PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
YAHIA FARES MEDEA UNIVERSITY
FACULTY OF SCIENCES

SPECIALTY PHYTOPATHOLOGY

Final dissertation in view of obtaining a master 2 in phytopathology

The effect of deferent concentration of Essential oil of *Melissa offisinalis* (lemon balm) and cloves against *Varroa destructor*

Presented by:
DAHMANI Aicha

In front of the jury:

RAHAL Mohamed	**MCA**	**Medea university**	**President**
DIF Sabrina	**MAA**	**Medea university**	**Examinator**
KEBOUR Djamila	**Professor**	**Blida university**	**Promoter**
MOSTFAOUI Houda	**MCA**	**Medea university**	**Copromoter**

Session 2021/202

Acknowledgements

First of all, I would like to thank Almighty God, who has given me the strength and patience I need to do this work.

I would like to thank Mrs KEBOUR Djamila for accepting being my director of memory.

I would like to express all my gratitude to my Director of Memory, MRs MOSTFAOUI Houda . I thank her for having supervised, guided, helped and advised me.

I would like to thank Mrs DIF Sabrina for reviewing my work.

I would like to thank Mr RAHAL Mohamed for being the President of the jury/

I would like to thank Mr KHADRAOUI Azzedine for his accepting me to do my internship in the CRAPC expertise.

I would like to thank Mr ABOU MOSTAFA Mohamed and his team for their encouragement and helping.

I would like to thank Mr SAHARI Missoum for accepting to do my experience in his apiary.

I would like to thank Mr DAHMANI Ali for his helping and encouragement.

I address my sincere thanks to all the professors, speakers and all the people who, through their words, writings, advice, and criticism, have guided my reflections and answer my questions during my research.

Dedication

I dedicate this work to my father, my mother for being by my side in every step

To my brothers and my sister for their supporting and helping

To my friends for their encouragement and caring

To Google scholar and Sci-hub and checking grammar site & biostatGTV

To me

The effect of deferent concentration of Essential oil of *Melissa offisinalis* and cloves against *Varroa destructor*

Abstract

The objective of our work is to highlight bioacaricidal activity of the essential oils of *Melissa ofissinalis* and clove against varroa. Essential oil of nails Cloves and melissa dried leaves was obtained by steam distillation using a Clevenger-type system. The investigation was designed to determine the chemical composition and acaricidal effects of oil,by using three concentrations 10%, 15%, 25% of our essential oil that was diluated with the tween 80, and a negative and a positive control ,the commercial product (organic naturel strip for varroa).the treatment application was in 15 beehives , 3beehives for one concentration. Our result shows a good effectiveness in varroa mortality comparing it by the commercial product (organic naturel strip for varroa). Commercial product with 146%, R10% concentration with 150%, R15 % concentration with 150%, and R20% concentration with 151%,

Keyword: essential oil , *varroa destrutor, melissa offisinalis,* clovs,*apis mellifera*.

L'effet de déférentes concentrations de l'huile essentielle de *Melissa offisinalis* et des clous de girofle contre *Varroa destructor*

Résumé:

L’objectif de notre travail est d'évaluer l'activité bioacaricide des huiles essentielles de *mélisse ofissinalis* et de clou de girofle contre le varroa. L'huile essentielle de clous de girofle et de feuilles séchées de mélisse a été obtenue par distillation à la vapeur d'eau à l'aide d'un système de type Clevenger. L'investigation a été conçue pour déterminer la composition chimique et les effets acaricides de l'huile, en utilisant trois concentrations 10%, 15%, 25% de notre huile essentielle qui a été diluée avec le tween 80, et un contrôle négatif et positif (le produit commercial (bande naturelle bio pour varroa). L’application du traitement a été dans 15 ruches, 3ruches pour chaque concentration. Notre résultat montre une bonne efficacité dans la mortalité du varroa en le comparant au produit commercial (bande naturelle bio pour varroa). Produit commercial avec 146%, R10% concentration avec 150%, R15% concentration avec 150% et R20% concentration avec 151%,

Mot clé : huile essentielle, *varroa destructor, mélisse oficinalise,* clou de girofle, *apis mellifera.*

تأثير التراكيز المختلفة للزيت العطري من ميليسا أوفيسيناليس والقرنفل ضد الفاروا المدمر

ملخص

الهدف من عملنا هو تسليط الضوء علي نشاط المبيدات الحيوية للزيوت الأساسية من ميليسا أوفيسيناليس والقرنفل ضد الفاروا .تم الحصول على الزيت العطري من القرنفل والأوراق المجففة للميليسا عن طريق التقطير بالبخار باستخدام نظام من نوع كلفنجر . تم تصميم البحث لتحديد التركيب الكيميائي وتأثيرات المبيدات القاتلة للزيت. باستخدام ثلاثة تراكيز 10٪ ، 15٪ ، 25٪ من الزيت العطري الذي تم تخفيفه باستخدام توين 80 ، وضبط سلبي وإيجابي (المنتج التجاري (الشريط الطبيعي العضوي للفاروا) تم تطبيق المعالجة في 15 خلية نحل ، 3 خلايا نحل بتركيز واحد أظهرت نتائجنا فاعلية جيدة في نفوق الفاروا مقارنة بالمنتج التجاري (الشريط الطبيعي العضوي للفاروا) منتج تجاري بنسبة 146٪ ، R10٪ تركيز بنسبة 150٪ ، تركيز R15٪ بنسبة 150 ٪ ، تركيز R20٪ بنسبة 151٪ ،

والمقارنة بين التركيزات الثلاثة 10٪ ، 15٪ ، 25٪ ,نتائجنا اظهرت تأثير جيد مقارنة مع الدواء المسوق.

الكلمات المفتاحية : الزيوت الاساسية, فاروا , الميليس, القرنفل ,النحل .

Table of Contents

Acknowledgements

Dedication

Abstract

Résumé:

ملخص

Introduction……..……1

1 Chapter I: Plants study 2

1.1 Entotoxic : 2

1.2 Extraction method: 2

1.3 Part one: Plant *Melissa officinalis L* 3

1.3.1 Historic : 3

1.3.2 Plant definition: 3

1.3.3 Plant classification: 4

1.3.4 Geographic Distribution: 4

1.3.4.1 In World: 4

1.3.4.2 In Algeria: 4

1.3.5 Chemical composition: 6

1.4 Part two: CLOVE 7

1.4.1 Historic: 7

1.4.2 Geographic distribution: 8

1.4.3 Classification: 8

1.4.4 Chemical composition : 9

2 Chapter II: *Varroa destructor* 11

2.1 Historic: 11

2.2 Definition: 11

2.3 Morphology: 11

2.4 Classification: 12

2.5 Geographic distribution: 12

2.5.1 In world: 12

2.5.2 In Algeria: 12

2.6 Life cycle: 12

3 Chapter III: Apis mellifera 14
3.1 Generality: 14
3.2 Classification: 14
3.3 Bee morphology: 14
3.3.1 Queen: 14
3.3.2 Workers: 15
3.3.3 Males: 15
3.4 Geographic distribution: 16
3.4.1 In world: 16
3.4.2 In Algeria: 16
3.5 Life cycle: 16
3.6 The critical phase between bees and varroa destructor: 17
3.7 Main treatment periods: 17
3.8 Damages: 18
Material and methods: 19
3.9 Presentation of Vegetal material: 19
3.9.1 *Melissa offisinalis*: 19
3.9.2 Cloves: 19
3.10 Extraction workplace Representation: 20
3.11 Apiary presentation: 20
3.12 Oil extraction: 21
3.12.1 First extraction of *Melissa offisinalis* in Blida: 21
3.12.2 The second extraction of the essential oil of Melissa officinalis in CRAPC: 21
3.12.3 Cloves extraction: 23
3.13 Oil identification by GS/MS : 24
3.14 Concentration: 25
3.15 Wooden tongue preparation 25
3.16 Method of treatment application 25
3.16.1 Estimation of varroa mite mortality: (A) 26
3.16.2 Estimation of daily varroa mortality: (B) 26
3.16.3 Estimate of the number of bees in a beehive: 26
3.17 Data analyses: 26
4 Results and discussion: 27
4.1 *Melissa offisinalis* extraction: 27

4.2 Cloves extraction: 28
4.3 Chemical composition of cloves essential oil 28
4.4 Oil identification: 29
4.5 analyses: 30
4.6 Data analyses 33
4.7 Insecticidal activity: 36
4.8 Apiary pest: 37
Conclusion: 38

Figure List :

Figure I 1 : plant of *Melissa officinalis* and flower (original) (Damous) 5
Figure I 2 plant of *Melissa offisinalis* (original)(Boumerdes) 5
Figure I 3: Cloves (original) 8
Figure II 4 : *Varroa* morphology (Duran, 2017) 11
Figure II 5 : *Varroa destructor* cycle life 13
Figure III 6 : Bee morphology(B) and *varroa destructor* (A) (apiculteur.ch, 2021) 15
Figure III 7 : the critical phase between bees and *varroa destructor* (ApiSavoir.ch) 17
Figure 8 : Vegetal material *Melissa offisinalis* 19
Figure 9 : Cloves 19
Figure 10 :Photo of the apiary (original) 20
Figure 11 : Extraction device (original) 22
Figure 12 : Extraction device (original) 23
Figure 13 : Reprsennt clove extraction and decantation (original) 24
Figure 14 : wooden tongue 25
Figure 15 :Chromatogram of cloves 28
Figure 16 : Varroa percent death in control population 31
Figure 17 : Varroa mortality in positive control for three beehives, treated with a biological product 31
Figure 18 : Varroa mortality in concentration 10% of essential oil 32
Figure 19 : Varroa mortality in concentration 15% for three beehives of essential oil 32
Figure 20: Varroa mortality in concentration of 25% for three beehives of essential oil 33
Figure 21 : varroa initial estimate 34
Figure 22 : corrected mortality 35
Figure 23 : paper which is cut by the bee and thrown out of beehive(original) 36
Figure 24 : cockchafer (original) 37
Figure 25 : bee and *varroa destructor* (original) 38
Figure 26 :GS/MS APPARATUS 43

Table list:

Table 1 : chemical composition of *Melissa oficinalis* (Feknous, 2014)..... 6
Table 2 : Chemical compounds of *Melissa oficinalis* essential oil (Feknous, 2014) 7
Table 3 : Composition and percentage of clove essential oil (*Eugenia caryophyllata*) obtained with GS-MS analysis (Chaieb, 2007)..... 10
Table 4 : cloves oil 29
Table 5 : p value of comparison between different concentrations..... 33
Table 6 : corrected mortality percentage..... 34

Abbreviation list

EO: essential oil

GC: Gas Chromatography

µl: microliter

µg: microgram

1 Introduction

Honeybees and wild bees play a key role in terrestrial ecosystems. Indeed, the majority of phanerogams could not complete their development cycle without the intervention of pollinators. This environment factor plays a major role in the reproduction of many plants by maintaining genetic diversity, and bio indicator role. (Adjlane, 2012)

During the last decay, Beekeepers noticed a huge rate of bees mortality caused by varroa destructor; the parasite dispersed worldwide and is currently considered as a major threat for apiculture. (Boubaker, 2019)

This threatens global food security and negatively affects vegetation, it needs more effort to control these phenomena. Actually, the use of the chemical product exhibits many negative effects on the apiculture as well as on the Beekeepers. (Robert, Böcker, Möhring, & Dalhaus, 2017)

The current strategy consists on the development of a new and safe product with an anti-acaricidal activity. In this optic, the biological treatment represents a great alternative. Biological product extraction method

Actually, the *Melissa offisinalis* is known to be affective on bee attraction to enter their beehives. In other hand, the clove considers as a powerful acaricidal effects.

That why, in this study, we aim to evaluate the acaridae activity of the two essential oil as a biological product extracted from plant '*melissa offisinalis* and clovs' on bee varroa's infection.

Bibliographic part:

Chapter I: Plants study

Chapter I: Plants study

1 Chapter I: Plants study

Essential oil: are the compounds results from the extraction of aromatic plant's deferent part (leaves, stem, seeds, flower), with deferent way, which is distillation (via steam or water) or mechanical methods such as cold pressing. They are also found in the resin, bark, and wood of trees (Leslev, 2005).

1.1 Entotoxic :

Due to essential oils, plants can adapt to their environment and have a means of defense against various agents, can intervene in the interactions between plant-plant (germination, growth, signaling) and plant-pathogen (plant-bioagresssor), certain plants have a repellent and anti-appetizing effect which can disturb the behavior of parasitic insects (Boubaker, 2019).

Biological control offers many possibilities which are currently being developed, by the isolation and identification of plant compounds which have antibacterial, antioxidant, antifungal, and insecticidal activity (Talbi, 2014).

The insecticidal effect of essential oils by contact or by injection or fumigation has been well demonstrated, several studies have been carried out on the efficacy of essential oils (Boubaker, 2019).

1.2 Extraction method:

The composition of essential oil (EO), even with in one specie, depends on many factors such as growing conditions, time, and place of the collection of raw material, drying and storage. The method of determination also has an impact on the qualitative and quantitative analysis of chemical compounds present in the tested EO. Like: Gas Chromatography, Mass Spectrometry, Chiral Column GC Analysis, Isotope Analysis, Organoleptic Evaluation, Fourier Transform Infrared Spectroscopy (FTIR), Heavy Metal Testing, Microbial Testing (Sutour, 2010).

Chapter I: Plants study

The most common method for obtaining the essential oil from the plant material is distillation. Most often it is carried out using glass Clevenger-type or Deryng apparatus (Tomaz BAJ, 2015) .

The Clevenger apparatus based on distillation is the most used method to determine the essential oil content of the fruit and has become the reference technique (Pingret, 2013).

1.3 Part one: Plant *Melissa officinalis L*

1.3.1 Historic :

It was previously known to the Greeks and Romans that used it in medicines, spice and also to make honey from it. Théophrast (de – 371 à – 286 av. J.-C.) describe it as medicine against insect bites, in menstrual disorders, abdominal pain and against rheumatic diseases, Iben Sina Bohara (Avicenna, 908-1036) describes the plant as a cardiac tonic. Paracelsus: who was interested in the extraction of active substance, (*Quinta essencia, arcanum*), extracted from this drug such an essence which "rejuvenates old ladies". This remark is echoed in data from 13th century England, according to which an individual who regularly consumes lemon balm tea for years can reach a very old age. Traditionally, lemon balm is used in the symptomatic treatment of digestive disorders and in the symptomatic treatment of neurotic conditions and as a neck massage product against rheumatic stiffness (Babulka, 2005).

1.3.2 Plant definition:

The Latin name ''Melissa'' (balm) has its roots in the Greek word ''meliteia'', from ''meli, melitos'' (honey) and refers to the great attraction of the plant for bees, the term ''officinalis'' was first added at the time of Linnaeus. It was first mentioned in a pharmacopoeia in France in 1733 (Herodez, 2002).

Melissa likes a light soil, a warm exposure, it is propagated by shrapnel in the fall it reseeds itself. We Harvest in May before flowering the leaves and the tops when dried quickly on racks in a current of air. Then, store it in a dry and dark place (chancrin, 1922).

Chapter I: Plants study

1.3.3 Plant classification:

Reign:	vegetal
Order:	lamiales
Phylum:	spermaphytic
Family:	lamiaceae
Class:	dicotyledonous
Genus:	Melissa
Species:	*Melissa officinalis*
Common name:	Lemon balm, Sweet balm and Common balm
Vernacular name:	Abrezezou (Tipaza); Habt lhlawa (Medea) and Ferziouza (Bouira) (Quezel, 1963)

1.3.4 Geographic Distribution:

1.3.4.1 In World:

This is spaces originated from Southern Asia Minor and southern parts of North America. Lemon balm population, distributed in all Mediterranean countries, including the coastal region of turkey and Northern Iran (Jafer, 2008).

1.3.4.2 In Algeria:

In Algeria, lemon balm is grown in the regions of Kabylie. Nevertheless, it is spontaneous in the mountains of Tell, where it is reported as quite rare until 1962. It is observed in the humid ravines of the mountains of Babors, Djurdjura and the Atlas Bliden, rubble, cool places, and forests, and around houses and cultivated in some place which apiculture use it (Feknous, 2014).

Chapter I: Plants study





Figure I 1 : plant of *Melissa officinalis* and flower (original) (Damous)



Figure I 2 plant of *Melissa offisinalis* (original)(Boumerdes)

Chapter I: Plants study

1.3.5 Chemical composition:

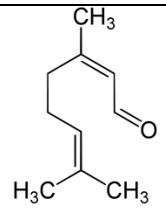
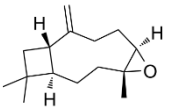
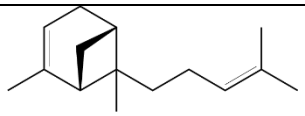
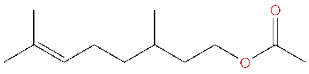
They identified a total of 22 chemical compounds in *Melissa officinalis* oil with GC- FID which are: Geranial 34.6%, nearl 26.0%, caryophyllene 7.5%, caryophyllene oxide 5.3% - pinene 5.3% and sabinene 3.6% (Fernández, 2020).

Table 1 : chemical composition of *Melissa oficinalis* (Feknous, 2014)

N°	Percentage (%)	Retention time **	Probable substance***
1	0,9	1002	sulcatone
2	3,6	1013	sabinene
3	5,3	1014	β-pinene
4	1,8	1054	β-Ocimene
5	1,5	1098	Linalool
6	0,2	1129	Citronelal
7	0,3	1139	Canfora
8	0,2	1147	Bomeol
9	0,4	1158	Pinocarvone
10	0,3	1165	Terpinen-4-ol
11	0,7	1176	α-terpineol
12	26	1236	Neral
13	34,6	1270	Geranial
14	0,9	1385	Acetato de geranita
15	7,5	1408	β-Cariofilene
16	0,7	1443	α-Bisabolene
17	0,3	1460	Humulene
18	2,6	1476	β-selinene
19	0,8	1486	γ-Gurjunene
20	1,8	1495	Alloaromadendrene
21	0,4	1507	A-selinene
22	5,3	1572	Cariofilene oxid
	4,0		other
% Monoterpenes	76,7		
% Sesquiterpenes	21,6		
%other	1,7		

Results obtnaid by CG-FID chromatogram, **retention index calculated kovats index,*** identification confirmed by CG-MS

Table 2 : Chemical compounds of *Melissa oficinalis* essential oil (Feknous, 2014)

Retention Time (min)	Majority compounds	Chemical structure
7.62	Néral (citral b)	 $C_{10}H_{16}O$
10.84	(-)-oxyde de caryophyllene	 $C_{15}H_{24}O$
11.86	Bergamotene	 $C_{15}H_{24}$
13.88	Citronellyl acetate	 $C_{12}H_{22}O_2$

1.4 Part two: CLOVE

1.4.1 Historic:

It is originated from the Moluccas Islands, it was very well known to the Chinese. Arabs used it and supplied Europe as spices; cloves were used in Rome and France and considered a luxury product. Then clove tree was introduced into other warm regions such as Cayenne, Saint Domingue and Martinique. Zanzibar become one of the most important production centers (late 18th century), then will come the turn of Madagascar and Indonesia. Nowadays, cloves are available in all supermarkets, at affordable prices (Barbelet, 2015).

Chapter I: Plants study



Figure I 3: Cloves (original)

1.4.2 Geographic distribution:

Cloves is originally from Indonesia, Moluccas Islands, and today it is found everywhere because of its production for its properties, Indonesia 68.2%, Madagascar 15.5%, Tanzania 9.7%, Sri Lanka 3.2%, Comoros 2.3%,Other countries1.1% (Amrani, 2018).

1.4.3 Classification:

Reign :	Vegetal
Class:	Angiosperm.
Order:	Myrtales.
Family:	Myrtaceae
Sous-family:	Myrtoideae.
Genre:	Syzygium Gaerth. (AIDAOUI, 2021)

1.4.4 Chemical composition :

Cloves are essentially made up of 15 to 20% essential volatile oils. Clove essential oil consists mainly of Eugenol (91.2%).) (Barbelet, 2015).

The major component of clove oil is usually considered to be eugenol, with β-caryophyllene and lesser amounts of other components such as benzyl alcohol, but the proportions vary widely (Chaieb, 2007).

Chapter I: Plants study

Table 3 : Composition and percentage of clove essential oil (*Eugenia caryophyllata*) obtained with GS-MS analysis (Chaieb, 2007)

N°	Percentage (%)	kovats index (HP-20M) **	Probable substance ***
1	0.93232	1172	2-Heptanone
2	0.66098	1232	Ethyl hexanoate
3	tr	1304	2-Heptanol
4	tr	1384	Menthyl octanoate
5	tr	1392	2-Nonanone
6	tr	1429	Ethyl octanoate
7	tr	1459	α-cubebene
8	tr	1491	Copaene
9	tr	1499	2-Nonanol
10	tr	1548	Linalool
11	tr	1588	2-Undecanone
12	1.38830	1595	β-Caryophyllene
13	tr	1619	Menthyl benzoate
14	tr	1647	Ethyl benzoate
15	0.19985	1668	α-Humulene
16	tr	1669	Menthyl chavicol
17	tr	1675	α-Amorphene
18	tr	1695	α-Terpinyl acetate
19	tr	1711	α-Muurolene
20	tr	1714	Benzyl acetate
21	tr	1731	Carvone
22	tr	1756	γ-Cadinene
23	tr	1826	2-Phenyiethyl acetate
24	tr	1827	(E)-Anethole
25	0.10538	1828	Calamenene
26	tr	1861	Benzyl alcohol
27	0.11437	1918	Calacorene
28	tr	1976	Caryophyllene oxide
29	tr	1985	Menthyl eugenol
30	tr	1986	Humulene oxide
31	tr	2018	Cinnamic aldehyde
32	tr	2072	Ethyl cinnamate
33	tr	2103	Benzyl tiglate
34	88.58535	2151	Eugenol
35	5.62086	2263	Eugenyl acetate
36	0.27527	2265	Humulenol
total identified	98.2769	98.2769	

Order of elution on HP-20M capillary , Identified by comparison of the mass spectral and Kovats index data, ** Kovats indices on HP-20M column, tr: trace (<0.1%).

ChapterII:*Varroa destructor*

2 Chapter II: *Varroa destructor*

2.1 Historic:

This mite was collected for the first time by the entomologist Edward Jacobson, on bees of the island of java, an acarologist made the first description of it in 1904 (Vidal-naquet, 2011).

2.2 Definition:

Varroa destructor is an external parasitic mite that contributes to the collapse of bee colonies, resulting economic losses problems related to the role of bees as the most important pollinators on earth (Koumad, 2015).

2.3 Morphology:

Varroa is a small flattened crab, (Clément, 2006) female that is usually found. Brown or dark brown in color and brilliant of ellipsoidal shape, it measures from 1 to 1.8 mm of length and 1.5 to 2 mm in width, visible to the naked eye on its host (Anderson, 2000).

Its weight is around 325 µg in the phoresis phase to 480 µg in the breeding phase, the females are very agile, and the ends of their legs are equipped with suction cups to hold on to the bees (Baker, 1984). Legs are short; body is covered with numerous bristles. Mouth has a biting-sucking device that allows it to pierce the bee's coating to suck up the hemolymph. Its flat shape is well suited to lodge between the pupa and the walls of the cells as well as on the body of the adult bee (Ellis, 2010).

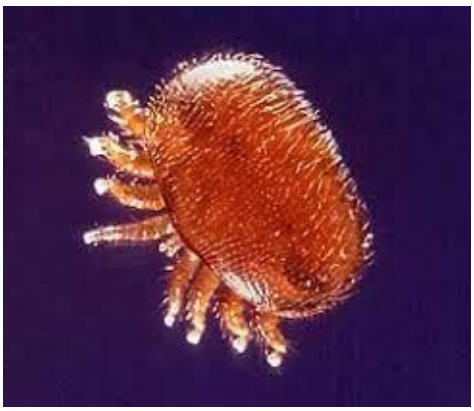


Figure II 4 : *Varroa* morphology (Duran, 2017)

Chapter II: Varroa destructor

2.4 Classification:

Reign:	Animalia
Under reign:	Metazoire
Embranchement:	Orthropoda
Under embranchement:	Chelicerata
Class:	Arachnida
Under class:	Acari
Super order:	Parasitiforme
Order:	Mesostigmata
Sous order:	Monogynaspida
Cohorte:	Gamasina
Under cohort:	Dermanyssiae
Super family:	Dermanyssoidea
Family:	Varroidae
Genus:	Varroa
Species:	*Varroa destructor* (Anderson, 2000)

2.5 Geographic distribution:

2.5.1 In world:

This mite native to Southeast Asia, nowadays in Asia, North America and Europe, there is no apiary escapes from this mite (Boudagga, 2003).

2.5.2 In Algeria:

The existence of *Varroa destructor* in Algeria has been reported for the first time in 1981, in the East in the apiary Oum Theboul El Kala (East Algeria). This parasite spread rapidly from there (Belaïd, 2010).

2.6 Life cycle:

The lifespan of the parasite is adapted to the life cycle of the bee. (Martin, 1998)

- Female begins oviposition about 60 hours after entering a brood cell, the development from egg to adult takes place in the operculum cell (Martin, 1998).
- Egg: 24 hours
- Free protonymph: 30 hours and first moult 24 hours
- Deutonymph: 48 to 60 hours
- Adult after an imaginable moult: 24 hours

Chapter II: Varroa destructor

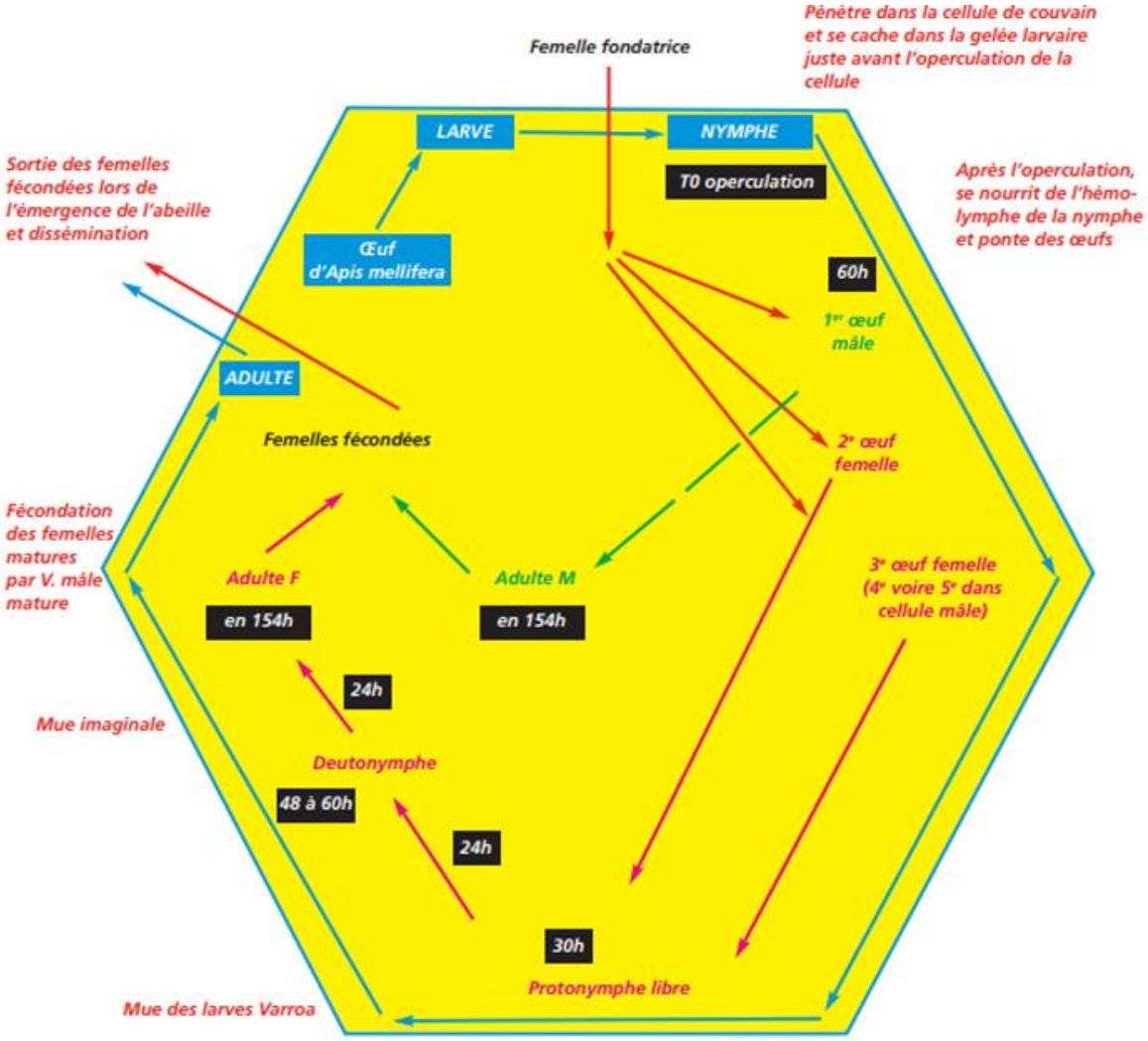


Figure II 5 : *Varroa destructor* cycle life

Chapter III: *Apis mellifera*

3 Chapter III: Apis mellifera

3.1 Generality:

Bees are insects, the genus *Apis* including several species. Some of them, such as *Apis mellifera* or honeybee, have been domesticated by man for their production of honey, pollen, royal jelly, propolis, wax and in some cases venom (Talbi, 2014).

The bee colony is monogynous because it contains only one queen. A domestic colony has 40,000 to 60,000 individuals during the summer season and cuts to 15,000 individuals or even 5,000 individuals in winter (Talbi, 2014).

3.2 Classification:

Embranchment:	Arthropoda
Under-embranchment:	Hexapoda
Class:	Insecta
Under-class:	Pterygota
Infra-class:	Neoptera
Super-order:	Endopteygota
Order:	Hymenoptera
Under-order:	Apocrita
Infra-order:	Aculeata
Super-family:	Apoidia
Family:	Apidae
Under-family:	Apinae
Tribu:	Apini
Genre:	Apis
Spices:	*Apis mellifera* (Douhet, 1977)

3.3 Bee morphology:

3.3.1 Queen:

The queen or the mother has a fundamental role in the life of the colony, young, vigorous and in good health. It measures 18 to 22 mm long and a thorax which reaches 4.2 in diameter with brick color or too dark, its abdomen is monochromatic and not striped, it

has short and toolless legs (longer than that of the worker bees),its mouth apparatus is reduced and its long is shorter (Talbi, 2014).

3.3.2 Workers:

The workers represent the very large majority of the population; have significant physiological and behavioral capacities, to ensure the different works within the hive. (Talbi, l'effet acaricide de l'huile essentile d'ecalyptus globulus sur varroa destructor, 2014). They measure about 12 to 13 mm long, thorax 4 mm in diameter, tongue length is 5.5 to 7 mm, legs equipped with brushes and a basket to collect the pollen, and the glands which produce the wax and make it possible to build the rays (Talbi, 2014).

3.3.3 Males:

Males they are unfertilized eggs laid by the queen, haploid n=16 (parthenogenetic reproduction). Eggs deposited in cells are larger than worker cells. Are approximately 15mm long and 5mm in diameter. This explains they are bigger than the workers and darker (Talbi, 2014).

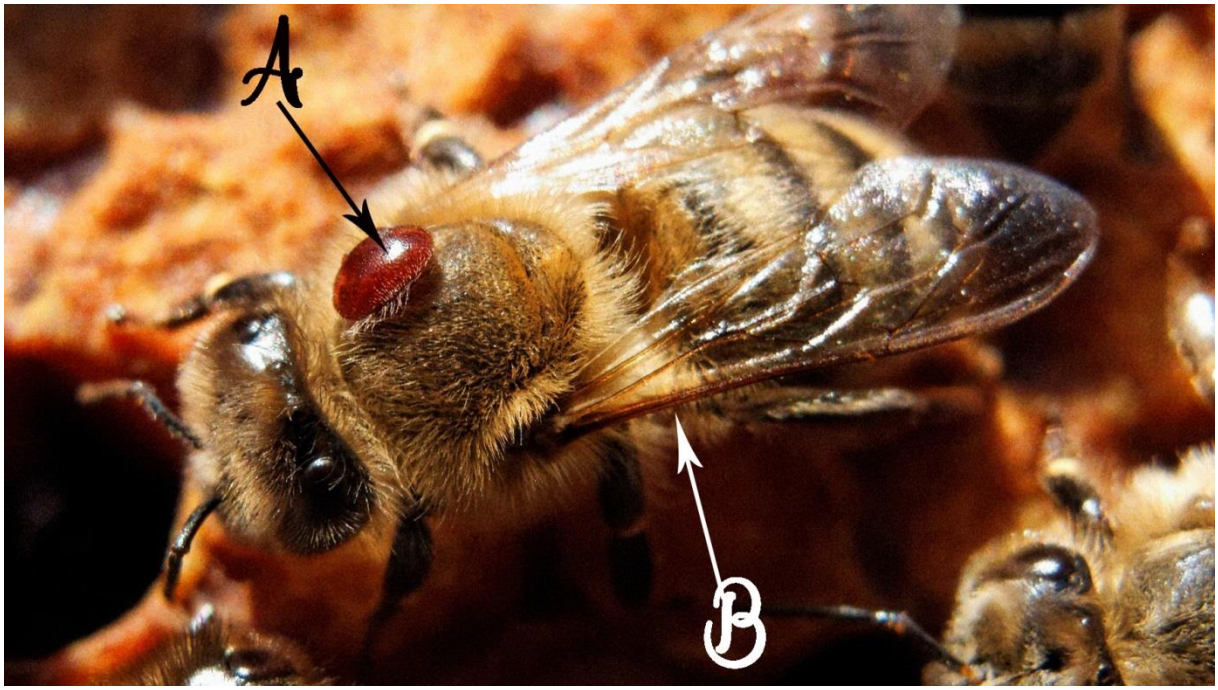


Figure III 6 : Bee morphology(B) and *varroa destructor* (A) (apiculteur.ch, 2021)

Chapter III: Apis mellifera

3.4 Geographic distribution:

3.4.1 In world:

The most widespread in the world is *Apis mellifera*, which extends from the southern tip of the African savannas, passing through the Mediterranean until reaching the limit of its expansion in northern Europe and southern Scandinavia. Such a variety of habitat, climatic conditions and flora, allowed the appearance of numerous subspecies or geographical races which are inter-fertile, each with its morphological and physiological characteristics adapted to each region (Adjlane, 2012).

3.4.2 In Algeria:

The race present in Algeria *Apis mellifera intermissa* is also called Tellian bee. *Apis mellifera sahariensis*, or Saharan bee, it is found in the south of Morocco and Algeria (Adjlane, 2012).

3.5 Life cycle:

The lifespan of the bee depends on pollen consumption. If young bees cannot consume this pollen, their lifespan is reduced (Maurizio, 1946).

The life cycle of the bee colony begins in late winter when the colony begins to grow. The main purpose of the colony is to reproduce in order to grow (Seeley, 2002).

During the winter, the bees gather in the center of the colony and form a cluster to maintain an adequate temperature for survival in the hive. At the end of the cold months, a hundred young workers are produced. During the spring, the colony has a maximum growth of up to 80,000 individuals (Free, 1977).

After fertilization of the queen by several males ،she becomes able to lay eggs in the comb cells. The eggs will give workers and queens ،and only diet determines the caste of these individuals (Conte, 2005).

3.6 The critical phase between bees and varroa destructor:

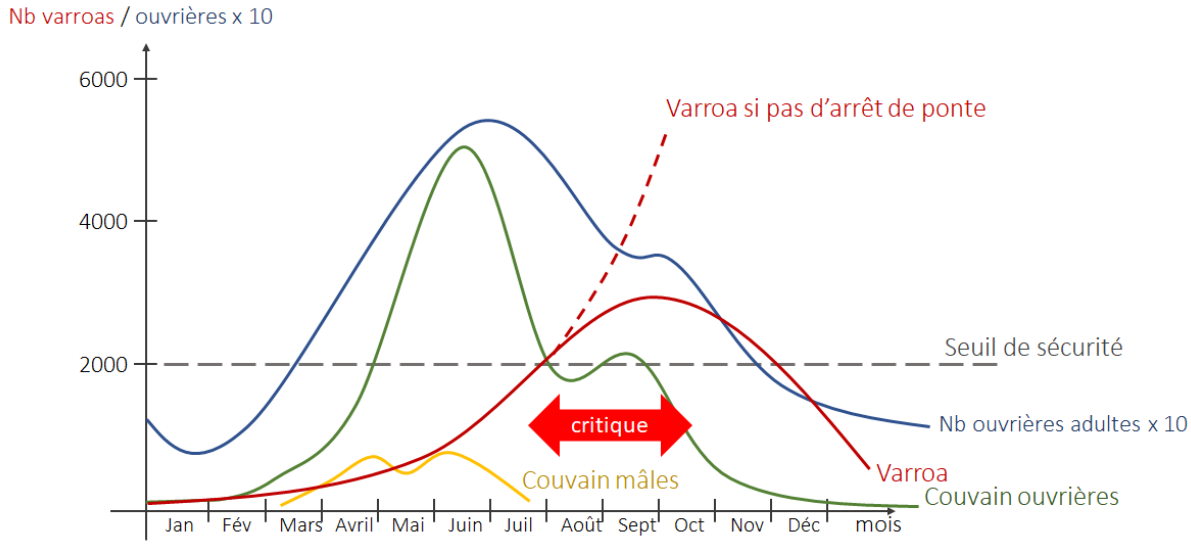


Figure III 7 : the critical phase between bees and *varroa destructor* (ApiSavoir.ch)

The development cycles of *Varroa destructor* and the bee are intimately linked

However, it remains dependent on the presence of bee brood to reproduce. In the absence of brood, the varroa population cannot develop and the individuals are then all in the phoresy phase. They are then accessible and vulnerable to acaricides. For these reasons, the search for a brood-free period is interesting in order to optimize the effectiveness of a treatment. In order to take advantage of the advantages of stopping egg-laying, it is advisable to carry out this operation in July. It is only when the queen is again in full laying, at the beginning of August (Imboden).

3.7 Main treatment periods:

Before the first honey flows, therefore in February – March. This treatment depends on the results of the surveys carried out in November / December: if the surveys show an infection of more than 50 varroa mites / beehive. Only products that are not subject to temperature conditions may be used (l'action sanitaire ensembe GDS centre, 2011).

The interpretation of infestation depends on the season. The treatment must be applied as soon as there is more than one varroa in the spring, while in summer, after the honey flows, the treatment must be applied if there are more than 25 varroa per day (l'action sanitaire ensembe GDS centre, 2011).

Chapter III: Apis mellifera

3.8 Damages:

We can consider the varroa destructor as the scourge of beekeeping because of serious damage to bee colonies (Boubaker, 2019).

Parasitized bee's colonies are weakened by the mite are predisposed to secondary infections such as viruses and bacteria: such as American or European foulbrood, and acute paralysis (Salem, 2013).

Physiologically:

- Parasitized bees show a reduction in hemolymph volume, varroa deprives the bee of several blood cells and proteins.
- The jelly produced by the nurse bees is then of lesser quality, which is detrimental to the good development of the brood.
- The number of hemocytes, one of the most employed measures of immune system compasant, is reduced (Salem, 2013).
- Etiological modification (loss of sense and direction) and reduction in flight ability.
- Reduction in the lifespan of the queen, sometimes leading to a stoppage of egg-laying.
- Reduction in size and malformation of the imagos.
- Reduction of male sexual potential (Conte, 2005).

Material & methods

Material and methods:

3.9 Presentation of Vegetal material:

3.9.1 *Melissa offisinalis*:



Figure 8 : Vegetal material *Melissa offisinalis*

Our vegetal material was the aerial parts of plants, it was collected in Boumredes

3.9.2 Cloves:



Figure 9 : Cloves

We just bought it from the supermarket

Material and methods:

3.10 Extraction workplace Representation:

The first extraction takes place in the laboratory of the experimental station of Blida

The second experience takes place in the CRAPC expertise: which is a center for carrying out analyzes on heavy metals and nutrients or contaminants in all living or non-living organisms, with, in addition to figure out, the use of high-tech equipment in analysis physico-chemical.

3.11 Apiary presentation:

Apiary is located in a large green area, which is surrounded by flowers of chamomile and field mustard, and a ravine (Boukmouri) passes by, full of *Tamarix* Tree that we can consider it as a melliferous tree.

The beehives made available to us are of the most common Langstroth model in Algeria, there are 50 beehives. Some of it with only five frames, some with ten frames and some with a beehive rise so the hive can hold 20 frames.



Figure 10 :Photo of the apiary (original)

Material and methods:

3.12 Oil extraction:

3.12.1 First extraction of *Melissa offisinalis* in Blida:

1. 350g of *Melissa officinalis* plant was weighed that is already dried for a week in the open air and away from light and cut into small fragments then we put under pressure cooker which is attached with an extraction system
2. we added about 3500ml of water
3. we lit the fire and waited for the extraction

3.12.2 The second extraction of the essential oil of Melissa officinalis in CRAPC:

1-50g of *Melissa ofissinalis* plant was weighted by us which was already dried for a week in the open air and away from light and cut into small fragments; we put them in the 2 L balloon

2- Fill the balloon with distilled water up to the two triremes with 1300ml and put it in the balloon heater

3- We use an extraction system: Clevenger.

The extraction lasts 3 hours after boiling the water

4- We recover the oil obtained in a vial using a solvent (ether d'ethylene)

5- We injected 1 µl with a syringe of the solution obtained in the GC / MS for analysis.

Material and methods:

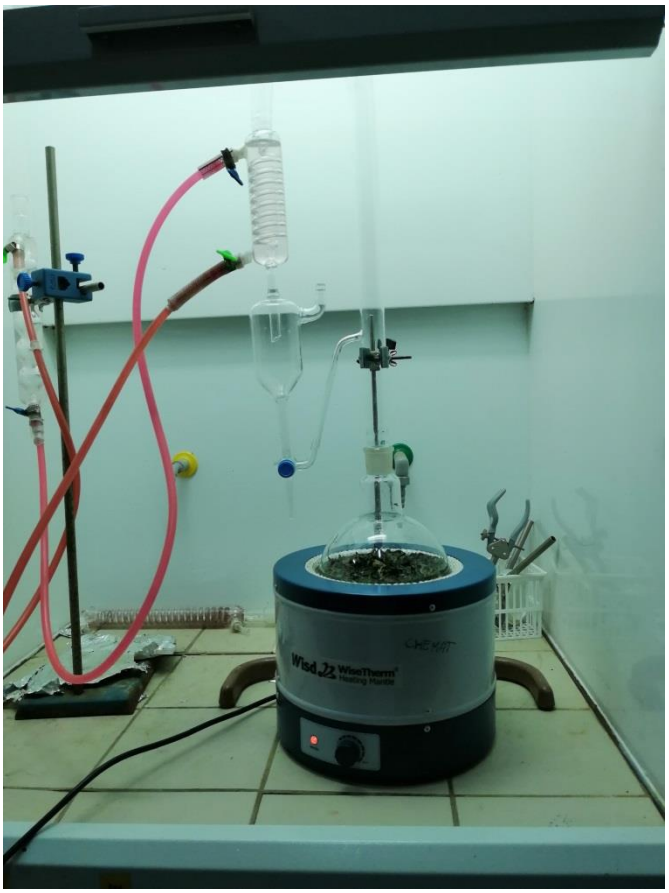


Figure 11 : Extraction device (**original**)

1. Dried plant was cut into small pieces

2. 80g of the plant was weighed by balance

3. We put them in a 2000ml balloon and was filled with 1400ml of distilled water

4. The extraction takes place for 3 hours with a Clevenger which has a small tube for the oil to condense and facilitate its recovery

5. Diethyl ether is used to recover traces of oil stuck to the edges

Material and methods:



Figure 12 : Extraction device (**original**)

3.12.3 Cloves extraction:

We change the plant: we use cloves

1. We weighed 85g of cloves then ground them into a powder and introduced them into a 1000ml balloon

2. Add 800ml of distilled water using a graduated cylinder

3. We carry out the hydrodistillation assembly and start the heating to the maximum at the beginning

Hydrodistillation lasts 3 hours

4. Pour the contents into a separating funnel

5. Add 5ml of dichloromethane solvent

6. Shake gently, degas, and then leave to decant with the cap open

7. We collect them in a labeled vial

Material and methods:



Figure 13 : Reprsennt clove extraction and decantation (original)

3.13 Oil identification by GS/MS :

Injector:

Temperature 250°C

Injection mode: split 1/20

Volume injected: 0.2 µl

Column :

Type: HP-5MS

Dimensions: length 30 m * D int 0.25 mm * film thickness 0.25 µm

Stationary phase: 5% Phenyl 95% dimethylpolysiloxane. (Other: Specify)

Oven temperature: 60°C for 8min, 2°C/min up to 250°C., isothermal for 10 min.

Analysis time: 113 min

Carrier gas: Helium Purity: N6.0; GV flow: 0.5 ml/min

Mass detector:

Analysis mode: Scan TIC (from 30 to 550)

Solvent delay: 3.5min

Interface temperature: 280°C

Type of ionization: Electron impact

Filament Intensity: 70 ev

Mass Analyzer Type: Quadrupoles

Material and methods:

Source temperature: 230°C

Equipment :

Chromatograph: Hewlett Packard Agilent 6890 more

Mass spectrometer: Hewlett Packard Agilent 5973

3.14 Concentration:

We aim to prepare three concentration 10%, 15%, and 25% of cloves essential oil by using the tween 80 dilute for 3%.

3.15 Wooden tongue preparation

We used it as a holder for beeswax mixed with our essential oils because it is inert to any interaction with bees



Figure 14 : wooden tongue

3.16 Method of treatment application

15 beehives are taken for the experiment, 3 as a negative control, 3 positive controls (using a biological product: organic natural strip for varroa), three for the first concentration of 10%, three for 15% and three for 25%.

Material and methods:

The procedure is to apply the treatment and visit the beehives twice a week for a month to count dead varroa mites and compare the results of the three concentrations with itself and with the biological product and the negative control.

We use wooden tongue depressor as a holder for the beeswax, which is mixed with the different concentrations of oil, the wax which allows the oil to be diffused in the hive and put it in under the frames at the bottom of the beehive.

3.16.1 Estimation of varroa mite mortality: (A)

The number of dead varroa mites divide on 30; it gives us an approximate number from real one (the estimate is made by dividing the daily mortality, this value by 90 days (maximum lifespan of varroa female). (Boubaker, 2019)

3.16.2 Estimation of daily varroa mortality: (B)

Estimated number of varroa in a beehive: B=A*90 (Boubaker, 2019)

3.16.3 Estimate of the number of bees in a beehive:

A frame can contain up to 2000 bees, if we have 10 frames in the hive then we get a number of 20 000 bees. (Boubaker, 2019)

CM % = (M-Mt*100)/ (100-Mt) (Boubaker, 2019)**.**

CM%: corrected mortality

M: Death percent in the treated population

Mt: Death percent in control population

3.17 Data analyses:

We use ANOVA test to analyze the data (variance analysis), the differences were considered significant at $p < 0.05$.

Results & discussion

4 Results and discussion:

The general problem of this research theme is about the quality, characterization, and valorization of the substances *Melissa offisinalis* and Cloves which are renewable resources contribute to the protection of the environment, the maintenance of biodiversity.

In order to find a solution, we carried out extractions of essential oils *Melissa offisinalis* and Cloves. Then we have the tests like biocides

4.1 *Melissa offisinalis* extraction:

In the beginning, we started by trying the extraction of the *Melissa offisinalis*, unfortunately, we failure to collect the oil by hydrodistilation as well as GS/MS technique. This problem is due probably to the low concentration of the oil in this plant or by using dichloromethane solvent we induce a higher dilution of our samples.

According to Bounihi and all study the extraction of Melissa officinalis by distillation procured 0, 4% of essential oil and 16, 5% of aqueous extract; these results are considered as a very low yield (Bounihi, 2016).

However, Feknous and al reported that the extraction of the essential oil from the plant, accomplished by the process of steam distillation prior to flowering, gave a yield of 0.17% (Feknous, 2014).

The use of gas chromatography coupled with mass spectrophotometry allowed us to know the qualitative molecular composition of the essential oil of this plant; It was previously described that the *Melissa offisinalis* oil is mainly composed by citral, caryophyllene oxide, bergamotene and citronellyl acetate.

The extraction of the non-volatile compounds by the Soxhlet gave 10.86% of the apolar compounds and 37.17% of the polar compounds.

The extraction of certain active ingredients by maceration made it possible to quantify a content of 7.6% tannins and 0.6% glycosides flavonic, thus giving this plant different therapeutic property.

The amount and range of chemical composition of EO plants of the same species in different regions may be caused by microclimatic, phytogeographic factors, plant genotypes and geographical and agronomic conditions, especially soil. However, as a

general rule, the main components remain the same, only varying their concentration levels (Kumar, 2011)

4.2 Cloves extraction:

The extraction of cloves gave a good yield in our experience.

The dried leaves, on hydrodistillation, gave 4.8% of the oil. (Raina, 2001), on the contrary (Kennouche, 2015) reported that Clove contains 14-20 % of volatile oil in their study which is a very high yield, same results were found in (Muchalal, 2017) study with 14,3% of yield for the Madagascar clove spices.

4.3 Chemical composition of cloves essential oil

This chromatogram represents the abundance of molecules in cloves oil

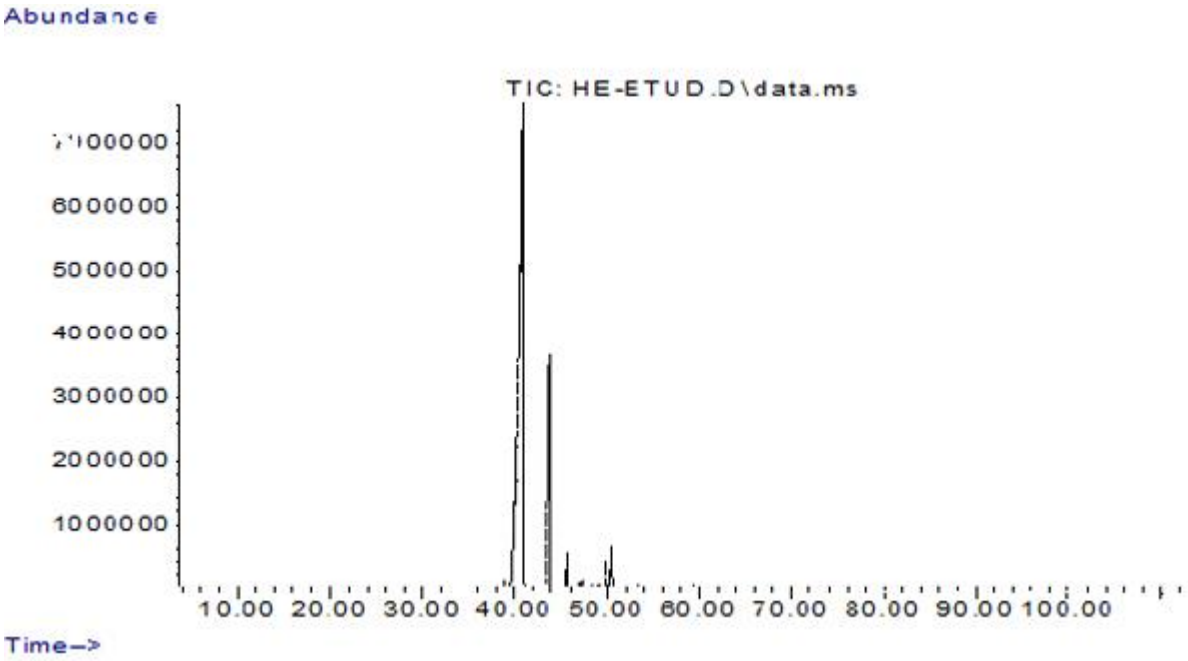


Figure 15 :Chromatogram of cloves

X axis represents time

Y axis represents the abundance of molecules in the oil

Each peak represents a molecule

The area under the peak represents the percentage of the molecule in the oil

The first high peak is the Eugenol (80.11%), follows with (E)-Caryophyllene (13.94%).

Results and discussion:

4.4 Oil identification:

Table 4 : cloves oil

RT	KI	Composé	%
28,41	**1199**	Methyl salicylate	0,04
38,95	**1355**	α-Cubebene	0,23
39,91	**1370**	Eugenol	80,11
41,8	**1399**	Siberene	0,04
43,02	**1419**	Methyl Eugenol	0,06
43,8	**1431**	(E)-Caryophyllene	13,94
45,44	**1458**	(Z) Methyl isoeugenol	0,03
45,61	**1461**	α-Humulene	1,16
46,03	**1467**	Allo aromadendrene	0,06
46,83	**1480**	Trans-Cadina-1(6),4-diene	0,18
47,04	**1484**	γ-Muurolene	0,13
47,29	**1488**	α-Amorphene	0,23
47,56	**1492**	Aristolochene	0,03
47,9	**1498**	ɣ-Amorphene	0,04
48,88	**1514**	δ-Amorphene	0,04
49,05	**1517**	ɣ-Cadinene	0,08
49,29	**1521**	δ-Cadinene	0,90
50,1	**1535**	Eugenol acetate	2,07
59,37	**1699**	2',4',6'-Trimethoxyacetophenone	0,11
Total			**99.460**

We identified a total of 19 chemical compounds in cloves oil in the GC/MS which are Eugenol (80.11%), (E)-Caryophyllene (13.94%), Eugenol acetate (2.07%) and others with trace

Results and discussion:

The major component of clove oil is usually considered to be eugenol, with β-caryophyllene and lesser amounts of other components such as benzyl alcohol, but the proportions vary widely. For example, (Prashar & Evans, 2006)found the content of eugenol to be 78%, with 13% β-caryophyllene, whereas (Pawar, 2006)found that the content of eugenol was 47.64%, with the concentration of benzyl alcohol at 34.10%.

(Chaieb, 2007) Reported that the chemical analysis resulted in the identification of 36 components, with a high concentration of eugenol (88.58%), eugenyl acetate (5.62%), β-caryophyllene (1.39%), 2-heptanone (0.93%), ethyl hexanoate (0.66%), humulenol (0.27%), α-humulene (0.19%), calacorene (0.11%) and calamenene (0.10%). These data are in agreement with the results from other studies.

4.5 analyses:

The data related to varroa mite mortality during the three outings allowed us to highlight the different quantitative variations in mortality between the positive control, the negative control and the concentration dilutions of the Cloves extracts at 10%, 15% and 20%.

During the three outings carried out during our study period, we quantified the evolution of varroa mite mortality, which is generally characterized by significant fluctuations in numbers (Figures 14, 15, 16, 17 and 18). During the first outing we observed significant mortality with the negative control. This is linked to the natural condition: their biological cycle on the other hand (T+, 10%, 15% and 20%), we recorded a low mortality rate which explains the contact with active materials which acts on the behavior and the physiology of the insect by disturbances of their life cycles. In addition, we observed fluctuations in mortality in all of our bio-controls and more accentuated with the T+ during the second outing. During the third trip to we noticed a significant mortality with the dilution at 15% of cloves.

Results and discussion:

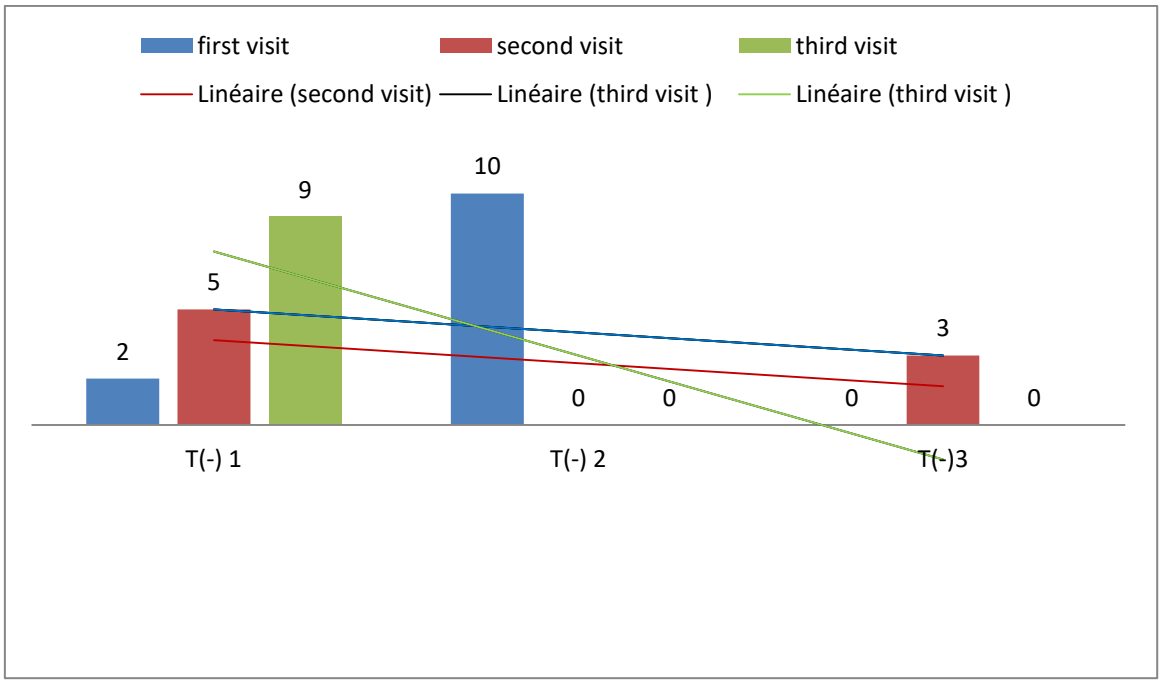


Figure 16 : Varroa percent death in control population

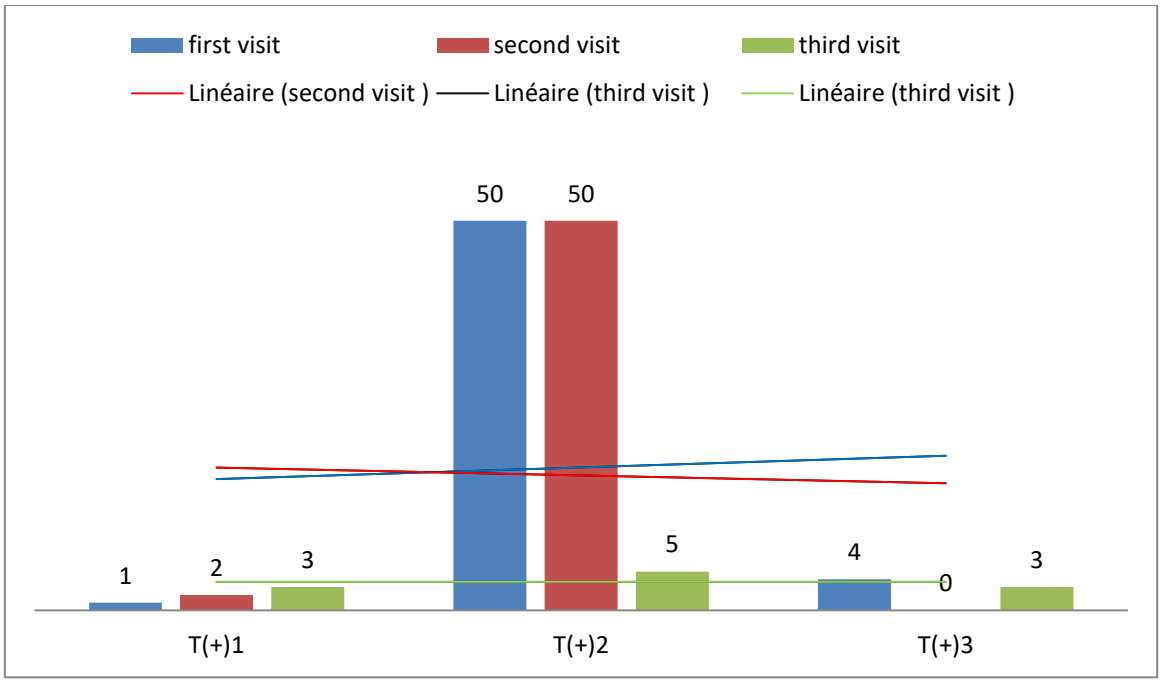


Figure 17 : Varroa mortality in positive control for three beehives, treated with a biological product

Results and discussion:

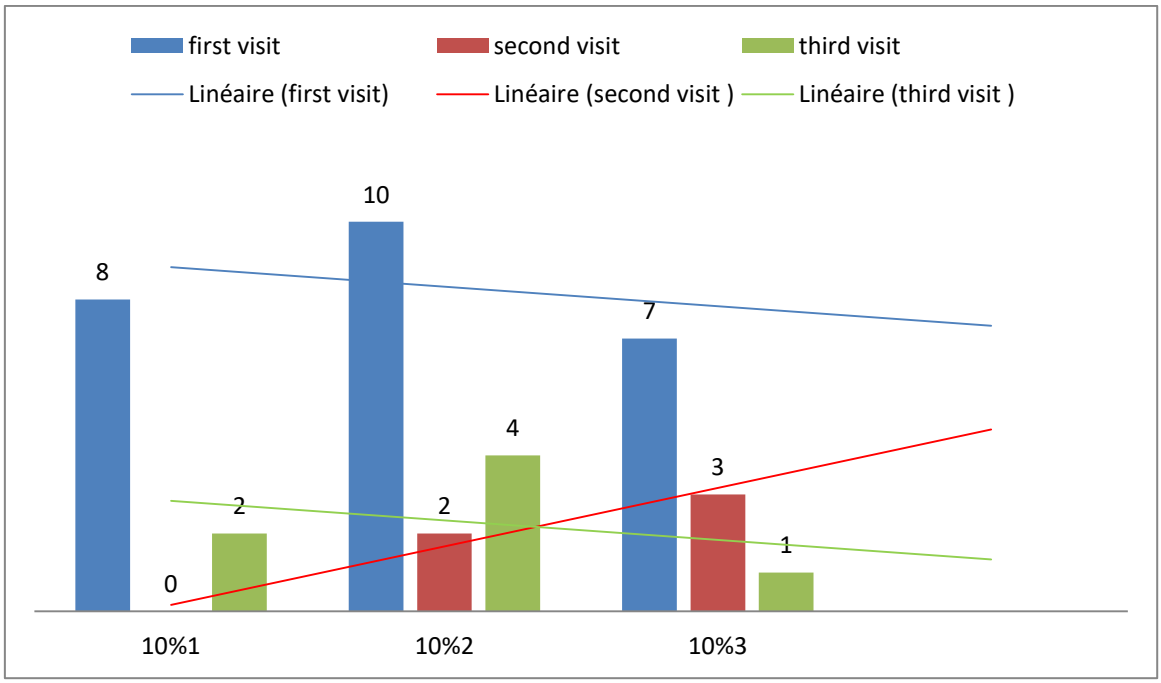


Figure 18 : Varroa mortality in concentration 10% of essential oil

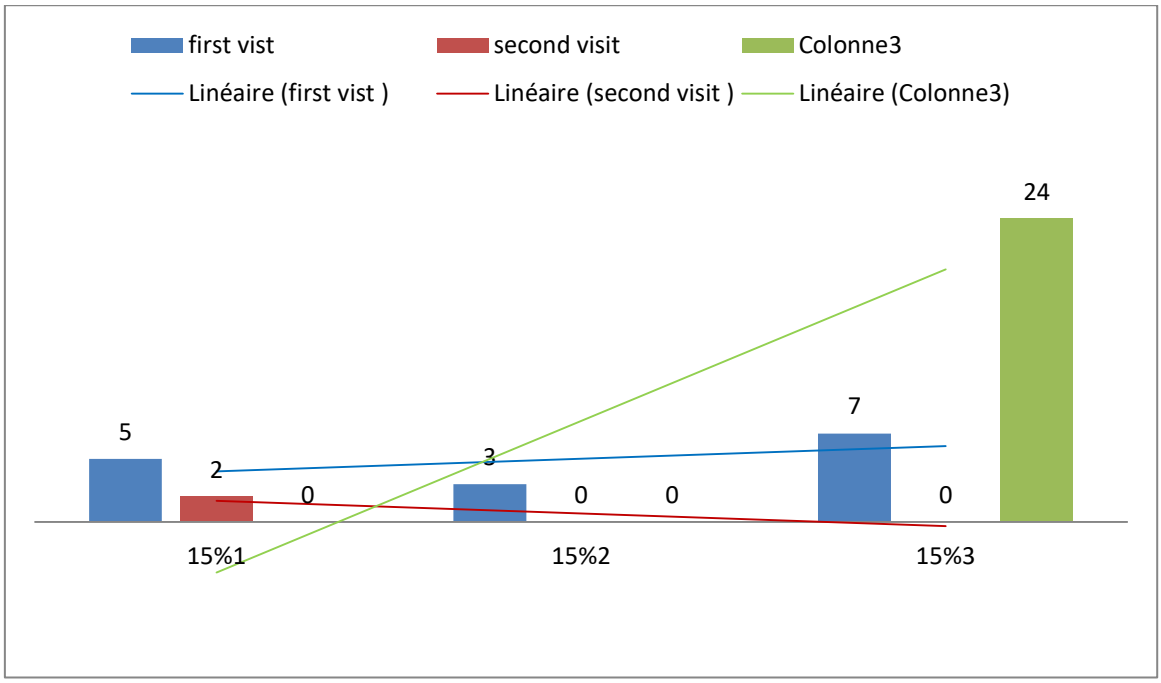


Figure 19 : Varroa mortality in concentration 15% for three beehives of essential oil

Results and discussion:

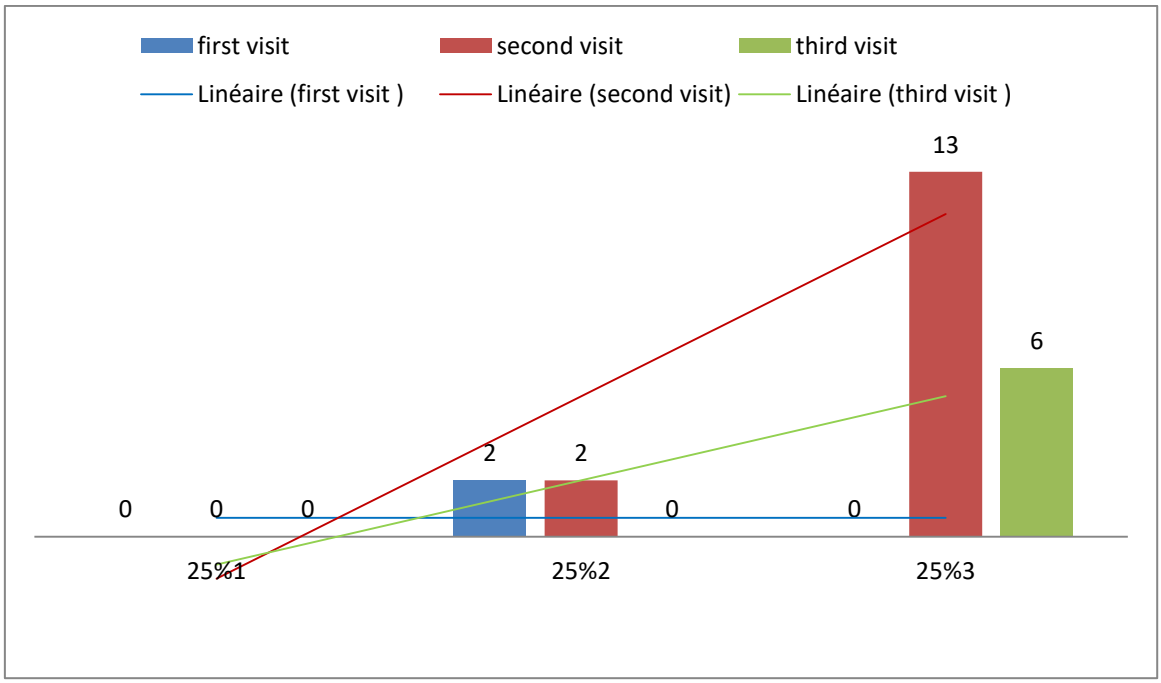


Figure 20: Varroa mortality in concentration of 25% for three beehives of essential oil

4.6 Data analyses

Table 5 : p value of comparison between different concentrations

	T-	T+	10	15	25
T-			0.285	0.695	0.786
T+	0.420		0.457	0.492	0.395

By comparing between the three concentrations to positive or negative control we noticed that p-value was generally not significant. This result due to the small number of varroa and limited duration of the experience (only 3 visits)

As we can see on table below, by comparing our oil extract effect to the commercial one we demonstrated that they have a quiet similar influence on the varroa. Also, this data was reproduced with the three tested concentration which confirmed that cloves oil is greatly effective on varroa even at low concentration.

Results and discussion:

Table 6 : corrected mortality percentage

	Ti	Vn	CM%
Rt	**0,0145**	**290**	
Rc	**0,059**	**1180**	**146,4211**
R10	**0,0185**	**370**	**150,6842**
R15	**0,0205**	**410**	**150,4737**
R20	**0,0115**	**230**	**151,4211**

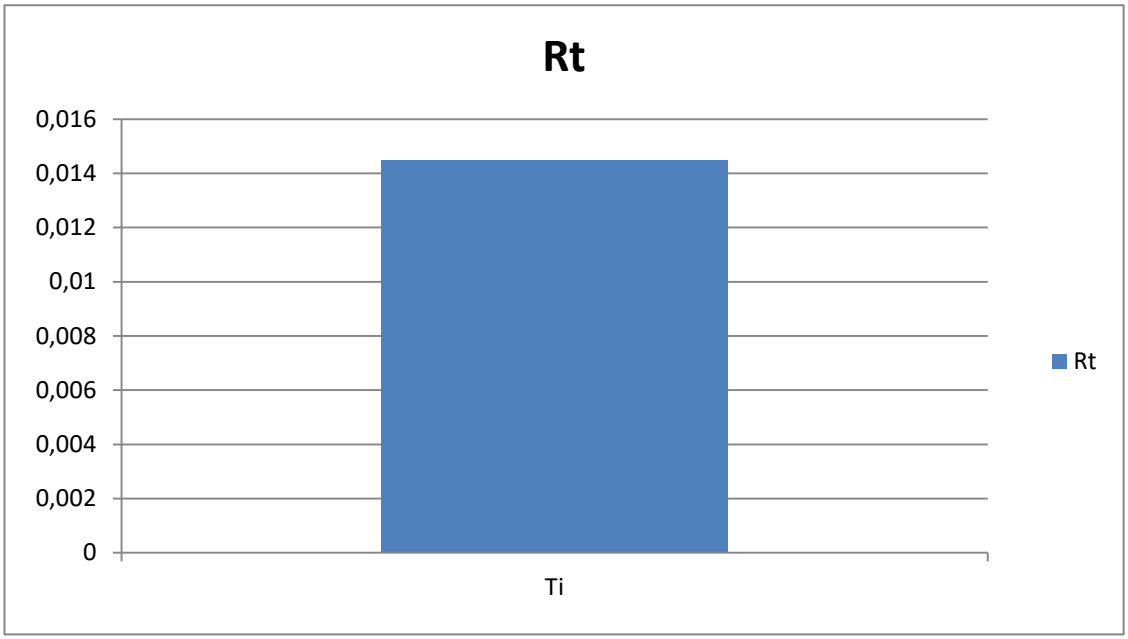


Figure 21 : varroa initial estimate

Results and discussion:

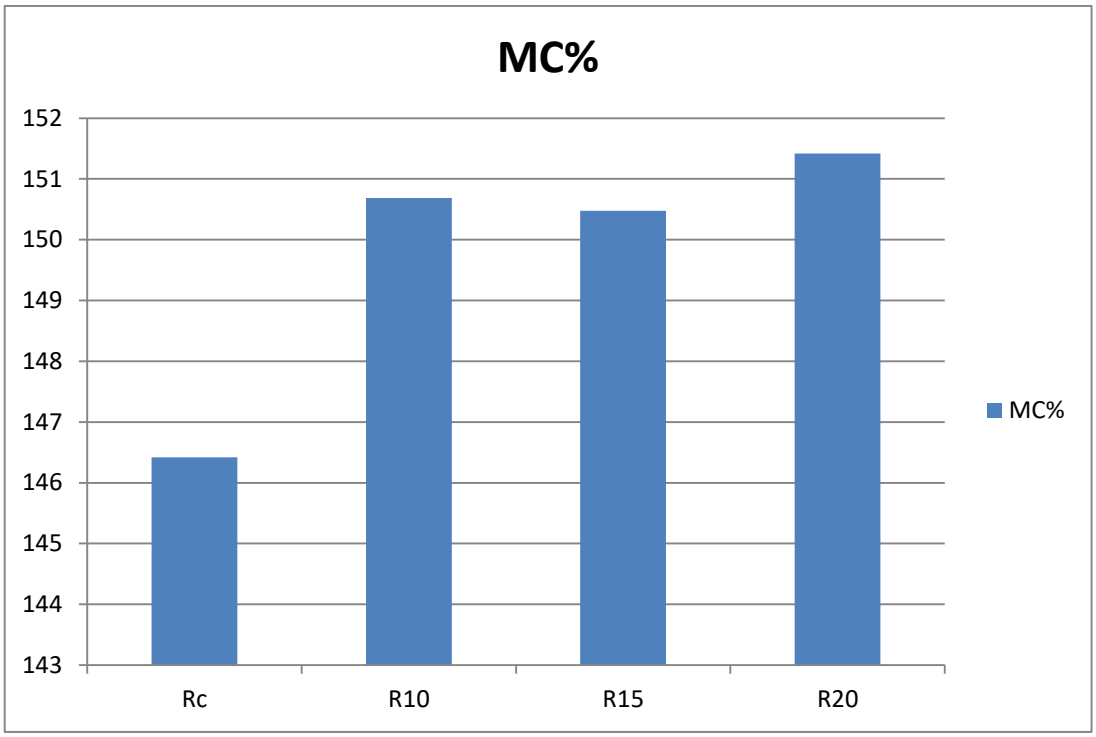


Figure 22 : corrected mortality

We obtained a total of 275 dead varroa, this small number due to some reason

Firstly, this is the first infestation of varroa in this apiary (there is not a large population of varroa)

The period of (April -may) is the beginning of the varroa's mites life cycle.

Due to rain, there has been a move in the days that we had to visit the apiary to count dead varroa.

The bees cleaned their beehives from the dead varroa mites, and from the papers that we put under frames so that the varroa mites fall in for counting

These causes that make the total number of dead varroa more than the number obtained.

Results and discussion:



Figure 23 : paper which is cut by the bee and thrown out of beehive(original)

4.7 Insecticidal activity:

The biological activity of *Eugenia caryophyllata* oil has been investigated against several parasites. It was shown to inhibit the emergence of *Culex pipiens* larvae. and to display insecticidal activity against *Pediculus capitis*. And some stored product insects and suppresses progeny development of *Tribolium castaneum* and *Sitophilus zeamais* with isoeugenol being particularly active. (Chaieb, 2007)

Clove essential oil has also showed acaricidal activity towards *Dermatophagoides farinae* and *D. pteronyssinus* with eugenol being identified as the acaricidal constituent of the oil. Eugenol congeners also exhibited potent acaricidal activity against both mite species, (Chaieb, 2007)

And (Perrucci, 1995)reported its effects against the causative organism of psoroptic mange, *Psoroptes cuniculi*, by direct contact and by contact only with the vapour phase. A more recent study has confirmed that clove oil could be used as a novel fumigant against Japanese termites (Park, 2005)

Results and discussion:

The efficacy of clove *Syzygium aromaticum* L oil as a treatment for V. destructor mites has been previously demonstrated both in the laboratory and in the field. In a 20-ml glass-vial residual test, 0.75 mg of clove oil caused a 96% mortality rate in V. destructor (Gashou, 2009)

A 2012 study by Su et al. showed that clove oil caused a 60% mortality rate in V. destructor mites at a dosage of 1.0 µl for 48 h, a level that is considered safe for bees. (Girisgin, 2014)

As an alternative to traditional acaricides, essential oil products present certain advantages, such as high toxicity to mites, low toxicity to bees, few residues in bee products and no drug resistance (Rosenkranz, 2010)

In Rashid at all study reported that The most effective combination was clove oil and tobacco extract when used in 5% concentration for 24 h. (Rashid, 2014)

4.8 Apiary pest:



Figure 24 : cockchafer (original)

According to Mr. Sahari, the cockchafer is a beetle harmful to the apiary, usually enters the beehive to feed on honey

Results and discussion:



Figure 25 : bee and *varroa destructor* (original)

Conclusion

Conclusion

Conclusion:

This result describes the known effects of clove oil and clarified its chemical composition by GC-MS and enabled the identification of 19components. The highest concentration was, as expected, of eugenol (80.11%), but the second most abundant compound was (E)-Caryophyllene (13.94%) followed by β-caryophyllene (2.07%), and 16 other molecule as trace with a total with 3.88%

In view of the known activities of the constituents and the variability of the oil, it is important that the composition is known. It has been shown that clove oil possesses acaricidal properties against the *varroa destructor*.

Our resultats analyses show a non-significant deference between the effect of the 3 concentrations we prepared(10%,15%,25%) from cloves with a safe effect on bees and an anti acaridial effect on varroa destructor

Also show a good effectiveness in varroa mortality percentage comparing it to the commercial product (organic naturel strip for varroa). Commercial product with 146%, R10% concentration with 150%, R15 % concentration with 150%, and R20% concentration with 151%,

By comparing our oil extract effect to the commercial one we demonstrated that they have a quiet similar influence on the varroa. Also, this data was reproduced with the three tested concentration which confirmed that cloves oil is greatly effective on varroa even at low concentration.

That allowed us to produce a biological treatment with the law concentration of cloves essential oil to minimalize the charge.

Bibliographic references

Bibliographic references:

(s.d.). *l'action sanitaire ensembe GDS centre.* (2011, novombre). Récupéré sur http://www.gdscentre.fr/index.php/abeilles/sanitaire/varroose

***apiculteur.ch.* (2021). Récupéré sur apiculteur.ch: https://www.apiculteur.ch/varroa-destructor**

Adjlane. (2012, décembre). Etude des principales maladies bactériennes et viralesde l'abeille locale Apis mellifera intermissa dans larégion médio-septentrionale de l'Algérie. Ecole Nationale Supérieure Agronomique – El-Harrach- Alger.

AIDAOUI,. (2021, Juillet). *Evaluation du pouvoir antiradicalaire des extraits de quelques épices (Cuminum cyminum, Curcuma longa et Syzygium aromaticum)*.

Amrani. (2018, Juillet). Etude de l'effet bio-insecticide de l'huile essentiellede Clous de Girofle (Eugenia aromatica) vis-à-vis d'un ravageur des denrées stockées (coléoptère;ténébrionidé) Tribolium confusum. Tizi-Ouzou.

Anderson,. (2000). Varroa jacobsoni (Acari: Varroidae) is more than one species. *experimental and applied acarology24*, 165-189.

anonyme. (2022, 03 02). *infoflora* . Récupéré sur Design and conception TWKS.CH, Info Flora: https://www.infoflora.ch/assets/db_doc/taxa_images/2012/06/20/20120620003525-dbd6ede6.jpg

***ApiSavoir.ch.* (s.d.). Récupéré sur Imanagement: https://www.2imanagement.ch/en/other/links/wwwapisavoirch/blocage-de-ponte-lutte-contre-le-varroa**

Babulka. (2005). la melisse(melissa ofisinalis L). *phytotérapie*, 114-117.

Baker. (1984). optomotor anemotax is polarizes self-steered zigzagging in flying moths. *physiological entomologie*.

Barbelet. (2015). *Le giroflier : historique, description et utilisations de la plante et de son huile essentielle*. Sciences pharmaceutiques: hal-01732523.

Belaïd,. (2010). effet du varroa destructor sur la morphométrie alaire et sur les composants du système immunitaire de l'abeille ouvrière apismellifera intermissa. *Lebanese Science Journa*, 83-90.

Boubaker, (2019, 07 08). *application des huile essentille sur le biocontrol du varroa parasite de l'abeilledomestique et conception d'un bioacaricide*.

Boudagga, (2003). Morphological identification of the Varroa species (Acari: Varroidae) colonizing Tunisian apiaries. *Systematic & Applied Acarology*, 97-100.

Bibliographic references

Bounihi. (2016, janvier 06). Criblage phytochimique, Étude Toxicologique et ValorisationPharmacologique de Melissa officinalis et de Mentha rotundifolia(Lamiacées).

Chaieb,. (2007). The Chemical Composition and Biological Activity of Clove Essential Oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): A Short Review. *Wiley InterScience*, 501–506.

(1922). la rousse agricole. Dans d. chancrin, *la rousse agricole* (p. 150). paris: imprimrie la rousse 17 rue montparnasse.

Clément. (2006). *les bons geste de l'apiculture.* Centre pour l'Environnement et le Développement.

Colin. b. (1999). Varroosis. In Bee Disease Diagnosis. 121–142.

Conte. (2005). *Apiculture : connaître l'abeille, conduire lerucher* (Vol. 698). Paris: Lavoisier, Tec & Doc.

Douhet, R. L. (1977). *L'abeille de A à Z embryologie, anatomie.* FNOSAD.

Duran, B. (2017, janvier 16). *futura planète* . Récupéré sur futura-sciences: https://www.futura-sciences.com/planete/dossiers/zoologie-surmortalite-abeilles-enigme-encore-non-resolue-1010/page/4/

Ellis, N. (2010). *universty of floruda.* Récupéré sur https://entnemdept.ufl.edu/creatures/misc/bees/varroa_mite.htm

Fauco, V. R. (1992). Diagnostic de la paralysie aiguë application à l'épidémiologie des maladies viralesde l'abeille en France en 1990. *apidologie*, 139-146.

Feknous. (2014). Extraction, caractérisation et identification de quelques métabolites. *Nature & Technology*, 7-13.

Fernández,. (2020). Chemical composition of essential oil of Melissa officinalis L. and antioxidant activity from Boa Vista-RR,brazil. *African Journal of Pharmacy and pharmacology*, 41-45.

Free. (1977). *The social organization of honey bees* (Vol. 124). London: Edward Arnold Ed.

Gashou,. (2009). Acute toxicity of essential oils and other natural compounds to the parasitic mite, Varroa destructor, and to larval and adult worker honey bees (Apis mellifera L.). *Journal of Apicultural Research* , 263-269.

Girisgin,. (2014). Determining the stability of clove oil (eugenol) for use as an acaricide in beeswax. *Journal Vet. Med*, 4.

Bibliographic references

***google maps* . (s.d.). Récupéré sur https://www.google.com/maps/@35.8368235,2.5043499,1155m/data=!3m1!1e3?hl=fr**

Herodez,. (2002). Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. *Food Chemistry*, 275–282.

Imboden,. (s.d.). *ApiSavoir.ch.* Récupéré sur Imanagement: https://www.2imanagement.ch/en/other/links/wwwapisavoirch/blocage-de-ponte-lutte-contre-le-varroa

Jafer. (2008). essential oil component in flower of leeon balm(melissa oficinalis L). *american journal of biochemistry and biotechnology*, 277-278.

Kennouche. (2015). Chemical Composition and Antimicrobial Activity of theEssential Oil of Eugenia caryophyllata Cloves Extracted by Conventional and Microwave Techniques. *Journal of Biologically Active Products from Nature*, 1 - 11.

Koumad. (2015). STUDY OF THE POPULATION DYNAMIC OF VARROA DESTRUCTOR IN ALGERIA. *BEST Journals*, 69-76.

Kumar, M. M. (2011). larvicidal and pupicidal properties of essential oils and their formulations against the housefly,Musca domestica. *Medical and Veterinary Entomology*, 302-310.

Leslev. (2005). Plantes aromatiques et médicinales. *l'oeil nature*, 112-197.

Martin. (1998). a population model of the ectoparasitic mite varroa jacobsoni in hony bee (apis mellifera) colonies. *Ecological modelling 109*, 267-281.

Maurizio. (1946). *Beodachtungen uber die Lebensdauer und Futterverbrauchgefangen gehaltenen Bienen* (Vol. 48). Bienen: Beihfte Schweis.

Muchalal, J. (2017). Composition de I'huile essentielled e clous de girofle. *Annales Techniques*, 255-258.

Park, S. (2005). Fumigant activity of plant essentia loils and components from garlic (Allium sativum) and clove bud (Eugenia caryophyllata) oils against the Japanese termite (Reticulitermes speratus). *journal of agricuture and food chemistry* , 4388–4392.

Pawar, T. (2006). In vitro efficacy of 75 essential oils against Aspergillus niger. *Mycoses*, 316 –323.

Perrucci. (1995). Structure/activity relationship of some natural monoterpenes as acaricides against Psoroptes cuniculi. *Journal of Natural Products*, 1261– 1264.

Bibliographic references

Pingret, S. (2013). An Improved Ultrasound Clevenger for Extraction. *Food Anal. Methods*.

Prashar, L., & Evans. (2006). Cytotoxicity of clove (Syzygium aromaticum) oil and its major components to human skin cells. *Cell Prolif*, 241–248.

Quezel, S. (1963). *Nouvelle flore de l'Algérie et des régions désertiques méridionales.* Paris.

Raina, S. A. (2001). Essential oil composition of Syzygium aromaticum leaf from Little Andaman, India. *FLAVOUR AND FRAGRANCE JOURNAL*, 334–336.

Rashid, A. S. (2014). Control of Varroa destructor (Acari : Varroidae) in Apis mellifera(Hymenoptera : Apidae) by using Plant Oils and Extract. *Zoological Society of Pakistan*, 609-615.

Robert, F., Böcker, T., Möhring, N., & Dalhaus, T. (2017). Taxe incitative sur les produits. *Recherche Agronomique Suisse 8(5)*, 176–183.

Rosenkranz, A. Z. (2010). Biology and control of Varroa destructor. *Journal of Invertebrate Pathology*, 96-119.

Salem. (2013). *l'effet acaricide de l'huile esentielle de citrus limonum sur varroa destructor parasite de l'abeille apis mellifera*.

Seeley. (2002). The effect of drone comb on a honey bee colony's production of honey. *Apidologie*, 75 – 86.

Sutour. (2010). etude de la composition chimique d'huile essentiel et d'extrait de manthes de corse et de kumquats. université de crose pascal paoli .

Talbi. (2014). *l'effet acaricide de l'huile essentile d'ecalyptus globulus sur varroa destructor*.

Tomaz BAJ, E. S.-M. (2015). EFFECTIVENESS OF THE DERYNG AND CLEVENGER-TYPE APPARATUS. *Polish Pharmaceutical Society*, 507-515.

Vidal-naquet, N. (2011). DISEASES OF THE REARED HONEYBEE APIS MELLIFERA L. 307-316.

annexes

GS/MS apparatus:





Figure 26 :GS/MS APPARATUS

Beekeeping tools and equipment:

Gloves, boot, honey bee suit: for protection during handling because this domestic species is extremely aggressive

Enfumoir: instrument that produces smoke to mask the pheromones of bees so as not to become aggressive, the smell of smoking marks as fire for bees, it is fed by leaves of juniper trees

Frame lifter: iron bar to detach the beehive frames

Wooden tongue depressor: use as a holder for beeswax with oils because it is inert to any interaction with bees.

Beeswax: use as a holder for essential oils.

Biological product: consider as positive control which is organic natural strip for varroa made of essential oil: thymol, peppermint oil, volatile laurel oil, eucalyptus oil, sunflower oil, 3mg fulimetrin.