, 1966

2.20. Biomarker assay

Adults in the control series and treated with EO (LC50) were assessed with a biomarker of detoxification, glutathione S-transferases (GST)and biomarker of oxidative stress catalase (CAT). At different periods after treatment: 24, 48 and 72 hours.

2.20.1. Determinationof glutathione S-transferases:

The activity of glutathione S-transferases (GSTs) is measured using the method of Habig et al., 1974. It is based on the conjugation reaction between the GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a cofactor, glutathione (GSH), and measured at a wavelength of 340 nm in a spectrophotometer.

Samples from the control and treated series of *D.melanogaster* are homogenized in 1 Mlphosphate buffer (0.1 M; pH 6). The homogenate is centrifuged at 1400 trs/ mn for 30 mn and the recovered supernatant will serve as a source of enzyme.

The assay consists of reacting 200 µL of the supernatant with 1.2 mL of the mixture CDNB (1mM)/GSH (5mM) [20.26 mg CDNB, 153.65 mg GSH, 1 mL ethanol, 100 mL phosphate

buffer (0.1 M, pH 6)]. The test is conducted with 3 repetitions each with 20 individuals with control sets. The absorbances are read every minute for 5 minutes at a wavelength of 340 nm against a blank containing 200 µL of distilled water replacing the amount of supernatant.Glutathione is estimated using the following formula:

$$\mathbf{X} = \frac{\Delta\ \mathrm{OD/mn}}{9{,}6} \times \frac{\mathrm{Vt}}{\mathrm{Vs}} / \mathrm{mg\ of\ protein}$$

X: millimoles of substrate hydrolyzed per minute and per mg protein (mM/min/mg protein).

ΔOD: slope of the regression line obtained after hydrolysis of the substrate as a function of time.

9.6: CDNB molar extinction coefficient (mM-1 cm-1).

Vt: total volume in the tank: 1.4 mL [0.2 mL supernatant + 1.2 mL of CDNB/GSH mixture].

Vs: supernatant volume in tank: 0.2 mL.

mg protein: amount of protein expressed in mg.

2.20.2. Catalase dosage

Catalase determination (CAT) is performed using the Claiborne technique (1985). This technique is based on the spectrophotometric measurement of the reduction of hydrogen peroxide (H2O2) to one oxygen molecule (O2) and two water molecules (H2O) in the presence of CAT at a 240 nm UV wavelength, according to the following reaction:

$$\mathrm{H2O2} \xrightarrow{\text{Catalase}} \mathrm{2\ H2O + O2}$$

Control adults and treated of *D.melanogaster*, are taken at different times (24, 48, 72 hours), the test is conducted with 3 repetitions each with 20 individuals. The fruit flies are homogenized in 1ml of tamponphosphate (100mM, pH 7.4), then centrifuged at 15000 trs/mn, for 10 mn. The recovered material will serve as an enzyme source.

The catalase activity is determined in a 25°C quartz spectrophotometer vessel on a 50 µL aliquot fraction of the supernatant diluted to between 1 and 1.5 mg protein/ml, or 0.05 to 0.75 mg in the vessel, to which 750µL phosphate buffer is added (100mM, pH 7.4). After stirring, the reading is carried out with the spectrophotometer. The reading of absorbances,is carried out after every 5 seconds for 30 seconds at a wavelength of 240nm against a blank with 800 µl phosphate buffer (100mM, pH 7.4), and 200 µL of H2O2.

The specific activity is calculated according to the following formula:

Seconds at a wavelength of 240nm against a blank with 800 µl phosphate buffer (100mM, pH 7.4), and 200 µL of H2O2.

The specific activity is calculated according to the following formula:

$$\mathbf{X} = \frac{\text{ODmax} - \text{Domin}}{0{,}04}\,\text{mg de proteins}$$

X: micromole of reduced substrate per minute and per mg protein (µM/mn/mg protein).

OD max: maximum optical density obtained.

OD min: minimum optical density obtained.

0.04: molar extinction coefficient of H2O2 (cm-1.mM-1).

mg protein: amount of protein expressed in mg.

2.20.3. Extraction and determination of biochemical constituents:

The control and treated individuals (LC50) were (adult male and female) preserved in 1 ml of TCA (trichloroacetic acid) at 20%. The extraction of the main biochemical constituents (proteins, carbohydrates and lipids) was performed using the process of Shibko*et al*., (1966)

2.20.3.1. Total protein assay

The Bradford technique (1976) is adhered for performing the protein test in a 100 µL aliquot fraction. 4 mL of Commassie Brilliant Blue (BBC) G 250 (Merck) reagent is added to the mixture. The BBC solution is prepared as follows: Homogenize 100 mg of BBC, in 50 mL of 95° ethanol, then add 100 mL of 85% orthophosphoric acid and complete to 1000 mL with distilled water. The shelf life of the reagent is 2 to 3 weeks at 4°C. This indicates that the blue colors contain proteins. At a wavelength of 595 nm, the absorbance is measured at the spectrophotometer. A 1 mg/mL beef serum albumin solution (Sigma) is used to create the calibration range (Table 4).

Table 5: Total protein assay in *D.melanogaster*: calibration range achieved.

Tubes	**1**	**2**	**3**	**4**	**5**	**6**
Standard albumin solution (µL)	0	20	40	60	80	100
Distilled water (µL)	100	80	60	40	20	0
BBC reagent(mL)	4	4	4	4	4	4
Amount of albumin(µG)	0	20	40	60	80	100

2.20.3.2. Determination of total carbohydrates

Total carbs were calculated in accordance with Duchateau &Florkin (1959). It consists of adding 100 µL of the supernatant contained in a test tube, 4 mL of the anthrone reagent and heating the mixture to 80 °C for 10 minutes, a green coloration develops whose intensity is proportional to the amount of carbohydrate present in the sample, the absorbance reading is made at a wavelength of 620 nm. The anthrone reagent is prepared as follows: Weigh 150 mg of anthrone; mix in 25 ml of distilled water and 75 mL of concentrated sulfuric acid. We are left with a transparent green solution that is kept in the dark. One milligram per milliliter (1mg/mL) of glucose is used as the calibration range.

Table 06: Determination of total carbohydrates in *D.melanogaster*: realization of the calibration range.

Tubes	**1**	**2**	**3**	**4**	**5**	**6**
Glucose stock solution (µL)	0	20	40	60	80	100
Distilled water (µL)	100	80	60	40	20	0
Anthronereagent (mL)	4	4	4	4	4	4
Amount of glucose (µg)	0	20	40	60	80	100

2.20.3.3.Determination of total fat

Total lipids were determined using the Goldsworthy *et al.*, 1972, method using the sulfo-phospho-vanillinic reagent. Lipid analysis is performed on aliquots of 100 µL of lipid extracts or standard range, to which 1 mL of concentrated sulfuric acid is added after the solvent has entirely evaporated. The tubes are then agitated and placed in a 100 °C sand bath for 10 minutes. After cooling, 200µL of this mixture is taken to which 2.5 mL of sulfo-phospho-vanillinic reagent is added. The optical density is measured in a spectrophotometer at 530 nm after 30 minutes in the dark. Lipids form hot with sulfuric acid, in the presence of vanillin and orthophosphoric acid, pink complexes. The reagent is prepared as follows:

Dissolve 0,38 g of vanillin in 55 mL of distilled water then add 195 mL of 85% ortphosphoric acid. This reagent can be stored for three weeks in the dark at 4°C. The following is how the stock lipid solution is made: In an Eppendorf tube, 2.5 mg of table oil (99% sunflower triglycerides) and 1 mL of chloroform ether (1V/1V) are added.

Table 07: Determination of total lipids in *D.melanogaster*: realization of the calibration range.

Tubes	**1**	**2**	**3**	**4**	**5**	**6**
Lipid stock solution (µL)	0	20	40	60	80	100
Solvent (ether/chloroform) (1V/1V) mL	100	80	60	40	20	0
Amount of lipids(µg)	0	50	100	150	200	250

2.21. Physicochemical characteristics of the soil

2.21.1. Soil sampling

The soils were sampled on October 20, 2023 at the Ouenza mountain site -Tebessa- (north of Algeria). The second sample made on October 21, 2023 on the mountain site Boulhef-edir -Tebessa- (north of Algeria). The sampling areas are the same recollection sites of the medicinal plant of this research (*R.montana*). Pits were dug at a depth of 20 centimetres (which corresponds to the average root length of the plant studied) 3 sampling points for each study area. The soil was taken from the target areas and placed in boxes containing 50 g of soil each for the analysis and study of the relationship between its components and the toxicity of the medicinal plant studied.







Fig 32: Collect of soil samples from the two stations at a depth of 20 centimetres.



Fig 33: Soil samplingfrom the two station areas.

2.21.2. Soil humidity test

Soil moisture or volume of water in the soil, it reflects the mass of water it contains, Soil moisture depends on the structure, texture as well as soil lithology to calculate soil moisture (Lunt *et al*., 2005 ;Demattê*et al*., 2006).

According to Gardner, 1965, the soil is weighed in the wet state then puted it in the oven for

48 hours to dehydrate them until it became completely dry then will be weighed.

Soil moisture determined by the following formula:

$$Hs = \left(\frac{Mh - Ms}{Ms}\right) * 100$$

Hs: soil moisture.

Mh: soil mass in wet condition.

Ms: soil mass in the dry state.

2.21.3. Electrical conductivity of soil

An estimate of the total dissolved salt content of a soil can be obtained from its electrical conductivity. It conveys an approximation of the sample's ionizable solute concentrations and degree of salinization (Corwin &Yemoto, 2020).

According to Rhoades, 1996, the electrical conductivity is determined from the extract of the saturated paste of the soil (a mixture of 250 g of the soil with distilled water until obtaining a wet paste not saturated with water).

Water extraction from the saturated paste is done by a vacuum cleaner under vacuum pump and the EC reading is done directly on a conductivity meter at 25°C with a soil-water ratio 1/5 (Pauwels *et al*, 1992).

Table 08: Scale of salure according to the E.C of the aqueous extract at 1/5 according to AUBERT, 1978:

E.C. (ms/Cm)	<0.6	0.6-1.2	1.2-2.4	2.4-6	>6
Appreciation	Unsalted	littlesalty	Salty	verysalty	extremelysalty

2.21.4. pH measurement of the soil for the two stations

The concentration of hydrogen ions in an aqueous solution may be used to express the pH, which is an acronym for "hydrogen potential" and indicates the acidity, neutrality, or alkalinity of the solution (Yehia & Said, 2021).

Weigh 10 g of dry soil sifted to 2 mm in a 100 mL beaker, add 50 mL of distilled water then shake and leave the solutuion in contact for 2 h. Then calibrate the PH-meter with buffer solutions. Finally, measure the pH of the solution using pH-meter (Mathieu &Pieltain, 2003).

Table 09: Water pH interpretation scale according to Ryser, 2001:

Ph	<5.3	5.3-5.8	5.9-6.7	6.8-7.2	7.3-7.6	>7.6
Agronomicassessment	stronglyacidic	Acid	weaklyacidic	neutral	weaklyalkaline	Alkaline

2.21.5. Granulometry of soil

NFP94-056, 1966, defines the method of granulometric analysis of soils by sieving. This standard is applicable to the determination of the granulometric distribution of soils, which is an important physical characteristic of soils, used for their classification and related to many geotechnical and geohydrological properties. Granulometric analysis is concerned with the mass distribution of the dimention of solid particles expressed as a percentage of the total mass of the material, and it can be carried out by sieving for grains with a diameter greater than 80 µm up to 100 mm.

Our analysis is performed by a laser granulometry; laser granulometry allows the granularity of a powder with a grain diameter between 1 and 128 microns to be quickly determined.

Following an explanation of the apparatus's operation, the authors report the findings of tests conducted on the granularity of bitumen emulsion, glass beads, and alumina powders, as well as an initial analysis of their application to natural soils (LAN & Dupas, 1973).



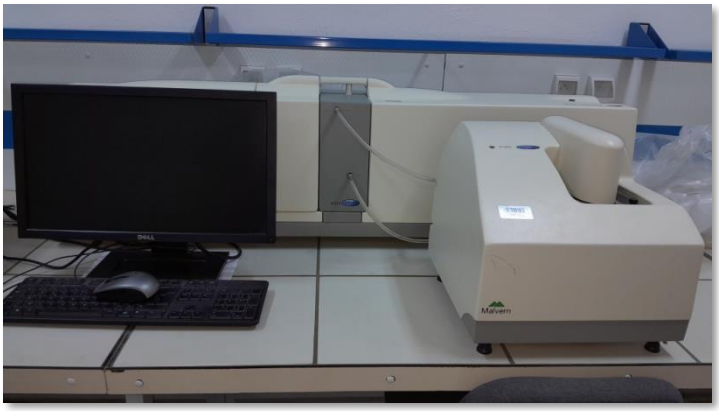


Fig 37: Lazer granulometry machine used to test Granulometry of soil (personal photo)

2.21.6. Determination of Organic Matter

After sieving the soil by a 2mm sieve, weighing one gramme of dirt and adding five millilitres of potassium bichromate, then attacked by 10 mL of concentrated sulfuric acid H_2SO_4.

Then leave at rest at about 12h to ensure the settling, the supernatant liquid will be put in test tubes for colorimetry, depending on (Yeomans & Bremner, 1988).

Table 10: Soil class by organic matter content (organic carbon), I.T.A, 1975:

Organic Matter content %	soil class
<1	poor souls
1-2	Poor
2-4	Average
>4	Rich

2.21.7. Heavy metals testing:

A sampling was carried out to enable the acquisition of data representative of the spatial variability of heavy metals in soils that cover the whole of the plain purpose of our study.

The calibration ranges are prepared from standard solutions of Iron and Zinc elements studied. The analysis was conducted using an ICP-MS (Inductively Coupled Plasma Mass Spectrometer) at the laboratory IRA LAB of Higher Institute of Applied Biology Medenine, Tunisia.

2.22. Statistical analysis

The results obtained were expressed (mean ± standard deviation), the comparison of the means was carried out using SPSS and toxicity tests and graphs using GRAPH PAD PRISM 7.

Results

3.RESULTS

3.1. Yield and phytochemical analysis of the plant

The EO obtained by hydrodistillationclevenger-type of the aerial parts of medicinal plant *R.montana*(P1) belonging to the Rutaceae family collected from zone 1 (Ouenza mountain) has a yield of 1.49±0.27% of the dry matter of the plant's aerial part, is yellow in colour, transparent, and has an attractive smell, these results are higher than those reported in EO obtained by hydrodistillation Clevenger-type of the aerial parts of medicinal plant *R.montana*(P2) belonging to the Rutaceae family collected from zone 2 (Boulhaf –Edir montain) whitchhas a yield of 0.75 ± 0.083% of the dry matter of the plant's aerial part, is yellow in colour, transparent, and has an attractive smell.

3.2. Chemical Composition of *R.montana*'s Essential oil of the first station (P1)

The analysis of *R.montana* (P1) EO has identified 22 compounds whose content is greater than or equal to 0.243%. These compounds represent 100% of the total oil(Table 11and Fig 40).

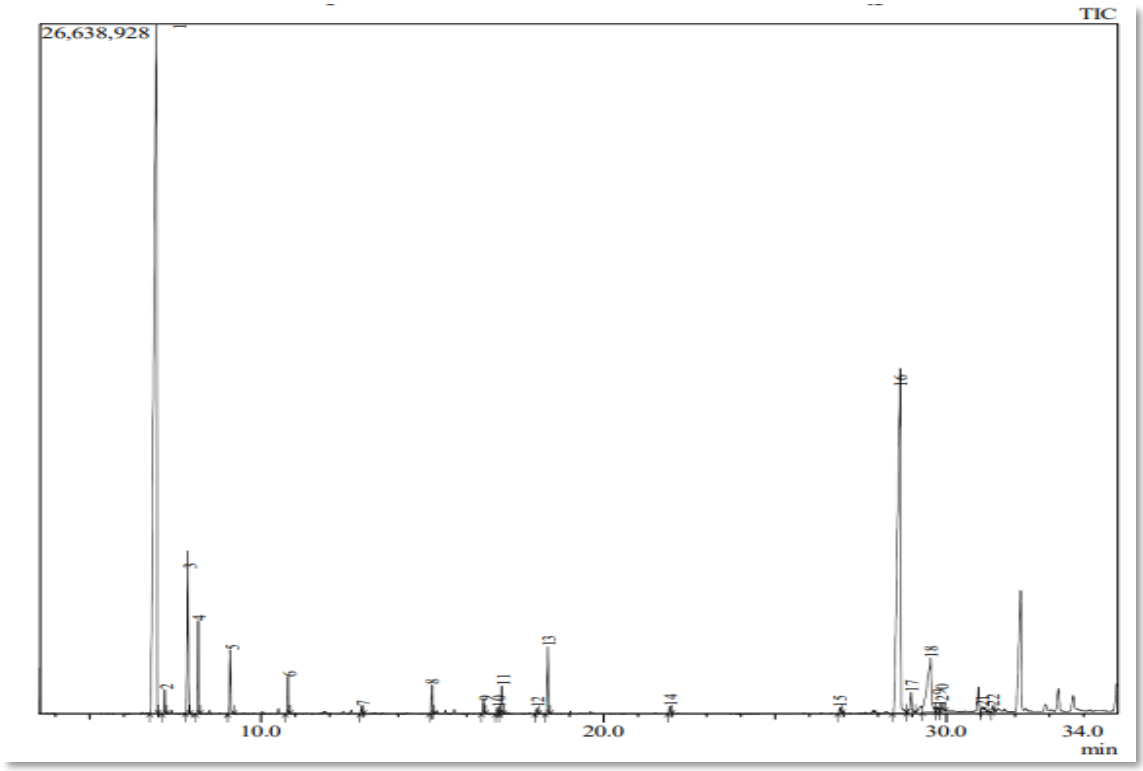


Fig38:GC-MS EO Chromatogram of *R.montana* (P1) EO (Abundance as a function of time in min).

Table 11: Chemical composition of the essential oil of *R.montana* (P1): retention time (TR) and concentrations (%) of the various constituents.

N	Compounds	Retention Time (min)	Concentrations (%)
1	**alpha-Pinene**	6.995	50.027
2	**Camphene**	7.236	0.638
3	**beta-Pinene**	7.915	4.744
4	**beta-Myrcene**	8.212	2.354
5	**Limonene**	9.142	2.094
6	**Linalool**	10.824	1.003
7	**alpha-Terpineol**	12.969	0.230
8	**Aceticacid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ylester**	15.018	0.781
9	**Eugenol**	16.520	0.483
10	**Geranylacetate**	16.921	0.164
11	**Cinnamicacid,methylester**	17.047	0.884
12	**alpha.-Farnesene**	18.065	0.157
13	**cis-beta-Farnesene**	18.393	1.865
14	**gamma-Muurolene**	21.940	0.237
15	**n-Hexadecanoicacid**	26.904	0.209
16	**Caryophylleneoxide**	28.649	26.461
17	**alpha-Farnesene**	28.970	0.909
18	**OleicAcid**	29.537	5.534
19	**Octadecanoicacid**	29.740	0.264
20	**1-Adamantaneaceticacid**	29.880	0.343
21	**Retinol**	31.042	0.376
22	**Viridiflorol**	31.353	0.243
	Total		100.000

The terpenic compounds identified in this plant represent 21 compounds, 87.43% of total composition of which 68.47% are monoterpenes (alpha-Pinene, Camphene, beta-Pinene, beta-Myrcene, Limonene, alpha-Terpineol, Eugenol and Geranylacetate) while 30.54% areterpenic hydrocarbonssesquiterpenes(gamma-Muurolene and Caryophylleneoxide) of total terpenic compounds.

alpha-Pinene (50.027%), Caryophylleneoxide (26,461%), OleicAcid (5.534%) and beta-Pinene(4.744%) were identified as the majority compounds of EO (P1).

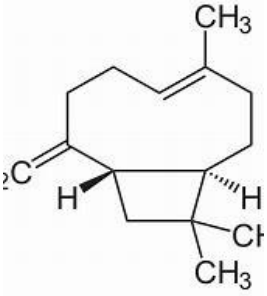
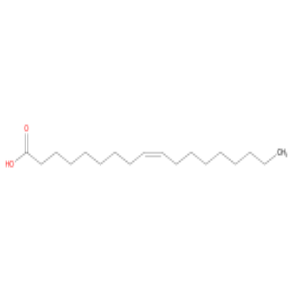
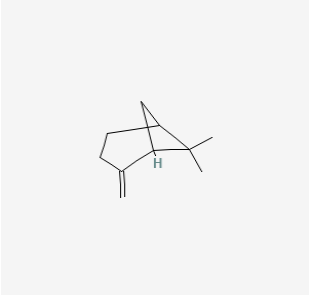
alpha-Pinene	Caryophylleneoxide	OleicAcid	beta-Pinene
			

Fig 39: Chemical structure of some major EO compoundsof *R.montana*'s from the first station (P1).

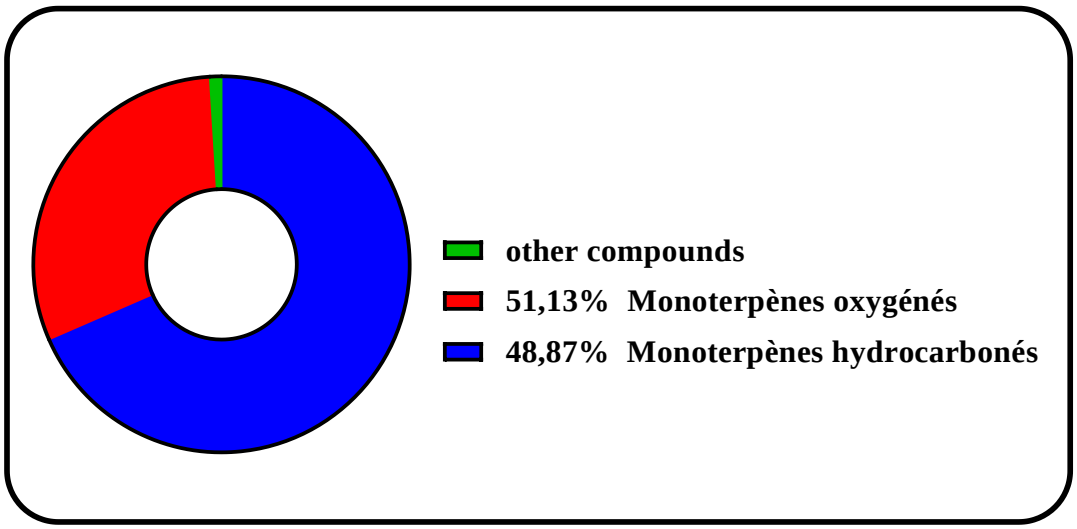


Fig 40: Chemical composant of *Rutamontana*EO from the first station (P1).

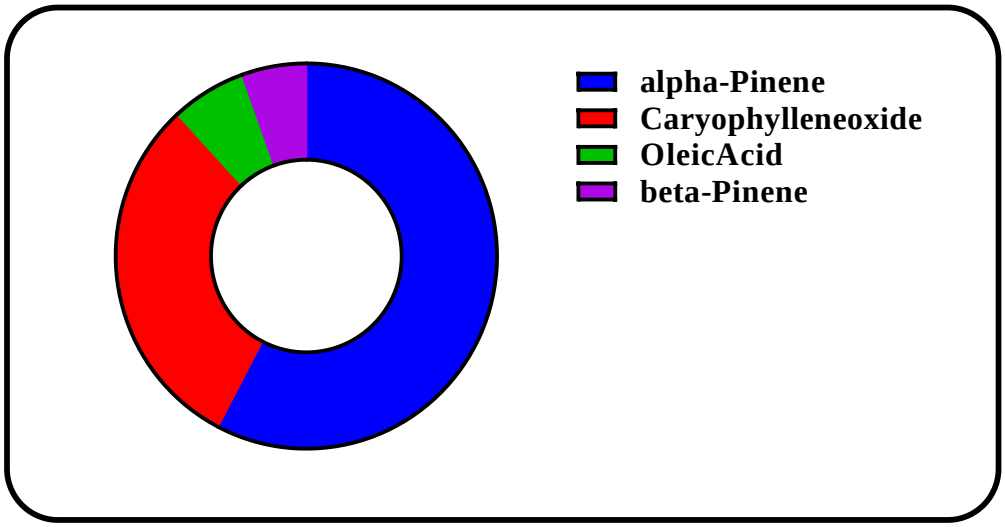


Fig 41: Distribution of major plant components of *Rutamontana* EO from the first station (P1).

3.3. Chemical Composition of EOof *R.montana* from the second station (P2)

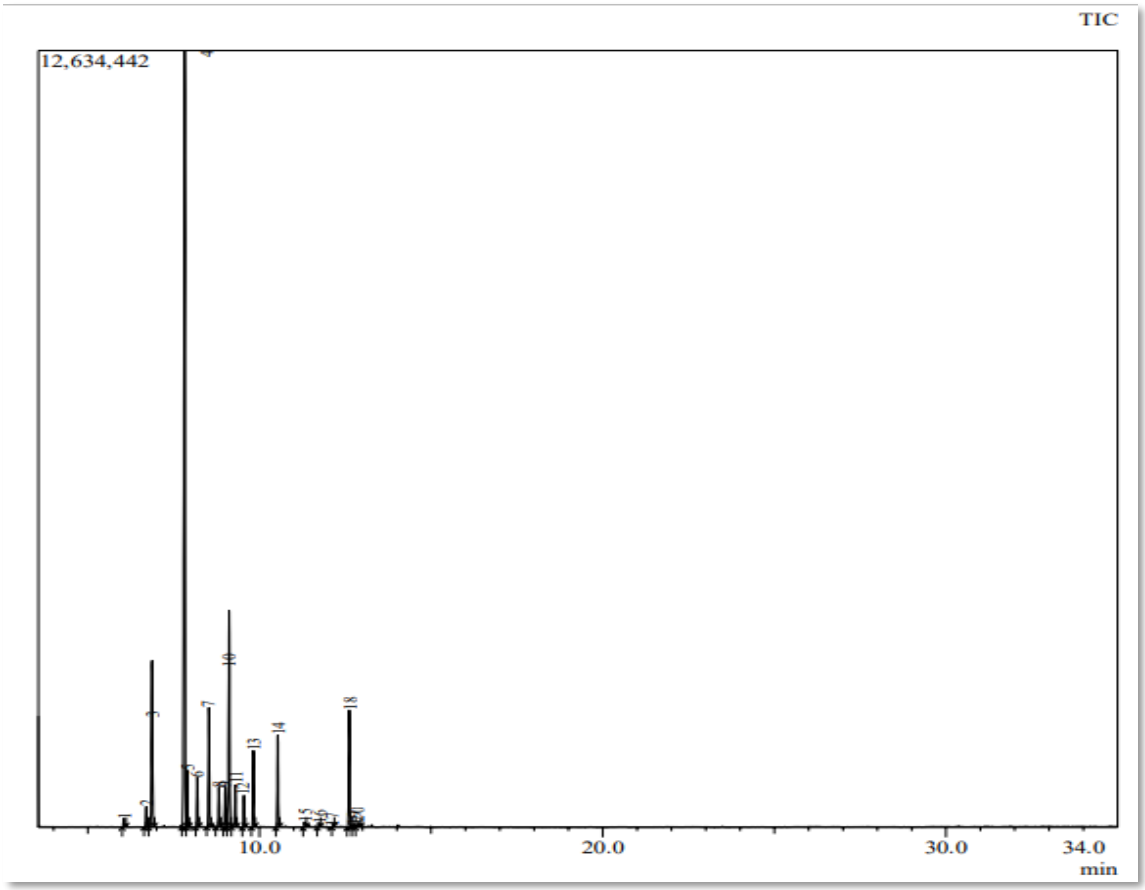


Fig 42:GC-MS EO Chromatogram of *R.montana*(P2) EO (Abundance as a function of time in min).

The analysis of *R.montana* EO (P2) by gas chromatography coupled with mass spectrometry revealed the identification of 20 compounds representing 91.38% of all oils. The percentage and retention time of identified constituents are presented in Table 12.

Table 12: Chemical composition of the essential oil of *R.montana*(P2): retention time (TR) and concentrations (%) of the various constituents.

N	**Compounds**	**Retention Time (min)**	**Concentrations (%)**
1	**Nonane**	6.047	0.465
2	**alpha-Phellandrene**	6.696	1.082
3	**(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene**	6.864	9.137
4	**(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene**	7.820	45.353
5	**beta-Pinene**	7.891	2.481
6	**beta-Pinene**	8.184	2.221
7	**alpha-Phellandrene**	8.524	5.829
8	**Cyclohexene, 1-methyl-4-(1-methylethylidene)-**	8.813	1.945
9	**Benzene, 1-methyl-3-(1-methylethyl)-**	8.999	1.996
10	**Limonene**	9.113	11.341
11	**(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene**	9.292	1.926
12	**1,3,7-Octatriene, 3,7-dimethyl**	9.542	1.451
13	**gamma-Terpinene**	9.821	3.633
14	**Cyclohexene, 1-methyl-4-(1-methylethylidene)-**	10.529	4.341
15	**Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-**	11.311	0.235
16	**Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-**	11.724	0.202
17	**2-Methyl-1-nonene-3-yne**	12.139	0.064
18	**Terpinen-4-ol**	12.614	5.962
19	**Benzenemethanol, .alpha.,.alpha.,4-trimethyl**	12.753	0.124
20	**L-alpha-Terpineol**	12.899	0.212
	Total		100.000

EO of *R.montana* (P2) consists of 17 compounds, 91.38% terpene derivatives, corresponding to monoterpenes while the rest 8.62% represents other chemical components (alkane, cymene, organic and aromatic compounds).

Four monoterpenes were identified as the major compounds:(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene 54.49% , Limonene 11.34 %, alpha-Phellandrene 5.829 and Cyclohexene, 1-methyl-4-(1-methylethylidene) 4.341 %. These compounds represent 71,66 % of the total oil.

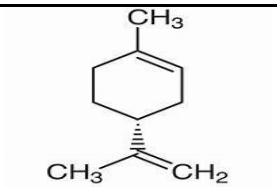
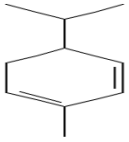
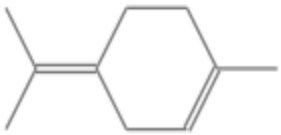
(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	**Limonene**	**alpha-Phellandrene**	**Cyclohexene, 1-methyl-4-(1-methylethylidene)**
			

Fig 43: Chemical structure of some major compounds of *R.montana* EO from the second station (P2).

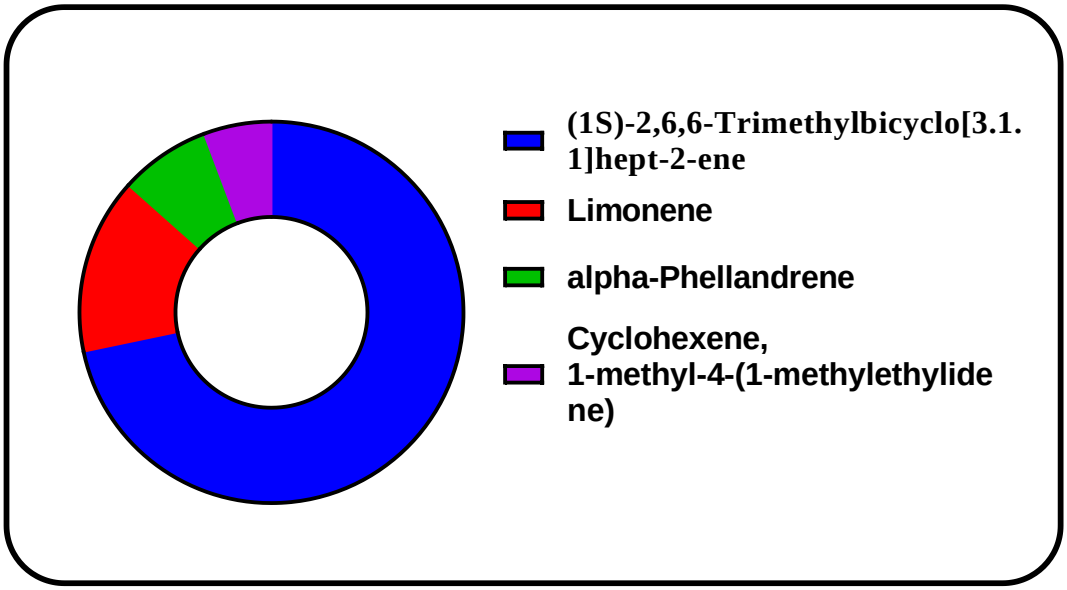


Fig 44: Distribution of major EO plant componentfrom the second station (P2).

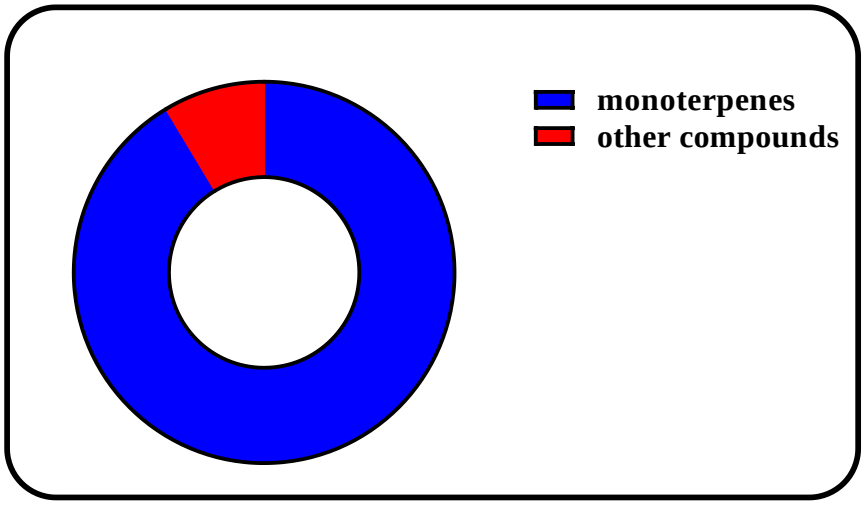


Fig 45: Chemical composant of*R.montana* EO from the second station (P2).

3.4.Antioxidant activities of *R.montana* Essential oil:

3.4.1.Free radical scavenging assay by DPPH

The antioxidant activities obtained by the DPPH method for EO extracted from the two plants *R.montana* are presented as IC50 in Fig.48.The analysis of variance revealed significant differences between the two Eos extracted. This activity was compared with that of ascorbic acid as a synthetic antioxidant. The results revealed that EO extracted from the first plant *R.montana* (P1) with an IC_{50} value of 22.58 (13.91 ± 32.25) µL/mL is an effective scavenger than EO extracted from the second plant (P2) *R.montana* and the ascorbic acid with an IC50 value of 41.96 (33.74 ± 51.32) and 1.501 (0.5061 ± 1.729) µL/mL respectively.

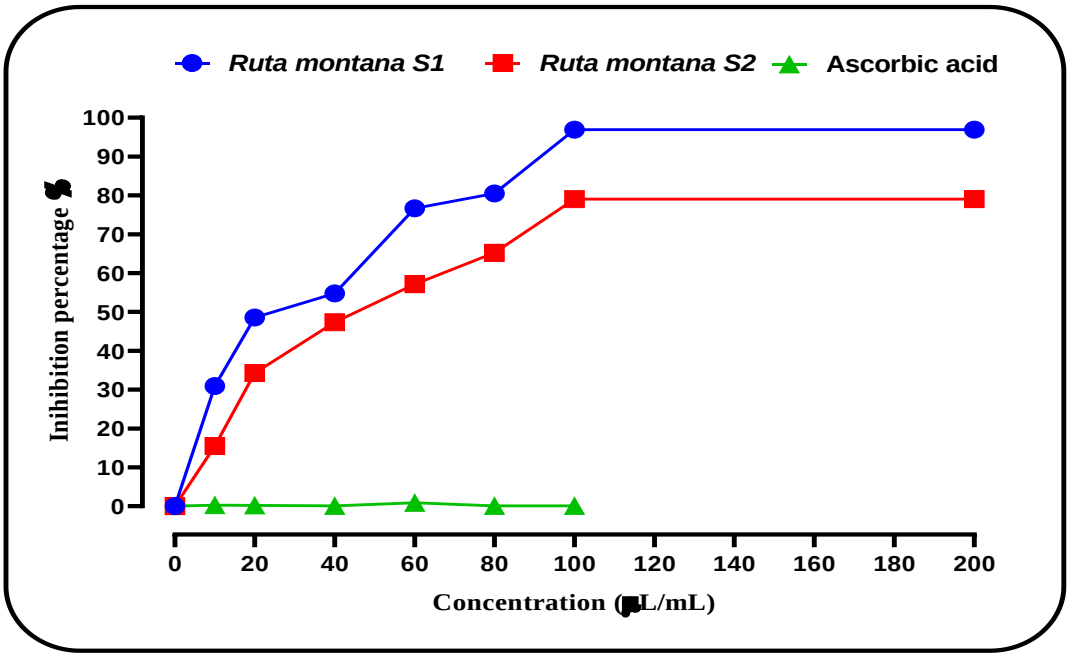


Fig 46: The IC50 values in the DPPH radical scavenging activity assay of the *R.montana* EO. Ascorbic acid was used as reference antioxidant.

3.4.2. Reducing power of iron (FRAP):

Linearity of FRAP (dose–response line) for standard solutions is shown in Fig 49. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability. The results of the FRAP assay are reported in figure 49.

There were notable variations between the two extracted Eos, according to the analysis of variance.This activity was compared with that of ascorbic acid as a synthetic antioxidant. The results revealed that EO extracted from the first plant *R.montana* (P1) with an IC_{50} value of 1.494 (1.120 ±1.687) µL/mL higher antioxidant activity than the second EO extracted from the second plant (P2) *R.montana* and the ascorbic acid with an IC50 value of 2.254 (1.801 ±1.876) and 1.501 (0.5061 ± 1.729) µL/mL respectively.

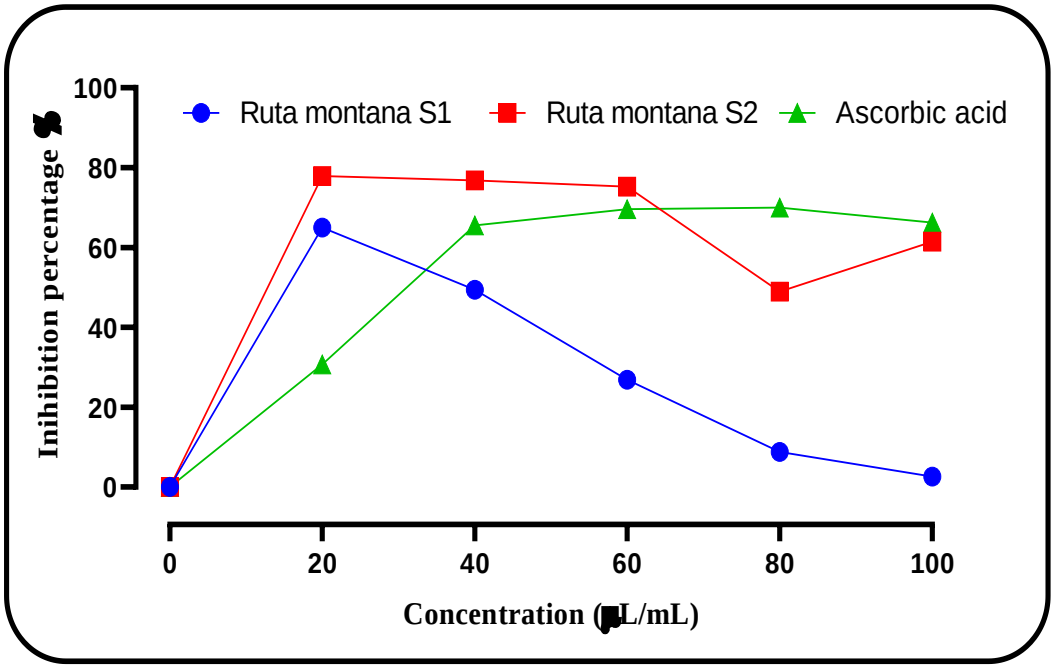


Fig 47: The IC50 values in the FRAP activity assay of the EO. Ascorbic acid was used as reference antioxidant.

3.4.3. ABTS decolorization assay of *R.montana*'s EO

The results of ABTS of the extracts are compared with TROLOX. Results were presented as IC50 (Fig 50). The analysis of variance revealed significant differences between Eos of the two plants *R.montana*. The results revealed that EO extracted from (P1) has high scavenging ability of the ABTS radical with an IC50 value of 46.14 (36.66 ± 56.44) µL/mL effective scavenger than the EO of second plant (P2) and TROLOX with an IC50 value of 61.73 (1.676 ± 1.907) and 74.49 (1.722 ± 2.050) µL/mL respectively.

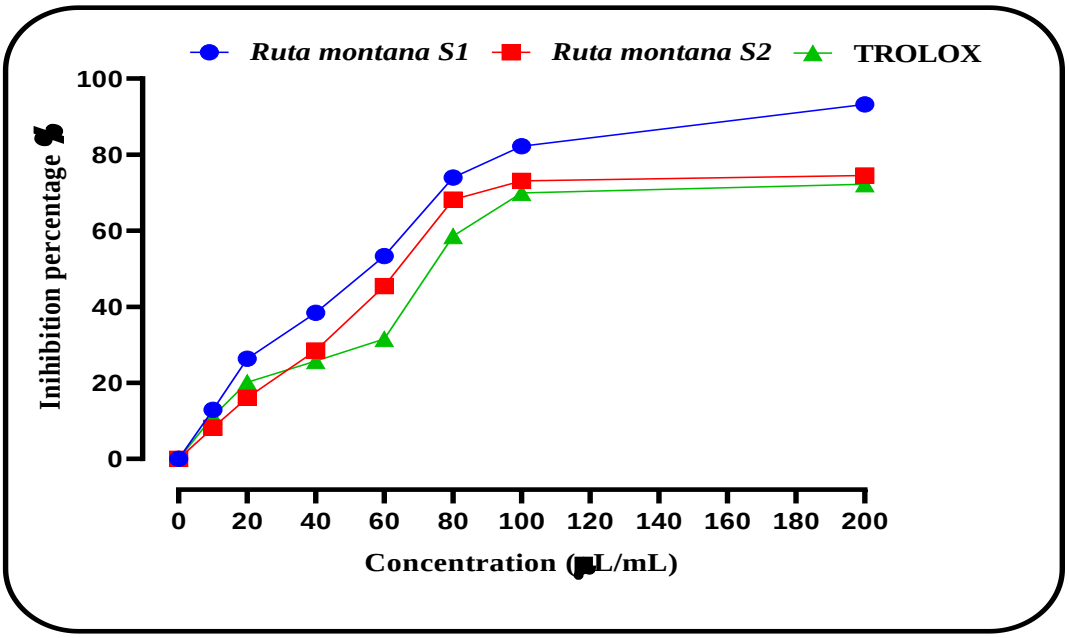


Fig 48: The IC50 values in the ABTS activity assay of the EOs. TROLOX was used as reference antioxidant

3.5. Essential oil insecticide testing for *D.melanogaster*

3.5.1. Larvicidal activity:

After a screening test, different concentrations of *R.montana* EO were applied to *D.melanogaster*over the three larval stagesL1, L2 and L3: from the lowest concentration 1µL/mL to the highest concentration 7 µL/mL for the first stage larveae, from the lowest concentration 1µL/mL to the highest concentration 9 µL/mL for the second stage larveae and from the lowest concentration 3µL/mL to the highest concentration 12 µL/mL for the third stage larveae. Negative control (water only) and positive control (water+1ml) ethanol) are produced in parallel. Among the two series witnesses, there was no mortality.

a. Larvicidal activity against larvae of the first stage of *D.melanogaster*

After a screening test, different concentrations of *R.montana* EO were applied to larvae of the first stage of *D.melanogaster* (1, 2, 3, 5 and 7 µL/mL). Negative control series (water only) and positive control series (water + 1ml ethanol) are performed in parallel. No mortality was observed in the two control series.

The corrected mortalities recorded during toxicity tests vary from5.83± 4.92 %at the lowest concentration, (1µL/mL)and 100.00±0.00% at the highest concentration (7µL/mL) in*D.melanogaster*.

The results show a higher mortality in *D. melanogaster* reflecting a greater sensitivity of this species to the action of the *R.montana* EO.

Analysis of variance to a classification criterion after angular transformation of mortality percentages reveals a highly significant difference ($p<0.001$)(table 13) between the concentrations used and this in *D. melanogaster*. The concentration-response curve expressing the percentages of mortality as a function of the applied concentrations (Fig 51 and 52) allowed the estimation of the values of the different lethal concentrations (LC) as well as their confidence intervals and the Slope (Table 14).

Table 13: Toxicity of *R.montana* EO (ppm), applied to 1st stage larvae of *D. melanogaster*: Corrected mortality (%) (m SD, n = 6 repetitions each with 20 individuals): Analysis of variance to a classification criterion after angular transformation of recorded mortalities (%).

Source of variation	SCE	Ddl	CM	Fobs	P
Treatement	50161.8	5	10032.4	370.43	0,000****
residualerror	812.5	30	27.1		
Total	50974.3	35			

****highly significant difference ($p<0.001$). SCE: sum of squares of deviations; ddl: degree of freedom; CM: mean square; Fobs: F observed; p: level of significance.*

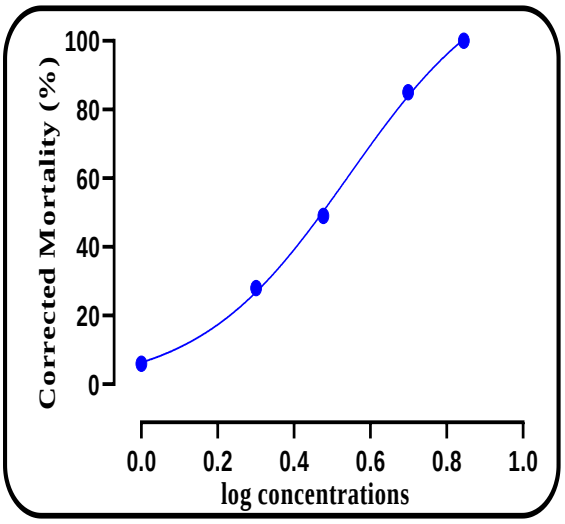


Fig 49: Effects of *R.montana* EO applied to 1st stage larvae of *D.melanogaster*: Concentration--response curve expressing percentage of mortality corrected for logarithm of concentration.

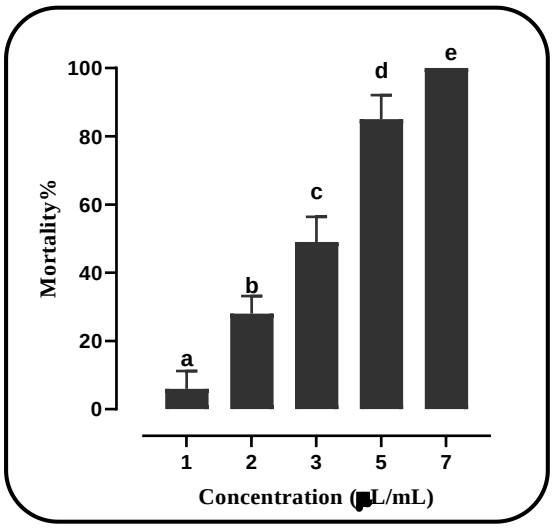


Fig 50: Effects of *R.montana* applied to 1st stage larvae of *D.melanogaster*: Concentration-response curve expressing percentage of mortality corrected for applied concentrations.(m±SD, n = 6 repeats each test)

EO of *R.montana* was applied to the concentration 1st stage larvae lethal (which cause mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 2.006, LC 50 = 2.871, LC 90 = 5.882 and a Slope of 3.064 (Table 14).

Table 14: Toxicity of *R.montana* EO applied to *D. melanogaster* 1st stage larvae: Determination of lethal doses and their confidence intervals (95%).

Stage	Hill slop	R^2	LC25 CI (95%)	LC50 CI (95%)	LC90 CI (95%)
L1	3.06	0.988	2.006 (1.548-2.453)	2.871 (2.457-3.328)	5.882 (4.423-8.402)

b. Larvicidal activity against larvae of the second stage of *D.melanogaster*

After a screening test, different concentrations of *R.montana* EO were applied to larvae of the second stage of *D.melanogaster* (1, 3, 5, 7 and 9µL/mL). Negative control series (water only) and positive control series (water + 1ml ethanol) are performed in parallel. No mortality was observed in the two control series.

The corrected mortalities recorded during toxicity tests vary from 10.83± 5.85 % at the lowest concentration, (1µL/mL)and 91.97 ± 4.08 % at the highest concentration (9µL/mL) in*D.melanogaster*.

The findings indicate a higher mortality rate in *D. melanogaster*, which suggests that this species is more susceptible to the effects of *R. montana* EO.

Analysis of variance to a classification criterion after angular transformation of mortality percentages reveals a highly significant difference ($p<0.001$)(table15 and 16)between the concentrations used and this in *D. melanogaster*. The concentration-response curve expressing the percentages of mortality as a function of the applied concentrations (Fig 53 and 54) allowed the estimation of the values of the different lethal concentrations (LC) as well as their confidence intervals and the Slope (Table 15).

Table 15: Toxicity of *R.montana* EO (µL/mL), applied to 2nd stage larvae of *D. melanogaster*: Corrected mortality (%) (mSD, n = 6 repetitions each with 20 individuals) : Analysis of variance to a classification criterion after angular transformation of recorded mortalities (%).

Source of variation	SCE	Ddl	CM	Fobs	P
Treatement	39861.8	5	7972.4	244.26	0,000****
residualerror	979.2	30	32.6		
Total	40841.0	35			

****highly significant difference (p<0.001). SCE: sum of squares of deviations; ddl: degree of freedom; CM: mean square; Fobs: F observed; p: level of significance.*

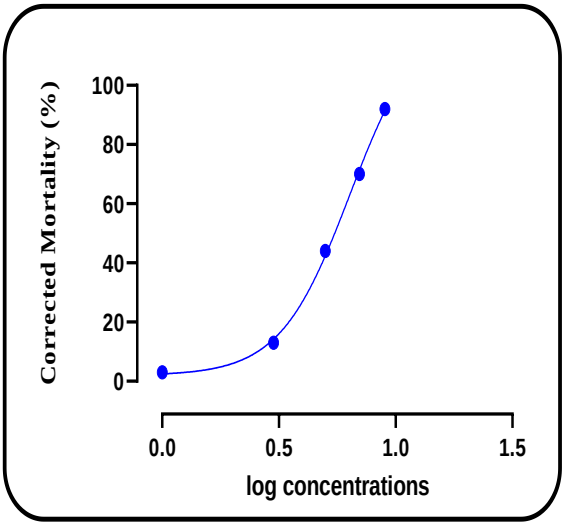


Fig 51: Effects of *R.montana* EO applied to 2nd stage larvae of *D. melanogaster*: concentration-response curve expressing percentage of mortality corrected for logarithm of doses.

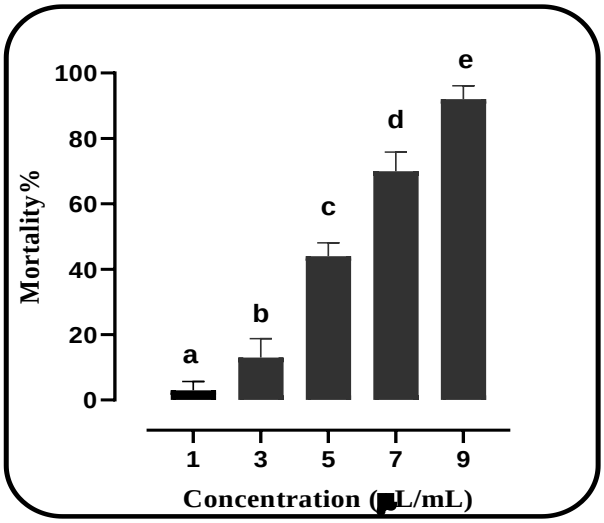


Fig 52: Effects of *R.montana* applied to 2nd stage larvae of *D. melanogaster*: concentration-response curve expressing percentage of mortality corrected for applied concentrations. (m±SD, n = 6 repeats each test)

EO of *R.montana* was applied to the concentration 2nd stage larvae lethal (which cause mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 3.931, LC 50 = 5.315, LC 90 = 9.717 and a Slope of 3.642 (Table 16).

Table 16: Toxicity of *R.montana* EO applied to *D. melanogaster*2ndstage larvae: Determination of lethal doses and their confidence intervals (95%).

Stage	Hill slop	R^2	LC25 CI (95%)	LC50 CI (95%)	LC90 CI (95%)
L2	3.64	0.992	3.931 (3.259-4.567)	5.315 (4.773-5.854)	9.717 (8.072-12.41)

c. Larvicidal activity against larvae of the third stage of *D.melanogaster*

After a screening test, different concentrations of *R.montana* EO were applied to larvae of the second stage of *D.melanogaster* (3, 5, 7, 9 and 12µL/mL). Negative control series (water only) and positive control series (water + 1ml ethanol) are performed in parallel. No mortality was observed in the two control series.

The corrected mortalities recorded during toxicity tests vary from 6.67 ± 4.08 % at the lowest concentration (3µL/mL)and 95.83 ± 3.76 % at the highest concentration (12µL/mL) in*D.melanogaster*.

Based on the data, it can be concluded that *D.melanogaster* is more susceptible to the effects of *R.montana* EO due to a higher death rate.

Analysis of variance to a classification criterion after angular transformation of mortality percentages reveals a highly significant difference ($p<0.001$) (table 17)between the concentrations used and this in *D. melanogaster*. The concentration-response curve expressing the percentages of mortality as a function of the applied concentrations (Fig 55 and 66) allowed the estimation of the values of the different lethal concentrations (LC) as well as their confidence intervals and the Slope (Table18).

Table 17: Toxicity of *R.montana* EO (µL/mL), applied to 3rd stage larvae of *D. melanogaster*: Corrected mortality (%) (m±SD, n = 6 repetitions each with 20 individuals): Analysis of variance to a classification criterion after angular transformation of recorded mortalities (%).

Source of variation	SCE	Ddl	CM	Fobs	P
Treatement	40120.1	5	8024.0	276.43	0,000****
residualerror	870.8	30	29.0		
Total	40991.0	35			

****highly significant difference (p<0.001). SCE: sum of squares of deviations; ddl: degree of freedom; CM: mean square; Fobs: F observed; p: level of significance.*

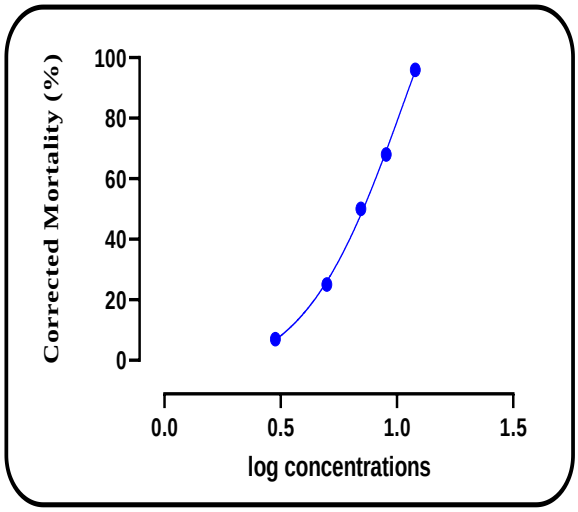


Fig 53: Effects of *R.montana* EO applied to 3rd stage larvae of *D. melanogaster*: concentration-response curve expressing percentage of mortality corrected for logarithm of doses.

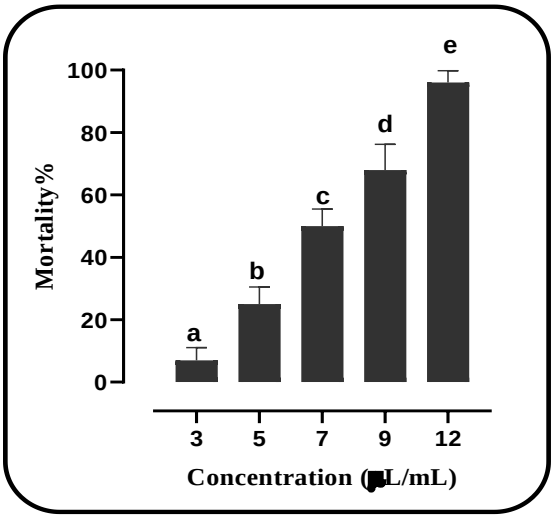


Fig 54: Effects of *R.montana*EO applied to 3rd stage larvae of *D. melanogaster*:concentration-response curve expressing percentage of mortality corrected for applied concentrations.(m±SD, n = 6 repeats each test)

EO of *R.montana* was applied to the concentration 3rd stage larvae lethal (which cause mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 5.128, LC 50 = 6.912, LC 90 = 12.56 and a Slope of 3.68.

Table 18: Toxicity of *R.montana* EO applied to *D. melanogaster*3rdstage larvae: Determination of lethal concentration and their confidence intervals (95%).

Stage	Hill slop	R^2	LC25 CI (95%)	LC50 CI (95%)	LC90 CI (95%)
L3	3.68	0.98	5.128 (4.025-6.144)	6.912 (6.033-7.811)	12.56 (9.949-17.75)

3.5.2.Pupicidalactivity

After a screening test,different concentrations of *R.montana* EO were applied to the *D.melanogaster*pupae (4, 5, 9, 12 and 15 µL/mL). Negative control (water only) and positive control (water+1ml) ethanol) are produced in parallel. Among the two series witnesses, there was no mortality.The results show a higher mortality in *D. melanogaster* reflecting a greater sensitivity of this species to the action of the *R.montana* EO.

Analysis of variance to a classification criterion after angular transformation of mortality percentages reveals a highly significant difference ($p<0.001$) (table 19)between the concentrations used and this in *D. melanogaster*. The classification of concentration by the Tukey HSD test.

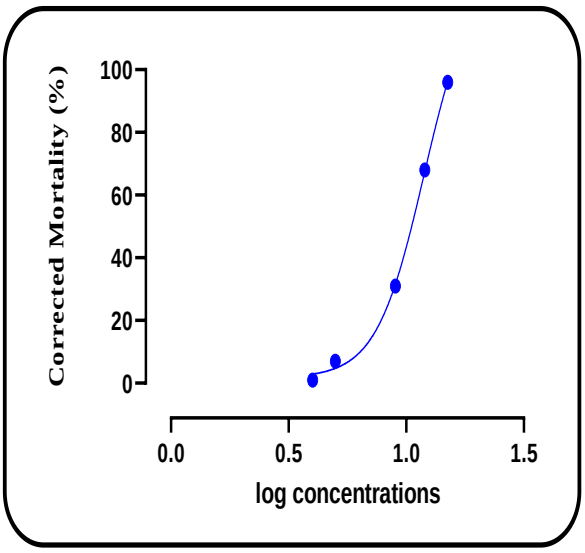


Fig 55: Effects of *R.montana* EO on pupae of *D.melanogaster*: Concentration-response curve expressing percentage of mortality corrected for logarithm of doses.

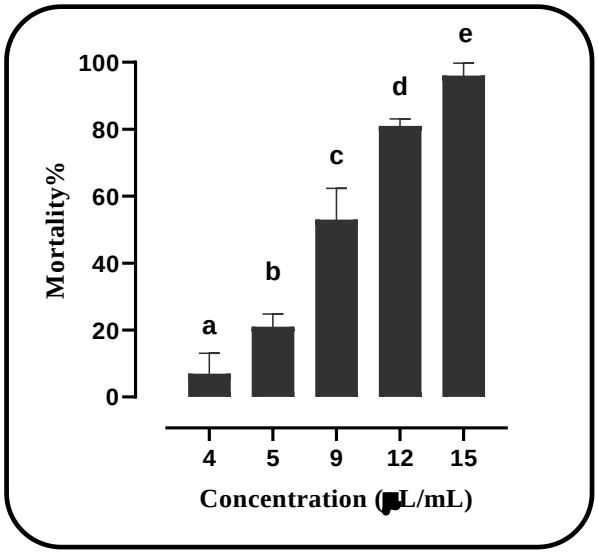


Fig 56: Effects of *R.montana* EO on *D.melanogaster* pupae: Concentration-response curve expressing percentage of mortality corrected for applied concentrations.(m±SD, n = 6 repeats each)

EO of *R.montana* was applied to the concentration pupae lethal (which causes mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 8.711, LC 50 = 10.36, LC 90 = 14.68 and a Slope of 6.318 (Table 20).

Table 19: Toxicity of *R.montana* EO (µL/mL), applied to the *D.melanogaster*pupae: Corrected mortality (%) (m±SD, n = 6 repetitions each with 20 individuals): Analysis of variance to a classification criterion after angular transformation of recorded mortalities (%).

Source of variation	SCE	Ddl	CM	Fobs	P
Treatement	40351.8	5	10082.4	375.23	0,000****
residualerror	833.8	30	29.1		
Total	41185.6	35			

Table 20: Toxicity of *R.montana*EO applied to *D. melanogaster*pupae: Determination of lethal doses and their confidence intervals

(95%).

Stage	Hill slop	R^2	LC25 CI (95%)	LC50 CI (95%)	LC90 CI (95%)
Pupae	6.32	0.993	8.711 (7.985-9.435)	10.36 (9.754-11.01)	14.68 (13.15-16.72)

3.5.3. Adulticidalactivity

After a screening test, different concentrations of *R.montana* EO were applied by fumigation on male and female adults of *D.melanogaster* (0.2, 0.4, 0.8, 1.6, 2.4, 4.2 and 5 µL/Lair).*R.montana* EO elicited significant (P<0.001) fumigant activity against *D. melanoaster*. Control series are performed. Negative control series for fumigation and positive control (ethanol) for ingestion are performed in parallel. No mortality was observed in the control series, so the solvent was not toxic to adult *D.melanogaster*.

The corrected mortalities recorded during fumigation toxicity tests vary from 0.09 % at 24 h up to 0.52 % at the lowest concentration (0.2µL/mL) and 2.85 % at 24h up to 14.56 % for the highest concentration (5µL/mL). These mortalities increase significantly depending on the concentration applied and the time after treatment in *D.melanogaster* treated by fumigation at 24 h after treatment.The results show that EO applied by fumigation and ingestion exerts an insecticide activity with a concentration-response relationship for *D.melanogaster*. The concentration-response curve expressing the percentage of deaths as a function of the logarithm applied concentrations(Fig 59) allowed the estimation of lethal concentrations (LC) as well as their confidence intervals and the Slope (Table 21).Mortality rate after 24 hours of *R.montana* EO exposure and susceptibility status against adults are shown in Fig 60.

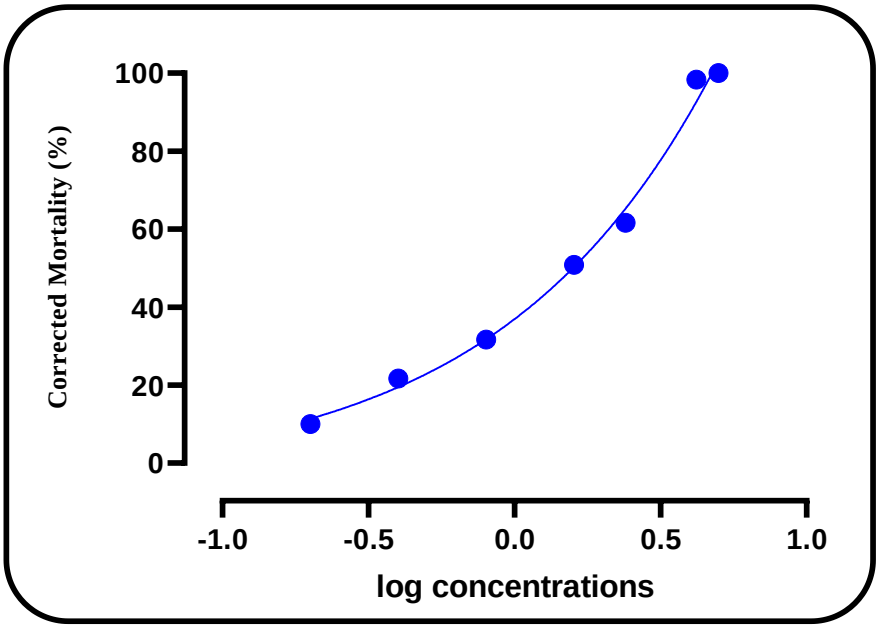


Fig 57: Effects of *R.montana* EO on adults of *D.melanogaster*: concentration-response curve expressing percentage of mortality corrected for logarithm of concentrations.

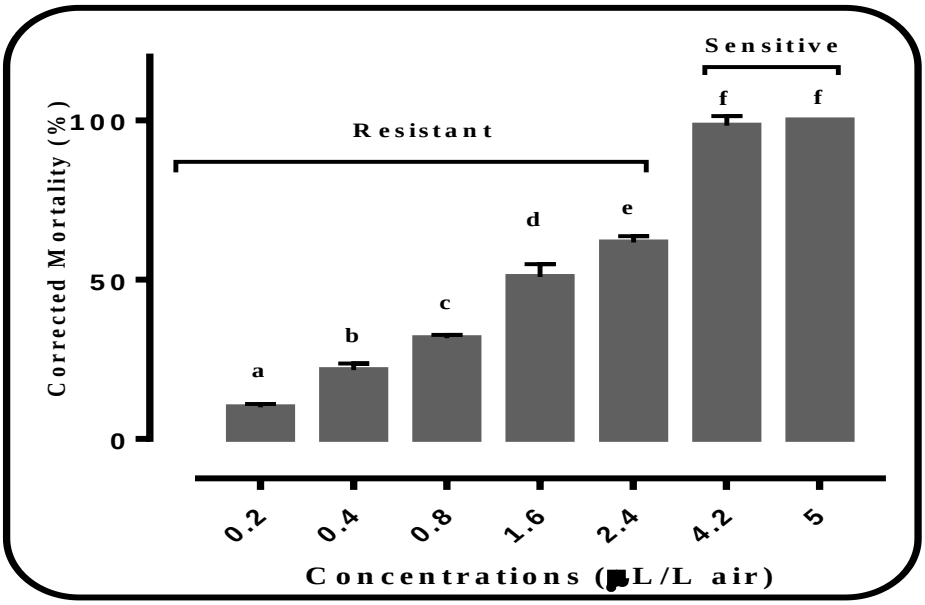


Fig 58: Efficiency of EO of *R.montana* applied on adults of *D.melanogaster*: corrected mortality (m±SD, n = 6 repeats each)

With rising concentration, *R.montana* EO's effectiveness rose noticeably. At a concentration of 5 µL/Lair, 100% of the adults died after receiving treatment with *R.montana* EO for 24 hours.Consequently, the results of Table 2 indicate that the biological experiments

demonstrated the significant adulticidal action of EO of *R.montana*, with an LC50 value of 1.31µL/Lair.

Table 21: Toxicity of essential oils of *R.montana*applied on adults of *D. melanoaster:* Determination of Lethal and sublethal concentrations (µL/Lair).

Time (hours)	R^2	Hill Slope	LC25 95% FL	LC50 95% FL	LC90 95% FL
24	0.932	1.56	0.649 (0.49 to 1.21)	1.311 (0.83 to 1.93)	5.36 (2.85 to 14.56)

Estimation of lethal concentrations (LC) and their confidence intervals and HillSlope (Table 8) was made by the PRISM GRAPH PAD 7.

3.6. Impact of *R.montana*'s Essential oil on growth

After the application of LC 25 and LC 50 on adults, the body weight was noted 24, 48, and 72 hours following EO exposure. Moreover, in *D. melanogaster* adults, the results mentioned in figures 61, 62 and 63show that the tow both concentrations of *R.montana* EO (LC25 and LC50) induces a significant decrease in body weight adults during the development of *D.melanogaster* by comparing to control series.

In addition, it is noted that the weight of adults *D.melanogaster* treated with LC50 and exposed 72 h to the EO are the most affected compared to the control series and other treated.

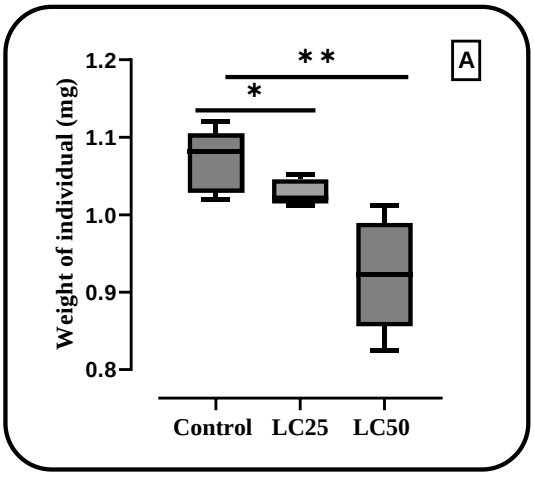


Fig 59 : Evolution of adult weight (mg) during the development of *D.melanogaster*24 h(m± SD; n = 5; for the same age, the averages followed by the same letter are not significantly different at p> 0.05)

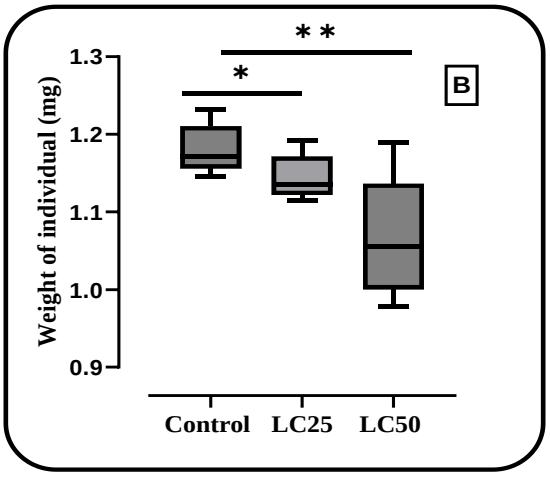


Fig 60 : Evolution of adult weight (mg) during the development of *D. melanogaster* 48 h (m± SD; n = 5; for the same age, the averages followed by the same letter are not significantly different at p> 0.05)

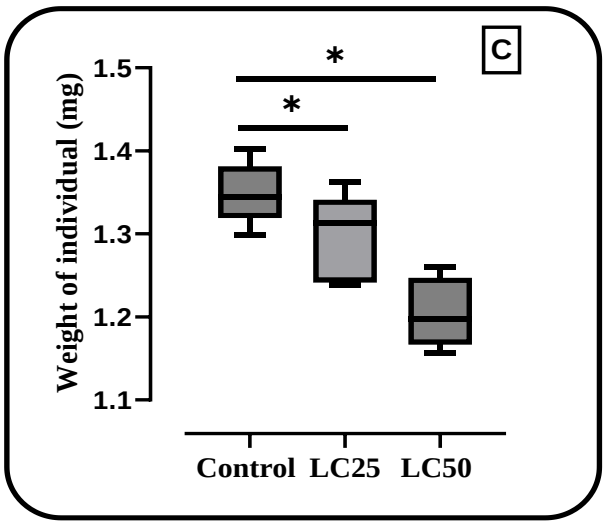


Fig 61: Evolution of adult weight (mg) during the development of *D. melanogaster* 72 h (m± SD; n = 5; for the same age, the averages followed by the same letter are not significantly different at p> 0.05)

3.7. Effect on development time

On recently enclosed adults of *D. melanogaster*, the EO of *R.montana* LC25 and LC50, 0.6 and 1.3 µL/Lair, respectively, was utilised to assess the length of development. The same concentrations are given to adults. Two subsequent generations' life cycles are evaluated in terms of their duration, compared with the control series (Fig 64, 65 and 66.). The results of the student's test indicate that the life cycle period for the first generation is considerably

increased by the two concentrations of *R. montana* EO (LC25: p = 0.020; LC50: p = 0.013) (LC25: p = 0.64 and LC50: 1.31 µL/1 Lair), respectively. For generation 2 (LC25: p = 0.013 and LC50: p = 0.004) and generation 3 (LC25: p = 0.009 and LC50: p = 0.002), the same observation was conducted during the second and third generations' cycle duration.

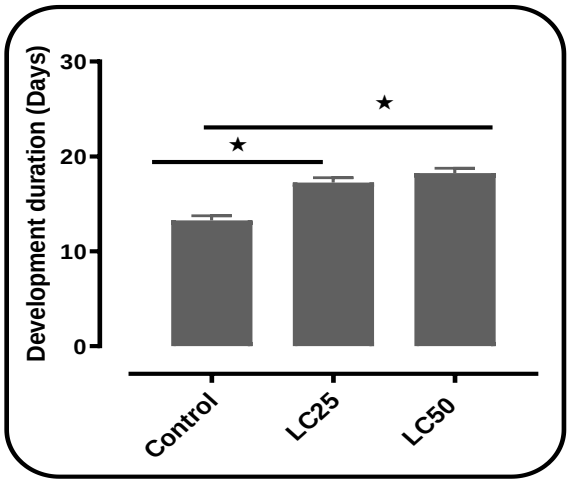


Fig 62: Effects of *R.montana* EO (LC25, LC50) ondevelopment duration of the first generation of *D. melanogaster*

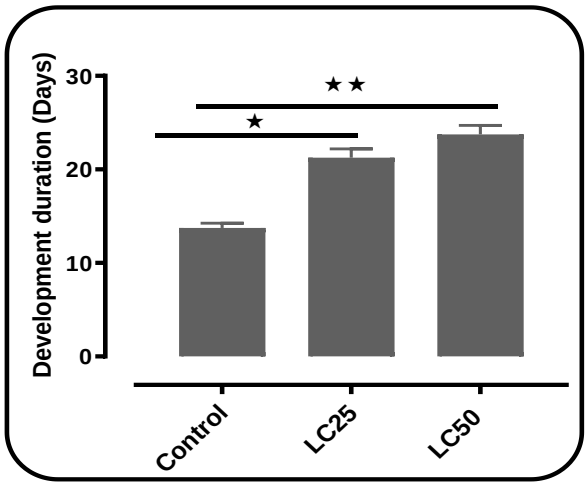


Fig 63: Effects of *R.montana* EO (LC25, LC50) ondevelopment duration of the second generation (B) of *D. melanogaster*

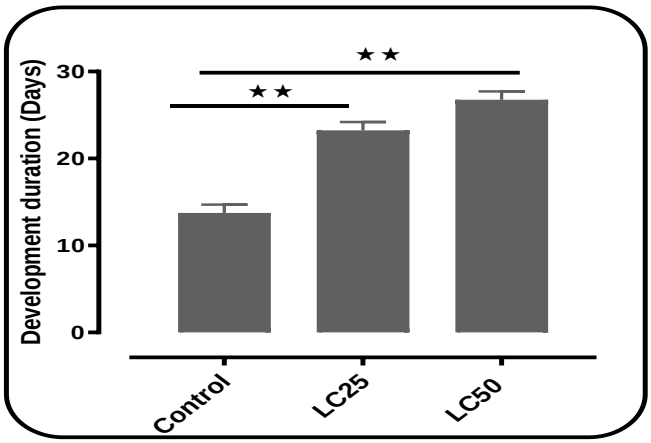


Fig 64: Effects of *R.montana* EO (LC25, LC50) ondevelopment duration of the third generation of *D. melanogaster*

3.8. Response time (LT50) bioassay against *D. melanogaster*

Once adult *D. melanogaster* flies were exposed to the highest concentration (5µL/Lair) using the fumigation method outlined, their survival was measured at 1, 3, 6, 12, 16, 20, and 24 hours later. We found that there was a higher mortality rate (Table 22). The data in Table 23 indicates that the lethal time (LT50) at which 50% mortality occurs is 9.25 hours, with a confidence interval ranging from 8.739 to 12.11 hours.

Table 22: Time-dependent mortality of *D. melanogaster* treated with EO of *R.montana* at 5 µL/L air.

Time (hours)	**1**	**3**	**6**	**12**	**16**	**20**	**24**
mortality%	5	13.33	31.67	53.33	71.67	90	100
ANOVA P value				**0.000**			

Table 23: Response time (LT10, LT25 and LT50) bioassay against *D. melanogaster*.

Response time	**R2**	**Hill slope**	**LT10 (95% FL)**	**LT25 (95% FL)**	**LT50 (95% FL)**
D. melanogaster	0.96	2.12	3.27 (1.40 to3.41)	5.50 (3.98 to 7.69)	9.25 (8.74 to 12.11)

3.9. Effect of *R.montana*'s Essential oil on sex and sex ratio

The sex ratio observed in *D.melanogaster* adults during 3 successive generations from adults treated with two concentrations LC25 and LC50 of the essential oil of *R.montana* (FIG. 5).Comparison of the means with the control series showed that the EO of *R. montana* at both concentrations revealed a significant effect with an increase in the number of adult females and a significant decrease in the number of males compared to the controls for the first generation (LC25; p=0.004, LC50; p=0.001), for the second generation (LC25; p=0.002, LC50; p=0.001), and the third generation with low concentration LC25p=0.000.

Table 24 shows the sex ratios of adults created in the laboratory under treatment with two concentrations LC25 and LC50. The sex ratio differed significantly between the control series and the treated series.

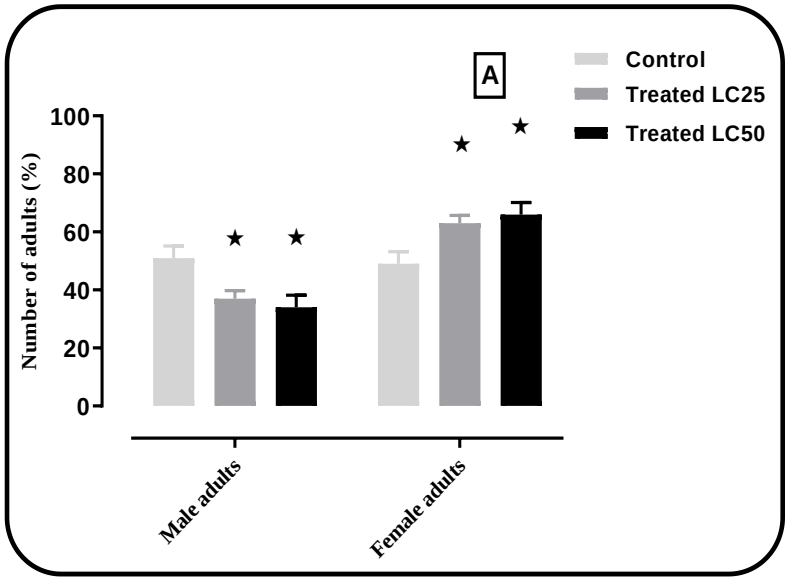


Fig 65: Effect of *R.montana*EO (LC_{25} and LC_{50}) on sex in adult's % of the first generation (A) of *D. melanogaster.*

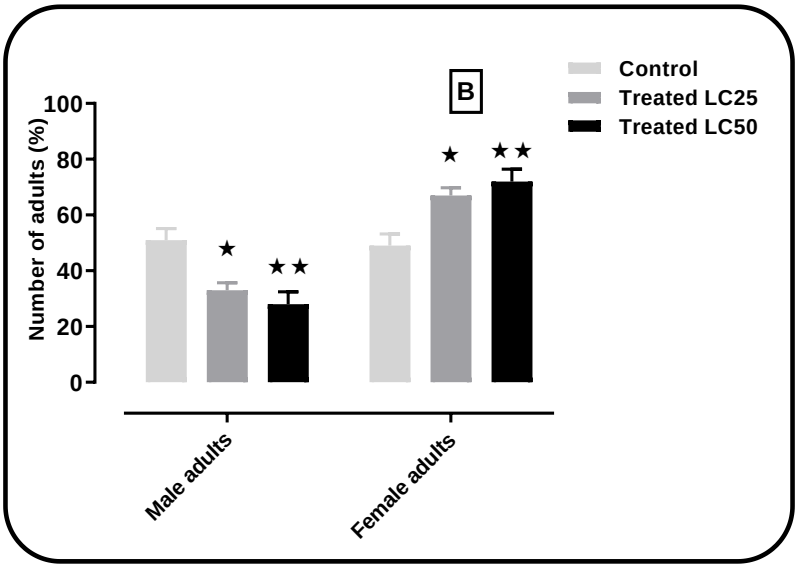


Fig 66: Effect of *R. montana*essential oil (LC_{25} and LC_{50}) on sex in adults % of the second generation (B) of *D. melanogaster*

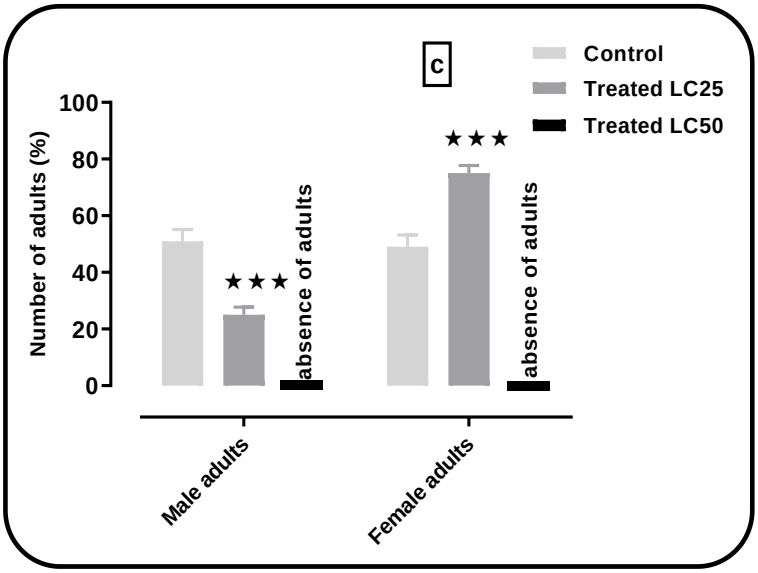


Fig 67: Effect of *R. montana*EO (LC_{25} and LC_{50}) on sex in adults % of the third generation(C) of *D. melanogaster*

Tabel 24: Sex ratio *of D. melanogaster*adults treated with *R.montana*.

Generation	Treatment	Sex ratio (M/F)	P value
G1	Control	1.05 ±0.17a	_
	LC25	0.59 ±0.07b	0.003
	LC50	0.52 ±0.09c	0.002
G2	Control	0.97 ±0.16a	_
	LC25	0.49± 0.06b	0.005
	LC50	0.39± 0.09c	0.002
G3	Control	0.96± 0.09a	_
	LC25	0.33± 0.06b	0.001
	LC50	/	_

Same letter on the same column means there is no significant difference.

3.10. Negative geotaxis behavior assay

Damage to the locomotor capacity of flies was recorded at two concentrations (LC25 and LC50) five hours of recording (1,3,6,12,24 hours). It was also noted that the first and second generations of treated flies exhibited a significant degree of motor disability, as shown in Fig 70, and that the tow concentrations applied after 24 hours were effective in significantly reducing locomotor ability during the times studied compared to the control.

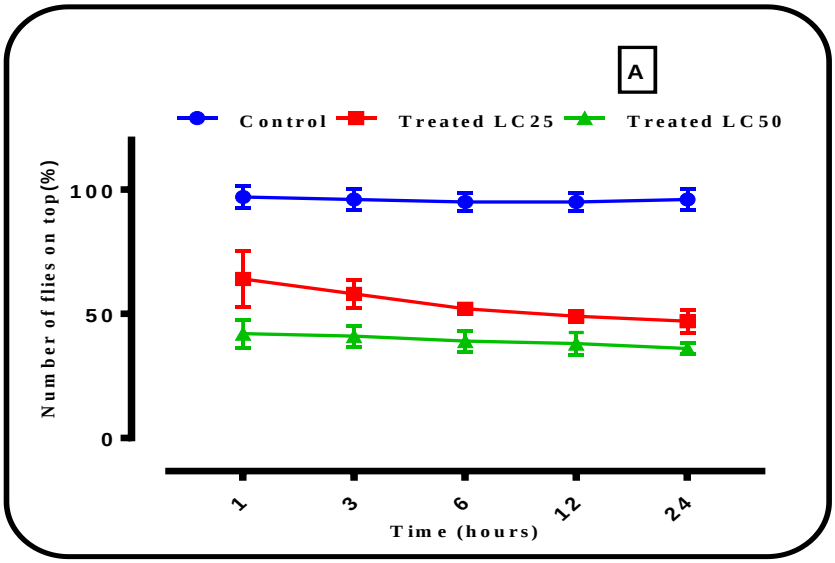


Fig 68: Effect of the *R. montana* essential oil on the locomotor ability of *D. melanogaster*, after 24h for the first generation (A).

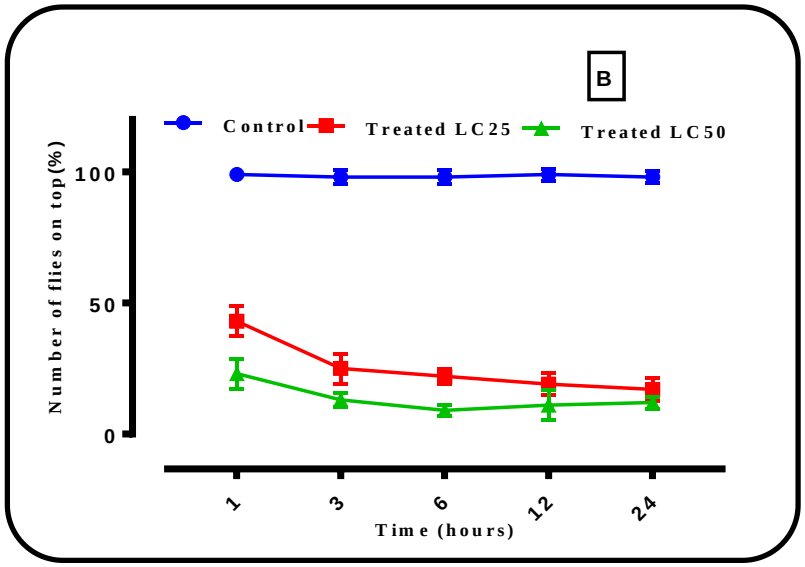


Fig 69: Effect of the *R. montana* essential oil on the locomotor ability of *D. melanogaster*, after 24h for the second generation (B).

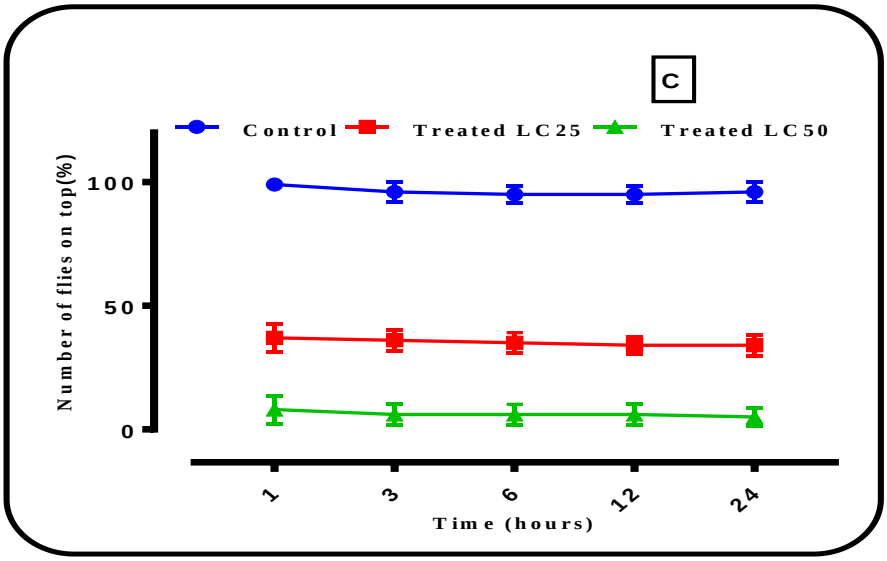


Fig 70: Effect of the *R. montana* essential oil on the locomotor ability of *D. melanogaster*, after 24h for the third generation (C).

3.11. Effect on the specific activity of glutathione S-transferases (GSTs)

The results of the specific activity of glutathione S-transferases (GSTs) are expressed innanomoles per minute per milligram of protein (µM/min/mg of protein). They arementioned in figure73.In *D.melanogaster*, the results show a significant decrease in activity Specific GSTs at 24 hours. The Tukey HSD test indicates a relationship dose-response at all times.From these data, it can be concluded that *R.montana*EO most affects the activity GSTs specific in adults of *D.melanogaster*.

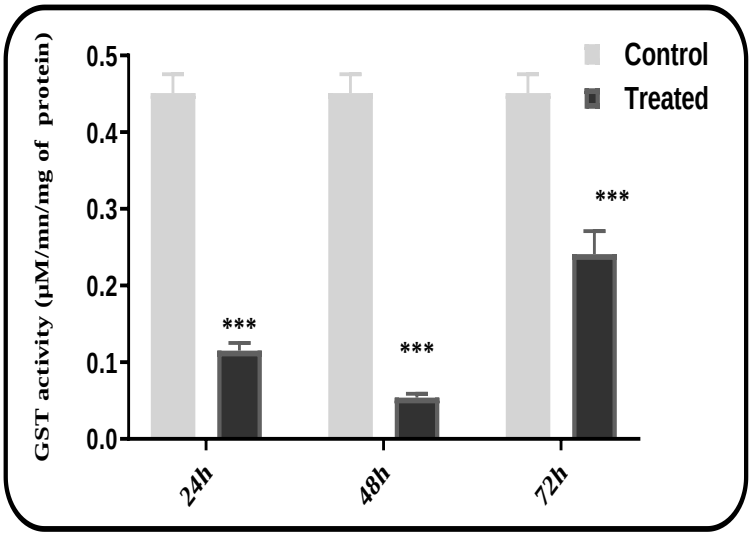


Fig 71: Effect of *R. montana* EO (LC50) on GSTs specific activity (µM/min/mg protein) in adults of*D.melanogaster* comparing to the control series (m SEM, n=6 repetitions each with 20 individuals): Tukey HSD test

3.12. Effect on the specific activity of catalase

Almost all living things exposed to the atmosphere contain the ubiquitous antioxidant enzyme catalase (CAT), which is involved in breaking down damaging hydrogen peroxide into oxygen and water.

The specific activity of catalase (µM/min/mg protein) in adults of *D.melanogaster* and treated (LC50) is recorded at 24, 48 and 72 hours after treatment.The comparison of means by the student's "t" test,reveals a significant decrease in catalase activity comparing to the control series at all times tested 24, 48 and 72 hours after treatment with *R.montana* EO(fig 74)

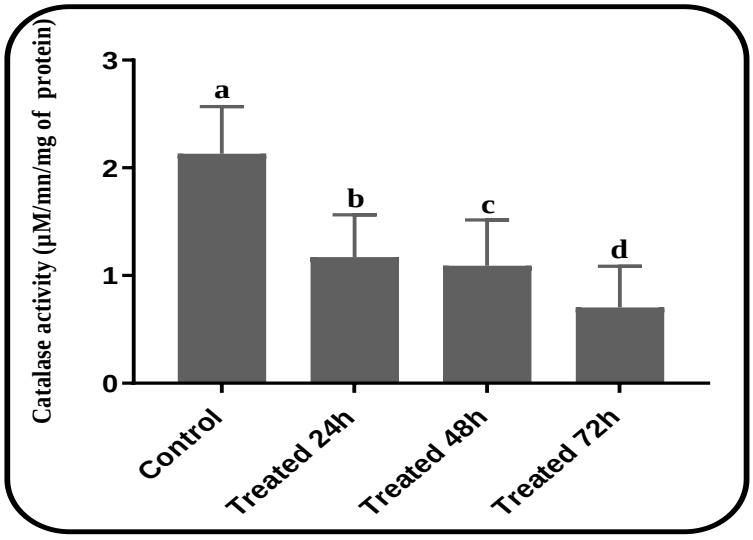


Fig 72: Effect of EOs (LC50) on catalase specific activity (µM/min/mg protein) in adults of *D.melanogaster* comparing to the control series (m SEM, n=6 repetitions each with 20 individuals): Tukey HSD test

3.13.Effect on chemical composition

3.13.1.Effect on total protein content

Total protein content was determined in adult control and treated males and femalesof species *D.melanogaster* with EO of *R.montana* (LC25, LC50) after exposing to 24, 48 and 72 hours. The comparison of mean values by the student "t" test shows that EO extracted from *R. montana* induce a significant decrease in the total protein content of *D.melanogaster* adults at 24 hour exposure LC25, LC50 (p=0.041, P= 0.014), at 48 h LC25, LC50 (p=0.013, p = 0.009), at 72 hours exposure (p = 0.004, p = 0.002) respectively.

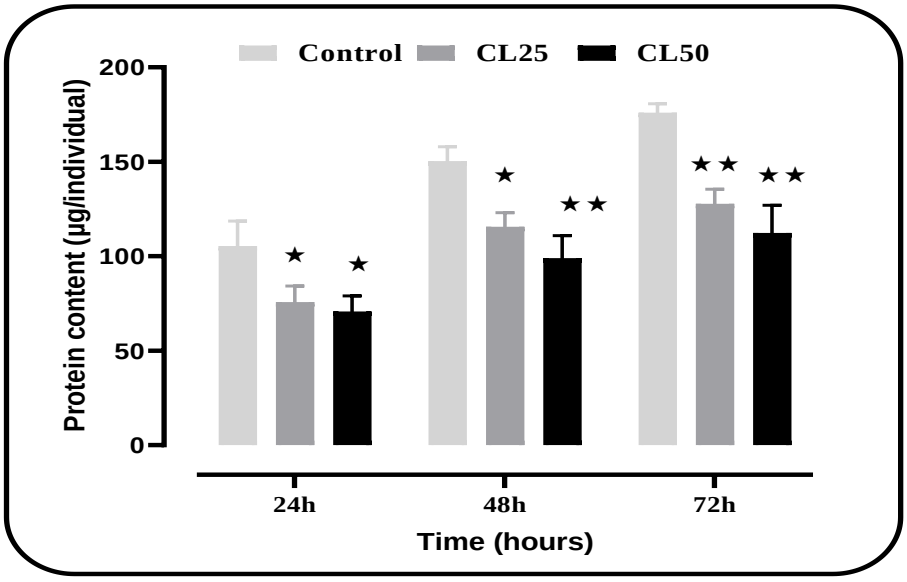


Fig 73: Effect of EOs (LC 25, LC50) on total protein content (µg/individual) of *D.melanogaster* individuals (m SD, n = 4 replicates with 20 individuals each)

3.13.2. Effect on total carbohydrate content

Carbohydrate content was determined in adult control and treated males and femalesof species *D.melanogaster* with EO of *R.montana* (LC25, LC50) after exposing to 24, 48 and 72 hours. Results presented in Figure 76, it is noted that treatment with EO extracted from*R.montana* has no effect in adults of *D.melanogaster* exposed to LC25 and LC50 for 24 h also in individuals exposed to LC25 for 48 h and 72 h respectively since no significant differences were reported (p>0.05). On the other hand, treatment with EO extracted from *R. montana*, (LC50) for 48 h and 72 h causes a significant decrease in carbohydrate content in adults of *D.melanogaster* (p=0.028; p=0.037 respectively).

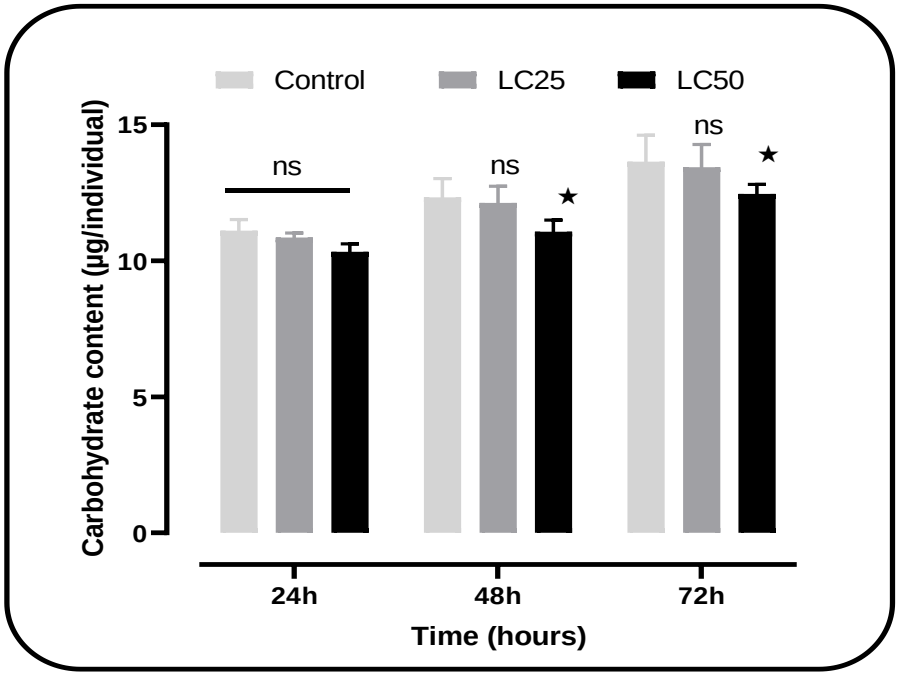


Fig 74: Effect of EOs (LC25, LC50) on total carbohydrate content (µg/individual) in *D.melanogaster* adults (m SD, n = 4 replicates with 20 individuals each). (ns Non-significant difference; * Significant difference (p< 0.05) between control and treated series).

3.13.3. Effect on total fat content

Total fats content was determined in adult control and treated males and femalesof species *D.melanogaster* with EO of *R.montana* (LC25, LC50) after exposing to 24, 48 and 72 hours.The comparative study by the «t» test of studies of control series and treated for *D.melanogaster*indicatesthat the EO treatment of *R.montana* causes a significant decrease in lipid content in individuals of the adults studied, LC25 for 24, 48 and 72 hours (p=0.023, p=0.045 et p=0.040) and LC50 pendent 24, 48 et 72 hour (p=0.007, p=0.005 et p=0.026) respectively.

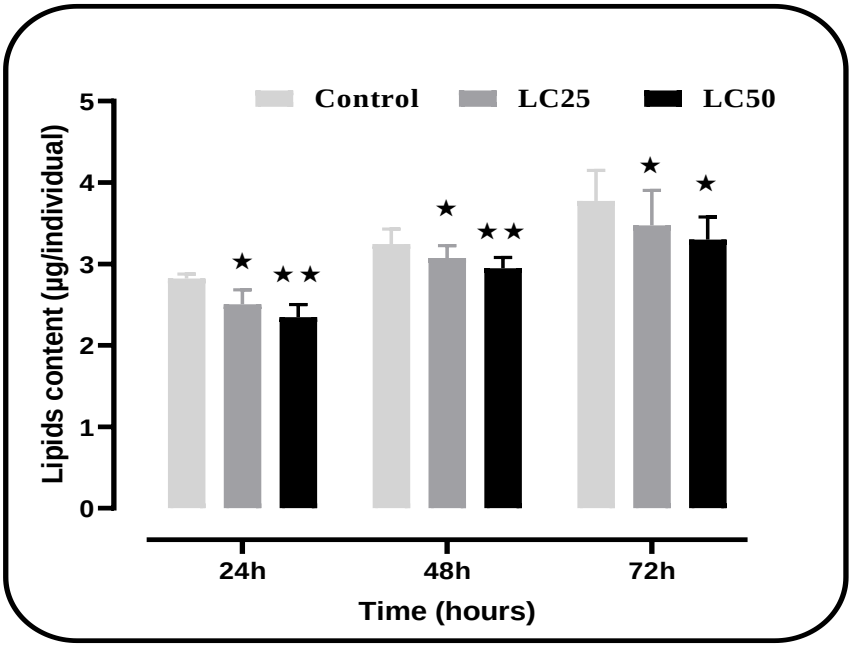


Fig 75: Effect of EOs (LC50) on total lipid content (µg/individual) of adult individuals of *D.melanogaster* (m SD, n = 4 replicates with 20 individuals each).

3.14. Residual efficacy

Residual potency of EO was measured to assess longevity of insecticidal activity after the application of *R.montana* EO with a concentration equal to 5µL/Lair by the fumigation method against twenty adults of *D.melanogaster* with 4 experiments. *R.montana*'s EO retained its insecticidal activity for about 5 days.The results of *R.montana*'s EO residual activity are shown in figure 78. They show a decrease in the sensitivity of adults of *D.melanogaster* to the treatments applied; this reduction is reflected in a reduction of the toxicity during the period tested (72 h).The sensitivity of adults of *D. melanogaster* decreases over time and varies with treatment applied. In addition, note a gradual decrease in the insecticide activity of treatments, reflecting a regular loss of biological activity over time.

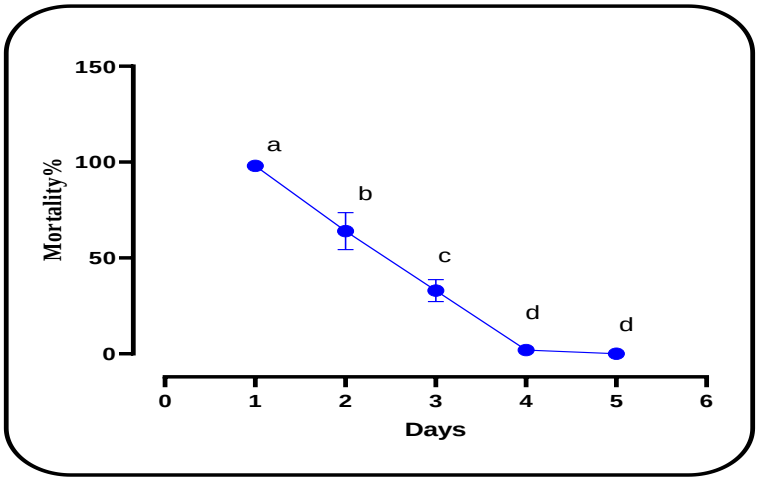


Fig 76:Residual potency of *R. montana* EO (5 µL/Lair) applied to *D. melanogaster* adults

3.15. Physicochemical characteristics of the soil

3.15.1. Physical and chemical soil analysis results:

In order for humans to meet all of their basic needs, soil is the most essential component. Plants are essential to all living things, and they grow in soil to meet their daily needs. Crops grow in soil to produce food and clothing. In addition to being crucial for agriculture, soil is also more beneficial to living things, so it is very important test the physico-chemical and ecological characterization of the same samples: Granulometry, organic matter, pH and electrical conductivity of soil of both plant's regions (P1) and (P2) as it is shown in the table.

Table 25: Soil physico-chemical and ecological characterization of the tow stations: P1 whitch belongs to plant 1 and P2 belongs to plant 2.

Characteristics		**Station 1(P1)**	**Station 2(P2)**
Deep (cM)		20 cM	20 cM
Humidity %		25.28 2.15	26.31 0.88
PH		8.49	8.61
E.C (dS /m)		138.14	182.73
Granulometry %	Clay	2.468	0.118
	Silt	22.307	12.469
	Sand	75.225	87.413
Organic Matter assay %		1.80	1.98

3.15.2. pH soil testing

The most significant property of soil is its pH level, its effects on all other parameters of soil.Our findings indicate that the range of values in the first station (mountain of Ouenza) varies between [8.35 to 8.62] at the average 8.94, while we see that the values vary between

[8.58 to8.63] average 8.60 in the second resort (Boulhaf-Edir mountain). In the variation of values of hydrogen potential (pH) there is not a significant difference between values for both station parameters (P=<0.001). According to Water pH interpretation scale according to Ryser, 2001, confirms that the soil belongs to both stations are alkaline.

3.15.3. Electrical Conductivity

Electrical conductivity is also a very important property of the soil; it is used to check the quality of the soil. It is a measure of ions present in solution.

The total amount of soluble salts that correlate to the salinity of the soil is defined by electrical conductivity. It is dependent upon the kind and quantity of salts that are found in the soil).It is observed that the first station's (the Ouenza Mountain) variance in values changes between [132.3 to146.24] at a median of 138.14. Although the values range from [180.2 to 187.6], the second resort's average is 182.73 (Boulhaf-Edir mountain). Scale of salure according to the EC of the aqueous extract at 1/5 according to AUBERT, 1978 confirms that the soil belongs to both stations are extremely salty (E.C. (ms/Cm) = >6).

3.15.4. Granulometry of soil

Granulometry considered being a modern and efficient technique to analyze the soil particle size distributions,the range of sizes of the particles assigned can differ depending on the specific classification. Nonetheless, the three types of particles clay, silt, and sand are included in all these classification schemes.

The representation of results of granulometry analysis on triangular textures shows that soils from first station (P1)show a dominance of sand and silt fractions (75.225% and 22.307%) from the clay fraction (2.468%). According to the triangle of textures classification, the type of soil is salty sand:

Table 26: soil particle size analysisbelongs to plant of the first station (P1).

Classification	terminals	%volume	Percentage
Clay	0-2	2.468	2.468
Silt	2-20	9.644	22.307
	20-50	12.663	
Sands	50-100	23.325	75.225
	100-200	25.349	
	200-2000	26.551	
	Total	100	100

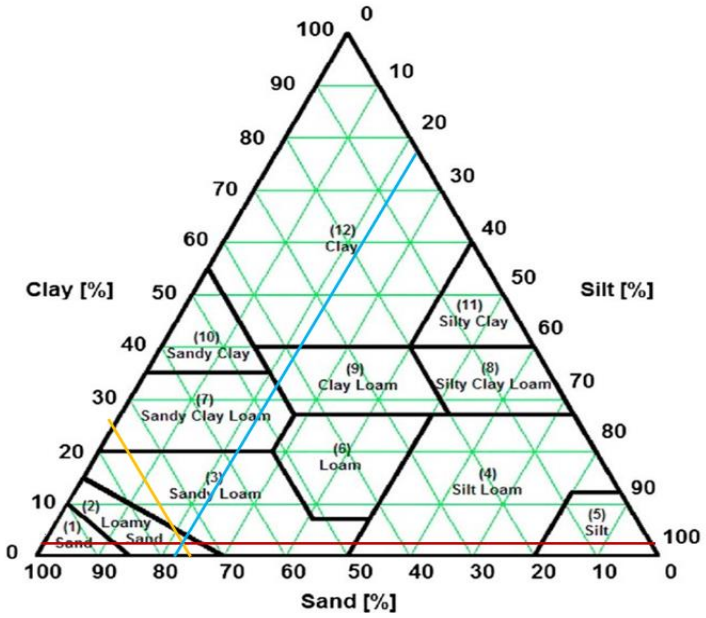


Fig 77: Triangle of textures the type of soil belongs to plant of the first station (P1)

The representation of results of granulometry analysis on triangular textures shows that soil from second station show a dominance of sand and silt fractions (87.413% and 12.469%) from the silt clay fraction (0.118%). According to the triangle of texturesclassification the type of soilbelongs to plant of the second station (P2) is sand:

Table 27: Soil particle size analysis belongs to plant of the secondstation (P2).

Classification	**terminals**	**%volume**	**Percentage**
Clay	0-2	0.118	0.118
Silt	2-20	4.198	12.469
	20-50	8.271	
Sands	50-100	17.448	87.413
	100-200	21.528	
	200-2000	48.437	
	Total	100	100

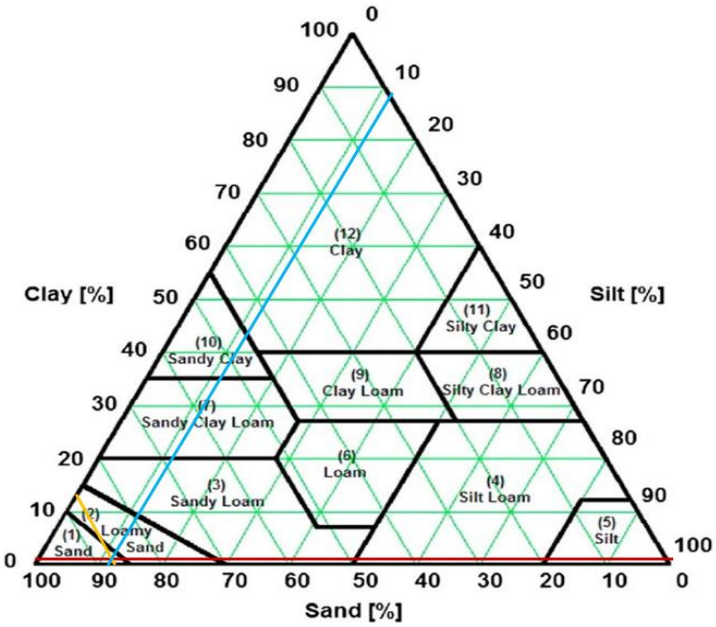


Fig 78: Triangle of textures the type of soil belongs to the plant of second station (P2)

3.15.5. Organic Matter (organic matter OM)

OM determining rates by the ratio of the difference between the weight of dry soil and soil weight cremated on the weight of dry soil; the latter is obtained by drying in an oven for 24 hours at 105°C.Determining the organic carbon concentration of a soil indirectly yields the organic matter content of that soil. The levels of organic matter (organic carbon) in each studied sample are shown in the following table:

Table 28: Organic matter content in soil samples of both soils of the two stations (P1 and P 2):

	Soil belong to P 1			**Soil belong to P 2**		
	R 1	**R 2**	**R 3**	**R 1**	**R 2**	**R 3**
%	1.74	1.78	1.87	2.07	2.04	1.84
M ± SD		1.80 ± 0.068			1.98 ± 0.12	

According to the classification of I.T.A (1975), soils are poor in organic matter (% = 1- 2).

3.15.6. Heavy metals

The process of testing soil for metal contamination is always changing in order to better identify the risks to the environment and public health that are related to heavy metals in

plants and soils. Analytical results show that the samples analyzed soils are characterized by an abundance of certain heavy metals: Iron and Zinc as it is shown in the table.

Table 29: Analytical results showed the abundance of certain heavy metals: Iron and Zinc in both stations (P1 and P2):

	Soil belong to P 1	**Soil belong to P 2**
Fe ppm	7385.88 and 1160.420	7.907 and 6.860
Zn ppm	114.737 and 23.323	11.066 and 0.590

We conclude that our study area first station (P1) is highly polluted or contaminated by iron that exceeds accepted standards, and it varies between 7385.88 and 1160.420 ppm with presence of Zinc 114.737 and 23.323 ppm while the second station (P2) is poor in metals with 7.907 and 6.860 ppm, 11.066 and 0.590 ppm of Iron and Zinc respectively.

Discussion

4. Discussion:

4.1. Yield and Essential oil

The EO obtained by hydrodistillationclevenger-type of the aerial parts of medicinal plant *R.montana*(P1) belonging to the Rutaceae family collected from zone 1 (Ouenza mountain) has a yield of 1.49±0.27% of the dry matter of the plant's aerial part,higher than those reported in EO of medicinal plant *R.montana*(P2) belonging to the Rutaceae family collected from zone 2 (Boulhaf –Edir montain) of 0.75 ± 0.083% with the same yellow in colour, transparent, and the attractive smell of both EOs.

According to Ammar et al. (2012), the same *R.montana* that was harvested from two distinct Algerian regions produced (4.5 ± 1.5%). The observed results were consistent with the findings reported by Mohammedi *et al.*, 2020), which demonstrated the variance in the EO composition of *R.montana* collected from seven distinct Algerian locales. Based on yields obtained in the wild from seven different places, the yield of *R.montana's* EO varied from 0.38% to 1.45% depending on the region from where it was gathered (1.45±0.38%). Nonetheless, a different yield in EO (0.37% ± 0.03%) was previously noted for (Benali *et al*., 2020). In the organs from the Rutachalapensis, the yield ranged from 0.06% in roots, 0.22% in stems, 3.37% in leaves and 3.86% in flowers. Previous research has demonstrated that the species, origin of the plant population, and the plant organs from which the essential oils were collected all affect the yield of Ruta plants (Barbouchi&Benzidia, 2021). Numerous variables, including harvest season and humidity, could be to blame for this variance in yields. Furthermore, EO output may be influenced by environmental variables like temperature as well as edaphic factors like height and soil characteristics (Mohammedi *et al*., 2020).

4.2. Chemical composition of essential oils CG/MS

Analysis of the chemical composition of EOs extracted from *R.montana* by CG/MS reveals thatThe terpenic compounds identified in EO of (P1) represent 87.43% of total composition in which the major compounds are monoterpenes68.47% (alpha-Pinene, Camphene, beta-Pinene, beta-Myrcene, Limonene, alpha-Terpineol, Eugenol and Geranylacetate) while 30.54% areterpenic hydrocarbonssesquiterpenes(gamma-Muurolene and Caryophylleneoxide) of total terpenic compounds while The essential oil of *R. montana* (P2) consists of 91.38% terpene derivatives, corresponding to monoterpenes ,the rest 8.62% represents other chemical components (alkane, cymene, organic and aromatic compounds).

Compared to published research, the findings of Benali *et al*., 2020 indicated that the primary constituents of *Achillea odorata susbsp.pectinata* were oxygenated monoterpenes, with the dominant constituents being camphor (45.01%), bornyl acetate (15.07%), borneol (11.33%),

and 1.8-cineole (eucalyptol) (2.96%). In contrast, the essential oil of *R.montana*EO was characterised by a rich presence of methylketone 2-undecanone (63.97%) as a major compound, trailed by camphor (3.82%) and cyclopropanecarboxylic acid (3.66%).

Screening was done on the essential oil of *Ruta graveolens* aerial parts. GC and GC–MS studies detected thirty-eight components. EO was primarily composed of 2-ketone compounds, namely undecan-2-one (46.8%) and nonan-2-one (18.8%) (De Feo et al., 2002).

After EO from the various parts of the herb were subjected to GC–MS analysis, Mejri et al., 2010 find thirteen major compounds. Of these, two are the most prevalent: 2-undecanone (77.18%), 2-decanone (8.96%), and 2-dodecanone (2.37%). It was found that 2-undecanone constituted the sole component (100%) of the essential oil extracted from the flowers of *R. chalepensis*.

Chaaban*et al*., 2019, explore the chemical composition of EO extracted from the medicinal plants Ruta graveolens, Mentha pulegium and Ocimumbasilicum, Linalool (29.23%), methyl cinnamate (18.97%), and eugenol (5.84%) were the main constituents of O. basilicum essential oil, followed by 1,8-cineole (5.74%), α-cadinol (5.69%), estragol (4.72%), and α-bergamotene (4.24%), while the main constituents of M. pulegiumEO were 1,8-cineole (14.60%), p-menthan-3-one (14.9%), piperitenone (11.4%), carene (10.19%), menthol (8.76%), trans-caryophyllene (6.64%), and menthyl alcohol (6.14%). Hydrocarbon ketones (52.5%) were notably abundant in EO isolated from *R. graveolens*, with the highest concentrations found in 1-nonene (19.4%), 2-undecanone (16.22%), and 2-nonanone (11.9%).

Essential oils of Algerian *R.montana* obtained from seven different areas defined by diverse environmental circumstances were examined by Mohammedi *et al*., 20120 for their chemical components, Based on GC-MS analysis, 40 components accounting for 87.8–99.2% of the total oils were identified. 2-undecanone (27.2–81.7%), 2-nonanone (1.9–39.5%), and 2-nonanyl acetate (tr – 24.8%) were the principal ingredients.

Comparing to results of El-Sherbeny*et al*., 2007, two-undecanone was the main component of EO, with 28 and 30 recognised components for the leaves and flowers, respectively, of *R. graveolens's* identified terpenic contents.

The quantity of components that have been identified varies and is dependent on the species and plant organ (Figueiredo *et al*., 2008; Haghighi *et al*, 2017).

4.3. Antioxidant activities of *R.montana* EO

4.3.1. Antioxidant activities of *R.montana* EO DPPH testing

The results revealed that EO extracted from the first plant *R.montana* (P1) with an IC_{50} value of 22.58 (13.91 ± 32.25) µL/mL is an effective scavenger than EO extracted from the second

plant (P2)*R.montana* and the ascorbic acid with an IC50 value of 41.96 (33.74 ± 51.32) and 1.501 (0.5061 ± 1.729) µL/mL, respectively.

To evaluate *in vitro* antioxidant activities of *R.montana* L. extracts, DPPH assayis performed, ethyl acetate extract demonstrated the higher scavenging capacity (IC50 = 0.044 ± 0.001 mg/mL) followed by methanol, aqueous and chloroform extract (Merghem &Dahamna, 2020) Also, the reaserch of shows that The highest DPPH radical scavenging activity (%) of Algerian *R. Montana* EO was recorded at 1M to be 8.16 % and 11.62 % for the two samples (EM), (EOEB) respectively compared to standard vitamin C (64%)(Amar *et al*., 2012). In Morocco, The evaluation of antioxidant activity of EOs and Phenolic Extracts from the Aerial Parts of *R.montana* using the 2,2-diphenyl-1-picrylhydrazyl test (DPPH) revealed that EO possesses a high potential for antioxidants, with an IC50 value of 548.5± 27.4 µg/mL (Drioiche*et al*., 2020).

The reaction between DPPH radicals and appropriate reducing agents results in the pairing off of electrons and a stoichiometric loss of colour in the solution, contingent upon the quantity of electrons assumed (Shirwaikar*et al*., 2006 ; Apak *et al*., 2016).

4.3.2. Antioxidant activities of *R.montana* EO FRAP testing

The results revealed that EO extracted from the first plant *R.montana* (P1) with an IC_{50} value of 1.494 (1.120 ±1.687) µL/mL higher antioxidant activity than the second EO extracted from the second plant (P2) *R.montana* and the ascorbic acid with an IC50 value of 2.254 (1.801 ±1.876) and 1.501 (0.5061 ± 1.729) µL/mL, respectively.

The antioxidant potential of *R.montana* extract was examined by ferric reducing power assays (FRAP), the highest ferric reducing power (66.66 ± 5.67 mg AAE/g of extract) (Benali *et al*., 2020). In the reaserch of (Belew, 2023),the antioxidant activity was measured using the FRAP radical scavenging assay. *Lippiaadoensis* showed the maximum radical scavenging effect, with IC50 values of 15.8 ± 1.7 (µg/mL). According to the tests of SafaeianLaein*et al*., 2021, the antioxidant potential was higher for lime pomace, radical savaging activity of pomace in the FRAP assay was 92.25 ± 2.45 µMtrolox equivalents/g dry weight.

The high antioxidant activity of medicinal plants' EOS is attributed to their abundance in phenolic compounds, primarily flavonoïdes of the flavonol type. It is well known from the literature that phenolic compounds have remarkable antioxidant capacity (Chrysargyris*et al*., 2021; Chrysargyris*et al*., 2020).

4.3.3. Antioxidant activities of *R.montana* EO ABTS+. Radical cation decolorization assay

The results revealed that EO extracted from (P1) has high scavenging ability of the ABTS radical with an IC50 value of 46.14 (36.66 ± 56.44) µL/mL effective scavenger than the EO

of second plant (P2) and TROLOX with an IC50 value of 61.73 (1.676 ± 1.907) and 74.49 (1.722 ± 2.050) µL/mL, respectively. Agrees with our present results, Elizondo-Luévano*et al*., 2023 evaluated the antioxidant activity of *R.chalepensis* (Rutaceae family). Results confirm it had potential to capture 2,2′–azinobis–3–ethylbenzothiazoline–6–sulfonic acid (ABTS) radicals, and evidenced antioxidant activity with IC50 values of 130.06 µg/mL.Also, BEN SGHAIER*et al*., 2018 found that at the maximum dose of 3 mg/mL, the results showed that methanol and aqueous extracts of *R.chalpensis* exhibit antioxidant effectiveness against the ABTS˙+ radical with 92.4 % and 87.5%, respectively.

The high concentration of various phenolic compounds and flavonoids found in extracts is responsible for the strong antioxidant activity tested by (ABTS) (Djeridane*et al*., 2006; Wojdyło*et al*., 2007). However, Due to its ability to assess the radical scavenging capability of both lipophilic and hydrophilic antioxidants, the ABTS.+ scavenging assay is preferred (Oboh *et al*., 2009 ; Çelik *et al*., 2010).

4.4. EO insecticide testing for *D.melanogaster*with *R.montana* essential oil

4.4.1. Larvicidal activity of *R.montana*essential oilagainst *D.melanogaster*

a. Toxicity on first instar larvae:

Our result show that EO of *R.montana* was applied to the concentration 1st stage larvae lethal (which causes mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 2.006, LC 50 = 2.871, LC 90 = 5.882and a Slope of 3.064

The susceptibility of early stage larvae of *D.melanogaster* and also confirmed byVan De Wouw*et al*., 2006 research of in whitchCyromazine exposure was found to cause significant increases in mortality only within the first 16 hours of the first instar stage of *D.melanogaster*. This suggests that cyromazine builds up in the larvae over time and that there is a delay between exposure and effect. It was noticed that adult eclosion occurred earlier in the early first instar stage of cyromazine exposure than in the later first instar stages. In the other hand, Kushalan*et al*., 2022 found that exposure of 500, 1000, and 2000 µg/mL of *C. orchioides A.L*. extract to Drosophila larvae did not cause any organismal death.

As well as the results of Yasur & Rani, 2015, the susceptibility of Drosophila first stage larvae to applied dose depond extracts and their antioxidant system.

b. Toxicity on second instar larvae:

Our reaserch found that EO of *R.montana* was applied to the concentration 2nd stage 8larvae lethal (which cause mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 3.931, LC 50 = 5.315, LC 90

= 9.717 and a Slope of 3.642. As well as our results, application of *Cleome arabica L.* (Capparidaceae) aqueous extracts by ingestion on second instar larvae (L2) of *D.melanogaster*. The results show that mortality rates can reach 90% after 15 days of treatment (Habbachi*et al*., 2020). However, the treatment was administered by ingestion to *D.melanogaster* larvae in their second instar (L2). Exposure to the aqueous extract of Solanum nigrum demonstrates that it has high insecticidal properties; at the end of treatment, 50g/l of the extract causes a 96.25% mortality rate in the larvae (Rahat *et al*., 2021).

Larvae in their second instar (L2) were treated with *Nicotiana glauca* extract, and the mortality rate was observed for a period of 15 days. Similarly, we applied a sub-lethal dosage of 25µg/mL to the larvae in their second instar (n = 50). Two days following the treatment, *D. melanogster* third-instar larvae are exposed to two nutritional media (control and treated with N. glauca), and the larvae's selection is recorded for an hour, the results demonstrate that *D. melanogaster* larvae can have up to 50% mortality rates after 15 days of treatment due to disruptions in gustatory and olfactory signals. Both treated and control larvae lost the capacity to smell their control medium and chosen the food preparation infused with *N. glauca* Graham's aqueous extract (Bouzar *et al*., 2022).

c. Toxicity on third instar larvae:

Due to our findings, *R. montana*EO was given at a concentration that was fatal for third stage larvae, which results in mortality rates of 25%, 50%, and 90% of the target population. It is established what the concentrations of LC25, LC50, and LC90 are: LC 25 = 5.128, LC 50 = 6.912, LC 90 = 12.56, and a slope of 3.679, respectively. Similary to this result, Bouabida and Dris, 2022 found that EO of *R.graveolens* showed larvicidal activity with LC50 and LC90 values (10.85µL/mL, 70.95µL/mL and 39.4µL/mL), (26µL/mL, 144.5µL/mL and 89.57µL/mL) against third instar larvae of *Drosophila melanogaster, Culex pipiens* and *Culisetalongiareolata* respectively.

Agree with finding of Pedroso *et al*., 2023, treatment of L3 larveae with EO of *Rosmarinus officinalis* increased the larval mortality and impaired the developmental success in *D. melanogaster.*

Five plant extracts were tested for *D. melanogaster* larvae (L3) mortality at different doses and exposure times, *E. prostrata*, *C. murale*, *A. indica*, *F. indicaP. hysterophorus*, after 72 hours, *E. prostrata* demonstrated a significant death rate (51.64%) at 30% concentration. When compared to mortality after 72 hours, mortality at concentrations of 10%, 20%, and 30% is quite low after 24 and 48 hours. After 72 hours of exposure, a death rate of 40.80% was noted at 30% for *C. murale*. Comparing mortality after 24 and 48 hours of exposure to that after 72 hours, low mortality was discovered at concentrations of 10, 20, and 30%. When

A. indica was exposed to concentrations of 10, 20, and 30% for 72 hours, the mortality rate was 42.45%, which is greater than the mortality rate after 24 and 48 hours. *F. indica* had the second-highest death rate of all five extracts at a concentration of 30% after 72 hours, at 43.92%. After 24 and 48 hours, mortality at concentrations of 10, 20, and 30% was significantly lower than that of 72 hours. After 72 hours, *P. hysterophorus* showed 41.87% mortality at a concentration of 30%. It was discovered that high mortality was produced by weed plant extracts at higher concentrations (Riaz *et al*., 2018).

4.4.2. Pupicideactivity of *R.montana* essential oil in *D.melanogaster*

The toxicity assessed from the mortality rate recorded after treatment, depends on the doses administered. Our study aims to test the toxicity of EO extracted from *R.montana* regarding the pupae of *D.melanogaster*, whose results show EO pupicide activity applied with a dose-response relationship.Our results show that EO of *R.montana* was applied to the concentration pupae lethal (which causes mortality of 25%, 50%, and 90% of target population). The LC25, LC50 and LC90 concentrations are determined, respectively LC 25 = 8.711, LC 50 = 10.36, LC 90 = 14.68 and a Slope of 6.318.

However, the results obtained by other works, in 2023, Malla & Chandra demonstrates that after 72 hours of exposure on *Diospyros montana*, 100% mortality was observed at 10 ppm in the Aedes albopictus pupicidal bioassay. With an increase in the concentration of the nano solution and prolonged exposure, the mortality rate of mosquito immatures (larvae and pupae) rose.Additionally, Abdel-Baki*et al*., 2021 confirm that fennel oil was shown to be poisonous to pupae of *Muska domestica*, as evidenced by the percentage inhibition rate (PIR), which varied from 23.3% to 100% six days after treatment and had an LC50 of 1.34%.

Following the search for Mahanta *et al*., 2017, *Citrus grandis*EO was tested for its toxicity against *C. quinquefasciatus* pupal stages at seven different concentrations. After a 24-hour period, the LC50 values were 441.42 ppm for EO extracted from the peels of the plant and 247.27 ppm for EO extracted from the leaves.

4.4.3. Adulticidal activity of *R.montana* essential oil on *D.melanogaster*

The results indicate that the biological experiments demonstrated the significant adulticidal action of EO of *R.montana*, with anLC10, LC25, LC50 and LC90 value of 0.321, 0.649

1.31 and 5.36 µL/Lair respectively, this effect depends on the concentration of EO. One of the plant groups with the most effective EO against insect pests, according to reports, is *R. montana*(Bouzeraa*et al*., 2019). The study of (Chaaban *et al*., 2019)shown that *R.montana* and *R. graveolens* plants could both be valuable as alternate fumigants for date conservation. The fumigant activity of *R. graveolens* EO against adults of *E. kuehniella* and *E. ceratonia* was found to be positive in both cases.Fumigation studies demonstrated that EO of *Citrus*

aurantium (Rutaceae) demonstrated an efficient control against *R.dominica* adults with the suppression of emergence and 100% death, according to research by Djebbi*et al*. (2023).(Wang *et al*., 2022) state that the *R. graveolens* EO and its main constituents exhibited a distinctly repellent activity at a concentration of 16 nL/cm^2, which was commensurate with the positive control's repellency. Furthermore, *Rutachalepensis* EO was found to be detrimental to *T.castaneum* adults and larvae (Majdoub*et al*., 2014). Regarding insecticidal action, the death rate of *R. graveolens* volatile oil against *R. padi* was 92.4%, while that of *C. distans* volatile oil was 56.4%. The fruit fly *D.melanogaster* is susceptible to insecticidal effects from both EO and ethanolic and methanolic extracts of Rutaceae plants, including *R. montana*, *R. graveolens*, and *Rutachalpensis* (Bouabida et Dris, 2022; Amrani *et al*., 2022).

Because of its chemical composition, this EO from the Rutacea family (*R.montana*, *R. chalepensis*, and *R.graveolens*) could be utilised as a substitute for synthetic insecticides for stable fly control. (Ali*et al*., 2013; Bouabidaet Dris, 2020; Wang *et al*., 2022).

4.5. Impact of *R.montana* EO on growth

Adults were treated with LC 25 and LC 50, and the body weight was recorded 24, 48, and 72 hours after EO exposure. Furthermore, by comparing a series of temoin, the results described in demonstrate that both of the concentrations of *R.montana* EO (LC25 and LC50) significantly reduce adult *D.melanogaster* body weight during development. Moreover, compared to the other treated and series of temoins, the weight of adult *D. melanogaster* exposed for 72 hours to the EO and treated with LC50 is the most affected.

Furthermore, Bouabida and Dris, 2020 used two lethal concentrations (LC25 and LC50) of *R.graveolens*EO to treat *Culisetalongiareolata* larvae in their fourth instar.
It is discovered that the substance inhibits the growth and decreases the volume of the body.With LC50 and LC90 values of 10.85µL/mL, 70.95µL/mL, and 39.4µL/mL, respectively, and (26µL/mL, 144.5µL/mL, and 89.57µL/mL) against third instar larvae of *D.melanogaster*, *Culex pipiens*, and *Culisetalongiareolata*, EO demonstrated larvicidal action. It also interfered with growth, causing a number of morphological abnormalities to be seen. Growth was also impacted (Bouabida and Dris, 2022).

4.6. Effect on development time

The student test findings show that the two concentrations of *R. montana* EO (LC25: p = 0.020; LC50: p = 0.013) (LC25: p = 0.64 and LC50: 1.31 µL/1 Lair), respectively, significantly lengthen the life cycle duration for the first generation. The similar observation was made for generation 2 (LC25: p = 0.013 and LC50: p = 0.004) and generation 3 (LC25: p = 0.009 and LC50: p = 0.002) during the course of their respective cycles.

In addition to this, Bouabida and Dris (2022) discovered in their research that exposure to the *R.montana* and *R. graveolens* extract prolongs the development time of *Culisetalongiareolata* larvae and pupae.Furthermore, ethanolic extracts of *Borago officinalis L.* have been shown to lengthen the duration of larval and pupae in a particular species of mosquito (*Culex pipiens*) (Draouet*et al*., 2020).Conversely, neither the length of the immature stages' development nor the weight of the pupae of Hypsipylagrandellaz were impacted by any of the *R.chalepensis* extract concentrations evaluated (Mancebo and others, 2001).

4.7. Response time (LT50) bioassay against *D. melanogaster*

In our study, after the application of the highest concentration (5µL/Lair) of *R.montana* EO on Drosophila adults the data indicates that the lethal time (LT50) at which 50% mortality occurs is 9.25 hours, with a confidence interval ranging from 8.739 to 12.11 hours. The results of Bachiri*et al*., 2018, show the application of EO of *Lavandula stoechas* (0.94; 1.25 and 1.57 µL / cm² respectively) on *T.castaneum* individuals, the results showed (LT50 17, 12, 9.16 days respectively). This demonstrates that the two species' EOs was active and had a significant insecticidal effect. In addition, It should be noted that during incubation times of 12, 24, 36, and 48 hours, respectively, the EO concentrations of *R.chalepensis* that result in 50% adult *Triboliumcastaneum*mortality are 0.447, 0.257, 0.20, and 0.176 µL/mL, Adults exposed to various EO concentrations have 50% survival durations that range from 11.75 to 40.73 hours, depending on the concentration (Najem *et al*., 2020). Indeed, (Bachiri *et al*., 2018) tested the concentrations of EO of two indigenous lavender species inMorroco (0.94; 1.25 and 1.57 µL / cm² respectively) to calculate the lethal time LT50 after which 50% of *Triboliumcastaneum* individuals are dying, It appears from the results that the fatal times differ for each species. For the application of EO of *Lavandula pedunculata* (0.94; 1.25 and 1.57 µL / cm² respectively) on *T.castaneum* individuals, the results showed (LT50 18.9, 12, 16 days respectively).

4.8. Effect of *R.montana* essential oil on sex and sex ratio

The odour of some host and nonhost plants causes male Mediterranean fruit fly (Ceratitis capitata (Wiedemann) males to become more competitive in mating, a characteristic that has been exploited in the sterile insect approach to increase the mating success of released, sterile males (Morató*et al*., 2015)

Our data showed that the EO of *R.montana* at both concentrations revealed a significant effect with an increase in the number of adult females and a significant decrease in the number of males compared to the controls for the first generation, for the second generation and the third generation with low concentration LC25. The sex ratio of insects can be influenced by both internal and external influences (Rosenheim *et al*., 1996).

4.9. Negative geotaxis behavior assay

Additionally, it was shown that the tow concentrations applied after 24 hours were successful in considerably lowering locomotor capacity at the times studied compared to the control, and that both the first and second generations of treated flies showed a high degree of motor impairment.

According to Kabir *et al*., 2008 and after 48 hours of treatment, *Seselidiffusum* extract caused a high degree of morphological abnormalities in larvae at a lower concentration of 100 ppm. After 48 hours of treatment, a high degree of growth and/or moulting disruption effect of extract against *Aedes aegypti* larvae was also detected, leading to the creation of larval–pupal intermediates.

4.10. Effect on GSTs specific activity

The GSTs are antioxidizing enzymes involved in the second stage of xenobiotic detoxification Cummins *et al*., 2011, By catalysing the reaction of these compounds with the endogenous glutathione thiol group (Baillie *et al*., 1991) The primary function of glutathione is to change lipophilic compounds into easily excretable hydrophilic molecules (Brattsten, 1988; Dekant, 2009). The development of resistance against chimiotherapeutic drugs, herbicides, insecticides, and microbial antibiotiques is made possible by GSTs. They have a significant role in the physiology of stress, intracellular transport, and the several pathways involved in biosynthesis (Petrussa*et al*., 2013 ;Bouamira*et al*., 2021), Glutathione S-transferases (GSTs) measure specific activity in nanomoles per minute per milligramme of protein (µM/min/mg of protein). The findings for *D. melanogaster* indicate a noteworthy decline in activity-specific GSTs after 24 hours, 48 hours, and 72 hours. At every tested time point, the Tukey HSD test reveals a dose-response connection. From these data, it can be concluded that *R.montana* EO most affects the activity GSTs specific in adults of *D.melanogaster*.The implementation of the detoxification procedure is a result of the increase in GST activity.

Furthermore, a significant decrease in the GST activity was observed after exposure of Black *Cutworm Agrotisipsilon*to Lemongrass EO at the LC50 (9.36 µmole/min/mg of protein) during a 24-hour period, although the activity was much lower at the LC15 and LC50 (8.80 and 7.00 µmole/min/mg of protein, respectively) (Moustafa *et al*., 2021).Additionally, the overproduction of enzymes may result from an alteration in a regulatory gene controlling the degree of enzyme expression as well as an increase in the number of copies of the gene encoding these enzymes. Moreover, a significant reduction ofGST avtivity was observed in the study of Tarigan&Harahap 2016, when the impact of EOsof cardamom, cinnamon and nutmeg oils Eos (LC50 0.01%, 0.132%, and 0.186% respectively) was tested on egg, larva, and adult of *Triboliumcastaneum Herbst* and *Callosobruchus maculatus F*. Further shows an

increase in GST activity (Cédric, 2008). The detoxication process cannot counteract these plant extracts' insecticidal effects. Increased acivity of GSTs reported in *Xanthogalerucaluteola* treated with neem oil (Valizadeh *etal*., 2013), when *Artemisia annua* methanolic extract is applied to *Glyphodespyloalis* (Khosravi *et al*., 2011),Furthermore, Shojaei *et al*. (2017) show a dose-dependent increase in GST activity in *Triboliumcastaneum* larvae treated to the LC30, LC50, and LC70 of *Artemisia dracunculus* EO. However, a decrease in GST activity has been observed in the larvae of *T. castaneum* and *C. maculatus* treated to EOs of cardamome, cannelle, and muscade (Tarigan*et al*., 2016); in the larvae of *Triboliumcastaneum* treated to oil of *Agastachefoeniculum* (Ebadoallahi*et al*., 2013); in the larvae of *Culex quinquefasciatus* treated to the EO of *Citrus grandis* (Mahanta *et al*., 2017); and in the larvae of *T. castaneum* treated to EOs of six plants: *Allium sativum*, *Mentha piperita*, Ocimum*basilicum*, *Thymus vulgaris*, *Sesamum Indicume* and*Chamaemelum nobile* (Abd El-Aziz & El-Sayed, 2009).The insecticide power may have its roots in this low degree of intoxication, which is reflected in a decrease in GST activity.

4.11. Effect on the specific activity of catalase

Catalase is essential for both the defence against oxidative stress and the removal of peroxyded hydrogen from cells (Seiberg, 2013; Hashem, 2014). The results obtained in adults of *D.melanogaster*and treated (LC50) is recorded at 24, 48 and 72 hours after treatment, reveal a significant decrease in catalase activity in the treated compared to the controls.

Our results are consistent with those of (Baş & Ersoy, 2020) whenEO of Hypericum *perforatum* was evaluated for 24 hours on *Tenebrio molitor* at different stages with LC50, LC90, and LC99 values, the results showed a decrease in antioxidant enzyme activities like catalase (CAT). Reduced CAT activity causes the cell to accumulate toxic H2O2, which causes the lipids in the membrane to peroxide (Cheng *et al*., 2021).

However, an increase in CAT activity was observed in*Musca* domestica larvae treated withBay, Lemongrass and Tea tree EOsat the exposure of LC50 concentration of EOs (Chintalchere*et al.*, 2021). Also, EOs of fertility tree and horseradish tree leaves at a final concentration of 10 µL/g for 24 hours of exposure produced an increase in CAT in *D.melanogaster* flies (Funmilola *et al*., 2020). This increase in CAT activity reflects an implementation of the detoxification process which is a form of insect defense (DeJong *et al*., 2007; Flasz*et al*., 2020).

4.12. Impact of EO on energy reserves of *D.melanogaster*

Determining total proteins and other chemical macromolecules, including lipids and carbohydrates, is crucial for physiological research (Dénie*et al*., 2020). Proteins are significant biochemical building blocks that the body needs to grow and develop in order to

perform its vital roles (Shadieva*et al*., 2020). The determination of biochemical constituents is carried out in the body of male and female adults of *D.melanogaster*, after treatment with EO of *R.montana*. Findings indicate that the treated group's protein content was lower than that of the controls. Additionally, after treating *Culisetalongiareolata* with *R. monata*, *R. graveolens*, and *Artemisia absinthium* hydro-methanolic extract, it dramatically lowers the body's protein content (Bouabida& Dris, 2022). According to total protein extraction and quantification studies ofDutra *et al*., 2020, EOs of *P. corcovadensis* and *P. arboreum* at all investigated dosages (LD30, LD50, and LD70) both encouraged a reduction in the amount of protein when compared with control in Spodoptera frugiperda pestwhereas for *P. marginatum* only increased the amount of total protein compared to control.

In insects treated with EOs and toxics, protein loss is a regular occurrence (Meng *et al*., 2020; Senthil-Nathan, 2020). This protein reduction may be attributed to one or more factors, such as decreased synthesis or increased degradation to detoxify the active ingredients present in plant extracts or Eos (Portha& Liu, 2023; Tu *et al*., 2023). In order to compensate for low energy levels brought on by stress, the breakdown of proteins into amino acids tries to make it easier for those amino acids to be incorporated into the Krebs cycle as ketones (Walker *et al*., 2021; Xiao & Guo, 2022). Furthermore, the decrease in protein stores can also result from the insect's physiological response to a stressful environment brought on by pesticides (González-Tokman*et al*., 2020; Kafel *et al*., 2021). In the other hand, the finding of Khater & El-Shafiey, 2015, shows that Eos may increase the content of protein in insect’s body essential oils extracts from *Wedeliatrilobata* (Fam.: Astracae) and *Melissa officinalis* (Fam.: Lamiacae) (LC50 of eatch EO) were assessed against the stored grain red flour beetle, *Triboliumcastaneum* (Herbst) and caused an insecticidal effect by increasing protein amount.

A class of very significant molecules is carbohydrates. Certain amounts provide live things with energy, either immediately usable (trehalose) or in the form of reserves (glycogen); others have a structural role (cellulose, chitin, acidehyaluronic) (Elbein, 2010; Finelli, 2019).

Glycogen and trehalose levels in tissues are closely related to physiological events such as theft, moulting, and reproduction (Harrison *et al*., 2012). The most significant amount of circulating sugars is trehalose. It is essential to the development cycle's metabolism (Paul *et al*., 2008; Zhang &DeBosch, 2020).

Carbohydrate content was determined in adult control and treated males and females of species *D.melanogaster* with EO of *R.montana* (LC25, LC50), our results indicate that treatment with EO extracted from *R.montana* has no effect in adults of *D.melanogaster* exposed to LC25 has no significant differences between treats and controls, On the other hand, treatment with EO extracted from *R.montana*(LC50) causes a significant decrease in

carbohydrate content in adults of *D.melanogaster*. This depletion could be explained by the dose-response relationship of the essential oils applied to the insect and its effect on the carbohydrate content in its body (Tarigan&Harahap, 2016). Previous work has shown the negative effects of essential oils on carbohydrate reserves (Borzoui*et al*., 2016; Dilworth et *al*., 2024). Similar results were obtained in*Culisetalongiareolata*treated with *Ruta graveolens*(Bouabida& Dris2020).

Lipids are the main source of energy for insects (Arrese &Soulages, 2010, dos Santos Aguilar, 2021) Lipids are carried by hemolymph to the user organs from the fat body, where they are synthesised and stored, particularly during vitellogenesis (Fruttero *et al*., 2011; Skowronek &Strachecka, 2021). Lipids increase during the feeding period and their amount varies according to the stage of growth and feeding conditions (Li *et al*., 2016). After being exposed to hazardous chemicals, lipids may be depleted as a result of changes in their synthesis, hormonal dysfunction affecting lipid metabolism (Horton *et al*., 1987; Begriche et *al*., 2011), or utilisation of this metabolic reserve (Brennan &Owende, 2010). Our comparative study of control series and treated for *D.melanogaster* adults with indicates that the EO treatment of *R.montana* (LC25, LC50) causes a significant decrease in lipid content in individuals of the adults studied. Under the influence of an insecticide, tissue lipid levels may drop as a result of lipoprotein synthesis (Ibrahim & El-Gamal, 2003; Rai *et al*., 2009), cellular damage repair (Astiz*et al*., 2009; Narra *et al*., 2017), and increased lipolysis for energy production (Gurushankara*et al*., 2007; He *et al*., 2020). Similar results were obtainedin*Culisetalongiareolata*treated with *R. Montana* and *R.graveolens* hydro-methanolic extracts (LC50 : 43.24 ppm)cuses a decrease in lipid content of insect (Bouabida& Dris2022). Also in other cases in whitch Eos of plants affects lipid amount such as treatment of *P. arboreum* EO(LD50 and LD70) against *Spodoptera frugiperda* reduced the amount of lipid compared to the control (Dutra *et al*., 2020). In opposite, EOs may not affect the amount of total lipids and affect other biochemical content as protein and carbohydrates as same as Ali *et al*., 2018 results in whitch Both tested oils Camphor oil (LC10) and castor oil (LC10) didn't affect the amount of total lipids but decreased total proteins and carbohydrates while targeted only carbohydrate content in 4th instar larvae of *Spodoptera littoralis*. Also, the finding of Dutra *et al*., 2020, all lethal doses tested (LC30, LC70 and LC 70) of *P. corcovadensis* EO did not differ in amount of *Spodoptera frugiperda* when compared to the control.

Furthermore, a number of studies have demonstrated that both synthetic and botanical pesticides have the ability to alter an insect's protein, lipid, and carbohydrate content (Assar *et al*., 2016; Souza *et al*., 2023). A significant reduction in protein content was observed in the results of Tarigan&Harahap, 2016, when impact of EOs on total carbohydrate, protein, fat

*R.montana*contents was evaluated by treating egg, larva, and adult of *Triboliumcastaneum Herbst* and *Callosobruchus maculatus F. Further* with cardamom, cinnamon and nutmeg EOs, Furthermore, all EOs reduce the total carbohydrate, protein, and fat contents, and cinnamon oil demonstrated to be the most effective among the three EOs. Lazarević*et al*., 2020, find that EO from *Thymus vulgaris* decreases protein and lipid levels in *Acanthoscelides obtectus*.

Ali *et al*., 2018, show similar results , Camphor oil (LC10) decreased total proteins and carbohydrates while castor oil (LC10) targeted only carbohydrate content in 4th instar larvae of *Spodoptera littoralis*, a serious pest on cotton in Egypt.

4.13. Residual efficacy of Essential oil

After the application of *R.montana* EO with a concentration equal to 5µL/Lair by the fumigation method against adults of *D.melanogaster*, results demonstrate a decrease in the adult*D.melanogaster*'s sensitivity to the administered treatments; this decrease is expressed in a decrease in the toxicity during the 72-hour test period. Adult *D.melanogaster* sensitivity varies with treatment and decreases with time. Furthermore, observe a progressive reduction in the insecticide efficacy of the treatments, which is indicative of a consistent decline in biological activity over time.To assess the effectiveness of EOs for protecting stored products, it is important to look at their residual activity (Campolo *et al*., 2018)

Jesser*et al*., 2017 evaluated residual potency of six EOs namely lavender (*Lavandula angustifolia Mill*.), peppermint (*Mentha piperita L*.), geranium (*Geranium maculatum L*.), palmarosa (*Cymbopogon martinii (Roxb.) Wats*), eucalyptus (*Eucalyptus globulus Labill*.) and bergamot (*Citrus bergamia Risso*) against adults of the Indian meal moth, *Plodia interpunctella*, the residues of EOs from palmarosa showed the highest residual activity followed by peppermint and geranium maintained the toxicity effects (25% mortality) until the 6th day, The EOs from bergamot, lavender and eucalyptus showed the lowest effect (the EOs produced 100% mortality at 0 day residual time), except eucalyptus which produced 66%, the toxicity order of EOs at 0.10% was palmarosa> peppermint = geranium > lavender = bergamot = eucalyptus at the highest concentration (0.15%).

Regarding the EOs' residual activity, the boiling point is the physicochemical parameter associated with residual activity.

4.14. Physicochemical characteristics of the soil

4.14.1. PH soil test

The most informative measurement available for identifying soil properties is most likely the pH of the soil (Bünemann*et al*., 2018). PH values of two to three in soil are indicative of the existence of free mineral acid, which is often H2SO4 (Osman & Osman, 2018).

In the variation of values of hydrogen potential (pH) there is not a significant difference between values for both stations (north eastern Algeria) parameters (P=<0.001). Water pH interpretation scale according to Ryser, 2001, confirms that the soil belongs to both stations are alkaline.

Ibtissem*et al*., 2021, discovered a few physicochemical traits that control the soil quality in the Annaba Willaya plain in northeastern Algeria. To ascertain the physicochemical characteristics of the soils in the Annaba plain, the findings indicate that the investigated soils had acidities ranging from 7.30 to 7.80 whitch confirm that the soil was alkaline.

Moreover, studies of wetland sites of the Oued Righ region, located in the northeastern Algeria, to determine the environmental factors controlling the composition and distribution of plant communities have shown thatSoil pH in the studied soils ranged from 6.88 to 7.75 and were neutral to alkaline (Koull&Chehma, 2016). However, soil pH fluctuated between 7.5 and 8.6 with an average of 8.1 ± 0.3were also neutral to alkaline (Neffar*et al*., 2016).

The dominance of the calcium cation and chloride anion in soil serves as what causes it to be alkaline (Rashad *et al*., 2010; Tavakkoli*et al*., 2015).

4.14.2. Electrical Conductivity

The prevalent technique for evaluating soil salinity in situ, specifically the electrical conductivity (EC) of the soil solution, involves determining the soil's apparent electrical conductivity and volumetric water content (Amakor*et al*., 2014; Visconti & de Paz, 2016) , followed by the application of calibrated or estimated EC curves. It is reasonable to determine salinity from apparent EC measurements as a soil solution's electrical conductivity and water content are the primary elements influencing that conductivity (Nadler, 2005; Rong-Jiang *et al*., 2007).

The range in values of the first station (Ouenza Mountain) is found to vary between [132.3 to 146.24] with a median of 138.14. While the numbers vary from [180.2 to 187.6], the average for the second resort (Boulhaf-Edir mountain) is 182.73. The soil from both sites is excessively salty (E.C. (ms/Cm) = >6), according to the AUBERT, 1978 scale of salure based on the EC of the aqueous extract at 1/5.Overall, paper aims of Aliat*et al*., 2016, was to describe the soils of 12 wetlands in the ecocomplex of wetlands in the Hauts Plateaux region in northeastern Algeria, ten of which are Ramsar sites. Each soil sample was analysed to determine electrical conductivity and other physicochemical propreties. The study wetlands were characterised by salty to very salty soils (electrical conductivity = $3 \cdot 46 \pm 2 \cdot 44$ dS m−1). Study of Neffar et *al*., 2016, provided a comprehensive quantitative floristic analysis of a salt ephemeral wetland “Sabkha Djendli” located in eastern Algeria. Soil was analysed for electrical conductivity (EC), Soil EC varied between 3.8 and 13.8 dS/m with an average of

10.0 ± 2.7 dS/m what has been classified as salty soil. Inversely, results indicate that soils of El Tarf area (Northeast Algerian) are low in electrical conductivity and not salty (Zaoui *et al*., 2016)

Soil of northeastern regions of Algeria were characterised by salty to very salty soils (Aliat*et al*., 2016). Many research indicated that salinity and EC is heavily influenced by Mg2+, Ca2+, SO42- (Issam &Boutoutaou, 2022).

4.14.3. Granulometry of soil

Particle-size distribution analysis, or granulometric analysis, refers to the process of quantifying ratios between elementary soil particles of various sizes without taking into account the mineralogical and chemical composition of the particles (Yudina *et al*., 2018).

Following our research, granulometry analysis of soil particle size analysis belongs to plant of the first station (P1) (the Ouenza Mountain), the type of soil is salty sand. Conversely, granulometry analysis of soil particle size analysis belongs to plant of the second station (P2) indicate that the type of soil belongs to plant of the second station (P2) (Boulhaf-Edir mountain) is sand. As stated of study of soils El Tarf area (Northeast Algerian) by Zaoui *et al*. (2016), the triangular textural projection of the granulometry analysis data indicates that the soils under analysis have a predominance of clay textures, with clay textures, sandy clay, and clay.loam.

As a consequence, the soils under study have a homogeneous texture. This soil's texture ensures that the pore water and nutrients required by plants are available to the plants and encourages permeability (Abukari*et al*., 2022).

4.14.4. Organic Matter (OM)

The organic matter (OM) maintains the reserve of nutrients and contributes to soil structure through ventilation and particle cementation (Fageria, 2012; Murphy, 2015). It originates from all living things that are either inside or on the surface of the soil(Kononova, 2012).

Plant litter, animal faeces, and root exudates are some of the living things that contribute to this organic matter. The rest of it is made up of microbial cells, dead animals, and decomposing plant matter (Ismail-Meyer *et al*., 2018).

Determining the organic carbon concentration of a soil indirectly yields the organic matter content of that soil. The levels of organic matter (organic carbon) in each studied sample are shown that soils from both stations are poor in organic matter. Also the results of soils study El Tarf area (Northeast Algerian) by Zaoui *et al*. (2016) indicate that soils are low in organic matter. Similar to other areas, the conversion of tropical regions for agricultural purposes take advantage of soil organic matter, namely nitrogen reserves (Adiaha, 2017). It is another important characteristic of soil. Soil erosion is accelerated when there is insufficient organic

matter in the soil. The soil is beneficial for agricultural techniques if it contains soil organic matter (Kekane*et al*., 2015).

4.14.5. Heavy metals

The health of humans and ecosystems is at danger from an accumulation of heavy metals and metalloids in soils and plants (Alengebawy*et al*., 2021).Our results indicat that our study area first station (P1) is highly polluted or contaminated by iron that exceeds accepted standards, and it varies between 7385.88 and 1160.420 ppm with presence of Zinc 114.737 and 23.323 ppm while the second station (P2) is poor in both metals with 7.907 and 6.860 ppm, 11.066 and 0.590 ppm of Iron and Zinc respectively. The very abundant element iron shows high levels compared to other metal (Zinc). Similar to our findings, Ten sites inside a wooded zone near an iron steel industrial plant (El Hadjar, northeasternAlgeria), were randomly chosen to assess the impact of vegetation on soil and found as contaminated by heavy metal fallout (lead (Pb), nickel (Ni), and zinc (Zn) (Kadem*et al*., 2004).

Conclusion

V.Conclusion and perspectives

The use of natural alternatives to synthetic insecticides with ecological and financial benefits is required due to the issues surrounding the use of chemical pesticides and their detrimental effects on human health and the environment.

Compared to manufactured insecticides, essential oils have highly fascinating insecticidal capabilities and a very sluggish rate of resistance development since they are aromatic compounds with a complex chemical composition.

The EOs of the two plants from different regions belonging to the Rutacea family has yields of 1.49% and 0.75% respectively of the dry matter of the aerial part of *R.montana*. GC-MS chemical analysis of EOs extracted from R.montana revealed an abundance of monoterpene compounds dominated by the presence of this plant contains 21 terpene compounds, accounting for 87.43% of the total composition. Of these, 68.47% are monoterpenes (alpha-Pinene, Camphene, beta-Pinene, beta-Myrcene, Limonene, alpha-Terpineol, Eugenol, and Geranylacetate), and 30.54% are sesquiterpenes (gamma-Muurolene and Caryophylleneoxide). *R.montana* (P2) EO is composed of 17 compounds, of which 91.38% are terpene derivatives, or monoterpenes, while the remaining 8.62% are various chemical components (alkane, cymene, organic, and aromatic chemicals). The Eos of both plants showed the best antioxidant activity confirmed with DPPH and FRAP test.

These EO were tested on *Drosophila melanogaster*, the most medically abundant insect species. Its actions have been evaluated on several aspects: toxicological, morphometric and biochemical.

Toxicological tests determined lethal concentrations (LC50 and LC90) of plant EO for larvae and adults of *D.melanogaster*. They reveal an insecticidal effect with a dose-response relationship. The EO of *R.montana* presents a very high larvicide power against *D.melanogaster*.

Despite, these oil cause a reduction in the specific activity of GST in the treated compared to controls. In addition, catalase was also affected by the application of lediminuating oil in the species studied, suggesting an induction of detoxification processes.

In addition, EO affects the growth of individuals by influencing the developmental cycle, reducing the body weight of *D.melanogaster*. Also, the energy reserves of individuals are also disturbed by the effect of essential oils. The results reveal a significant decrease in protein, carbohydrate and fat content in individuals of different stages studied. However, The sensitivity of adults of *D. melanogaster*to EO decreases over time and varies with treatment applied.

Also ecological characterization of soil related to plant's regions were tested: Granulometry, organic matter, pH and electrical conductivity

Also the results indicate that our soils belongs to both plants regions (P1 and P2) are low in organic matter, have low electrical conductivity and salty. Soil pH has an alkaline character very alkaline,characterized by an abundance of certain heavy metals especially for iron and zinc with different quantities.

This study based on the use of aromatic plants as an insecticide, opens wide perspectives in the field of fundamental knowledge on the one hand and in the field applied on the other. In the future, it would be interesting to complete this work by:

- Evaluation of the effect of the active ingredients (majority components) of the EO of plants and their formulation for their application as biocide.
- Evaluation of the antibacterial, antifungal and antioxidant activity of these EO.

At the end of this study we make the following recommendations:

- Researchers: We recommend further work on plant extracts whose larvicide and adulticide activities have been proven in this study. Investigations must be conducted to discover other larvicide and adulticidal substances from our plant heritage. The use of extracts from our plants will help reduce the cost of insecticides and preserve our environment.
- To the authorities:

  - To invest in the promotion and promotion of medicinal plants,

  - Health decision-makers to strengthen scientific research networks by providing them with substantial means to achieve their objectives.

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Annexes

Annexes :

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