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COMPOSITION OF THE ESSENTIAL OIL OF THE LEAVES OF *ARTEMISIA HERBA ALBA* ASSO (ASTERACEAE) AND ITS INSECTICIDAL ACTIVITY ON *CALLOSOBRUCHUS MACULATUS FABRICIUS* (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

In order to develop a strategy for the safe management of insect pests in stored agricultural commodities, the essential oil of *Artemisia herba alba* Asso. was tested against *Callosobruchus maculatus Fabricius* raised on seeds of *Cicer arietinum* (L.) at 20-30°C and 65±5% relative humidity under storage conditions. Chickpea seeds were fumigated with concentrations of essential oil and then infested by young adults of bruchids. On each treated or control lot, 10 newly emerged pairs were released. The number of dead bruchids and number of eggs hatched and unhatched from the seeds were counted. At the end of their development, adults emerged from treated and untreated seeds were counted separately by sex and the success rate was calculated. Three replicates were conducted for each treatment.
The essential oil extracted from *Artemisia herba alba* Asso was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). The chromatogram of the essential oil of *Artemisia herba alba* Asso shows 15 signals relating to the 15 compounds of which camphor is the major constituent with a percentage of 96.15%. The results of the biological tests obtained showed that the essential oil of *Artemisia herba alba* Asso exerts repellent and toxic effects on *Callosobruchus maculatus*. It causes a high mortality of adults and affects its fertility, fertility, and success rate in a very significant way compared to controls. These show that fumigation of stored commodities with the essential oil of *Artemisia herba alba* Asso against insect pests can be considered under storage conditions without risk to consumers and the environment.

Keywords: Essential oils, GC/MS, *Artemisia herba alba* Asso, *Callosobruchus maculatus*, *Cicer arietinum*, Bioassay

INTRODUCTION

Legume seeds are the main source of protein in many developing countries, unfortunately, they suffer considerable losses during storage (Pérez Mendoza *et al.*, 2004).
Between harvest and consumption, more than 30% of production is lost, this proportion is higher in the Sahel region due to the long storage period. During storage, mushrooms, beetle rodents (Bruchidae and Curculionaides) attack the seeds causing considerable loss (Delobel and Maurice, 1993).
Callosobruchus maculatus Fabricius is one of the pests of infestation and destruction of stored grain. Because of its high incidence, synthetic insecticides have been used to control it, but the potential damage to human health and the environment caused by these insecticides is now considered a real problem. Methyl bromide and phosphine are toxic to a broad spectrum of insect pests and have been commonly used to control beetles harmful to stored commodities (Mueller, 1990). Although these fumigants are effective and economical, they have led to unexpected side effects such as ozone depletion, environmental pollution, pest resistance, and harm to the organism (Jembere *et al.*, 1995; Okonkwo and Okoye, 1996).
The problems of resistance and harmfulness of synthetic insecticides have led to the need to find more effective and healthier alternatives. Thus, essential oils are the most tested products currently (Pérez *et al.*, 2010; Andréa *et al.*, 2011; Hany, 2012; Khani and Asghari, 2012; Sharifian *et al.*, 2012; Titouhi *et al.*, 2017; Sabbour, 2019). These natural insecticides, known as plant insecticides, have several advantages over synthetic compounds because of their rapid biodegradation and reduced environmental risks.
The aim of this paper is verifing and evaluating the effet of the essential oil extracted from the leaves of *Artemisia herba alba* Asso on *Callosobruchus. maculatus* which develops at the expense of several species of mature and dry leguminous plants.
Artemisia herba alba Asso is a species of the Asteraceae family. It grows in Morocco, where it has a great reputation in traditional medicine because of its benefits on human health, but in high doses, it can cause some intoxication.

MATERIALS AND METHODS

Plant used

Artemisia herba alba Asso was harvested in the region of Errachidia (31° 55′ 55″ north, 4° 25′ 28″ west) of Morocco in April 2018.

Drying of plant material

The harvested organs are dried in the laboratory for seven days. The raw material is spread out in thin layers and turned frequently at room temperature 25°C.

Extraction of the essential oil of *Artemisia herba alba* Asso

The extraction of the essential oil was carried out by hydro-distillation of 100g of the dry plant matter in a volume of 1.5L of distilled water brought to 100°C in a Clevenger type essencier (Clevenger, 1928). The distillation lasts three hours after recovery of the first drop of distillate. The essential oil is dried with anhydrous sodium sulphate and stored at 4°C in the dark. The yield of essential oil is expressed in relation to the dry matter (in mL/100g dry matter).

Liquid chromatographic analysis at atmospheric pressure of the essential oil of *Artemisia herba alba* Asso

The crude *Artemisia herba alba* Asso essential oil is chromatographed on a silica gel column (60G) at atmospheric pressure. The eluent used was initially made using hexane, then by increasingly polar mixtures (hexane/ether).
The fractions obtained were identified by ultrasound GC gas chromatography using a VB5 type column (50% phenyl, 95% methylpolysiloxane) coupled with the Polaris type mass spectrum (EI 70ev, 10-300Uma) under the following conditions:
- Oven temperature: variation from 50°C to 250°C at a rate of 5°C/min and from 250°C to 300°C,
- Carrier gas: helium, with a flow rate of 1mL/min,

- Injection temperature of 250°C.
The identification of the various constituents was based on their mass spectra and retention indices in the stationary phase compared to those of the synthetic standard compounds in the database.

BIOASSAYS

The biological study is focused on an insect pest of stored legume seeds *Callosobruchus. maculantus.* It is a Bruchidae beetle whose larvae develop at the expense of ripe, dry grains of food legumes.
The breeding of *Callosobruchus maculatus* has been carried out on chickpea seeds (*Cicer arietinum*). The seeds used as a carrier for the bioassay were purchased in a supermarket and were free of infection for an extended stay in the freezer, without any chemical treatment.
The strain of *Callosobruchus maculatus* used in the bioassays was made from insects that emerged from chickpea seeds from a storage warehouse in Meknes, Morocco.
Mass rearing takes place in Petri dishes where several pairs of bruches are placed, in the presence of healthy chickpea seeds for 6 days in desiccators containing 66.3mL distilled water and 33.7mL concentrated sulphuric acid to have a humidity of about 65% and to limit attacks and the establishment of mites in the rearing.
The rearing is carried out at a temperature of 20-30°C in daylight (July-September 2018).
After oviposition, the dead adults are removed and the contaminated seeds are kept under the same conditions until the emergence of the offspring that were used to maintain the strain. Second generation insects were used for 24 hours after emergence for bioassay.

Impact of essential oils of *Artemisia herba alba* Asso leaves

For each trial, 50 chickpea seeds were placed in Petri dishes, three replicates for each concentration and control consisting of 50 seeds was also repeated three times. All boxes were infested with 20 insects (10 pairs) 24 h old, sexed adults. Each concentration was poured into a watch glass using a Pasteur pipette. Three boxes were placed in a desiccator with a volume of 4.6L of air and a cup containing the corresponding essential oil. The latter was placed in the center of the desiccator, then the assembly was tightly closed and an untreated control was also hermetically sealed.
The concentration for each of the essential oils of *Artemisia herba alba* Asso are summarized in the following table:

Table 1 Concentration of essential oil of *Artemisia herba alba* Asso used in biotest

Concentration (g/4,6L of air)	*Artemisia herba alba* Asso
Witness	0.00
Dn/2	0.31
Dn	0.62
D2n	1.24

- Dn: The normal concentration which is the quantity of oil (in g) obtained per 100g of the vegetable matter,
- Dn/2: Half the concentration (Dn),
- D2n: Double the concentration (Dn).

Impact of the essential oil of the leaves of *Artemisia herba alba* Asso on the mortality of adults of *Callosobruchus maculates*

The counting of dead insects was carried out every day for a period of- 10 days every 24 hours.

Impact of the essential oil of the leaves of *Artemisia herba alba* Asso on fecundity, fertility, and the emergence rate of *Callosobruchus maculates*

After 10 days of counting dead insects, we counted the hatched and unhatched eggs with a binocular magnifying glass at the first emergence, we removed the adults as they appeared until they stopped completely.

Analysis of the data

For the determination of the fertility of adults of *Callosobruchus maculatus*, counting of hatched and unhatched eggs was carried out using a binocular magnifying glass after 15 days of exposure and after counting dead insects.

$$\text{Fertility} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs laid}} * 100$$

The success rate of adults of *Callosobruchus maculatus* is calculated as the percentage of adults that emerged in relation to- the total number of eggs laid.

$$\text{Success rate (\%)} = \frac{\text{Number of insects emerged}}{\text{Number of eggs laid}} * 100$$

RESULTS AND DISCUSSIONS

Extraction yield of the essential oil of *Artemisia herba alba* Asso

After the hydrodistillation of 100g of the leaves of *Artimisia herba alba*, the essential oil is light yellow in color. The yield we obtained after three repetitions is 0.62%.

Chemical composition of the essential oil of *Artemisia herba alba* Asso

After the hydrodistillation of 100g of *Artemisia herba alba* Asso leaves, the essential oil obtained is analyzed by GC/MS.

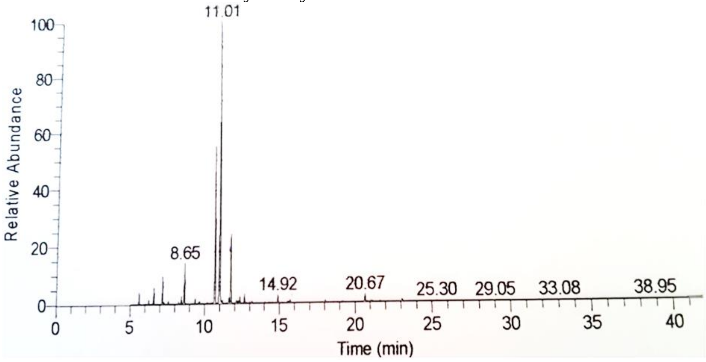

Figure 1 Chromatogram derived from GC/MS of the essential oil of *Artemisia herba alba* Asso

Chromatogram analysis showed that the essential oil of *Artemisia herba alba* Asso recorded 15 peaks (15 compounds) with a high relative abundance and each peak has its own m/z mass spectrum.
To separate these compounds, 2g of the essential oil of *Artemisia herba alba* Asso was chromatographed on silica gel using hexane as the eluant and diethyl ether in different percentages. 4 fractions F_1, F_2, F_3 and F_4 were isolated with proportions of 20.50%; 22.50%; 16.50% and 16.50% respectively.
The four fractions F_1, F_2, F_3, and F_4 of the essential oil of *Artemisia herba alba* Asso were analyzed by gas chromatography coupled with mass spectrometry (GC/MS) (Table 2).

The analysis of the chromatograms of the four fractions F_1, F_2, F_3, and F_4 showed that the essential oil of *Artemisia herba alba* Asso consists mainly of Camphor (96.15%), Caryophyllene oxide (29,45%), 10,12-Octadecadienoic acid (20.68%), Chrysanthyl acetate (16.82%), Santoline alcohol (22.56%) and other constituents with percentages ranging from 3.07% to 11.71%. This composition is characterized by the high content of Camphor, the essential oil studied is rather close to the Camphor chemotype. Work has been carried out on 303 samples that are taken from 91 stations of the Moroccan Atlas (Lamiri *et al.*, 1997a,b). They were able to identify 16 chemotypes including α-Thujole, β-Thujol and Camphor which are the majority after a study of 303 samples.

Effect of the essential oil of *Artemisia herba alba* Asso on *Callosobruchus maculates*

Effect on longevity

Except concentration 0.31g/4.6L of air, the essential oil of *Artemisia herba alba* Asso affects the longevity of *Callosobruchus maculatus* adults in a very significant way (Table 3). The longevity of bruches ranges from 1 to 5. For the control, this parameter ranges from 1 to 10 days. There is a concentration- and sex-dependent toxic effect. However, it should be noted that there is a high individual variability ranging from 23.71% to 63.05%. In general, within each batch, males live longer than females (Figures 2 and 3).

Table 2 Chemical composition of the fractions obtained by CPL on silica gel of the essential oil of *Artemisia herba alba* Asso

Compounds	F_1	F_2	F_3	F_4
Caryophyllene oxide	29.45	-	-	-
10,12-Octadecadienoic acid	20.68	-	-	-
Trans-3,3-heptadien-2-one	5.25	-	-	-
para-Cymen-8-ol	5.24	-	-	-
1-Benzyloxymethyl-1-hydroxymethyl-2,5-cyclohexadiene	-	18.78	-	-
Chrysantethyl acetate	-	16.82	-	-
Sabinyl acetate	-	4.72	-	-
Myrténÿl acetate	-	3.90	-	-
Bicyclo [3,1,1] heptane-6,6-dimethyl-3-methylene	-	3.82	-	-
1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl) cyclohexane	-	3.35	-	-
Camphor	-	-	86.15	-
Bicyclo [3.1.1] heptan-endo-6-ol, syn-7-bromo	-	-	4.90	-
Santoline alcohol	-	-	-	22.56
cis-Sabinol	-	-	-	13.45
3-Cyclohexene-1-ol, 4-methyl-1-(1-methylethyl)	-	-	-	11.71
5-Methyl-3(1-methylvinyl)-1,4-hexadiene	-	-	-	8.53
Myrtenol	-	-	-	4.82
cis-Pinene hydrate	-	-	-	4.06
Phenol, 2-(1-methylethyl)	-	-	-	3.67
2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl)	-	-	-	3.11
cis-Sabinene hydrate	-	-	-	3.07

Table 3 Longevity in days of *Callosobruchus maculatus* on chickpea seeds treated with different concentrations of the essential oils of *Artemisia herba alba* Asso

Concentration (g/4.6L of air)	Gender	Effective	Average ± Standard deviation	Minimum	Maximum	Coefficient of variation
0.00	Males	30	6.97±2.17	1	10	31.19
0.00	Femeles	30	7.63±1.81	1	10	23.71
0.31	Males	30	4.50±2.37	1	10	52.77
0.31	Femeles	30	4.17±2.63	1	10	63.05
0.62	Males	30	2.60±1.35	1	5	52.09
0.62	Femeles	30	1.50±0.86	1	5	57.40
1.24	Males	30	1	1	1	0.00
1.24	Femeles	30	1	1	1	0.00

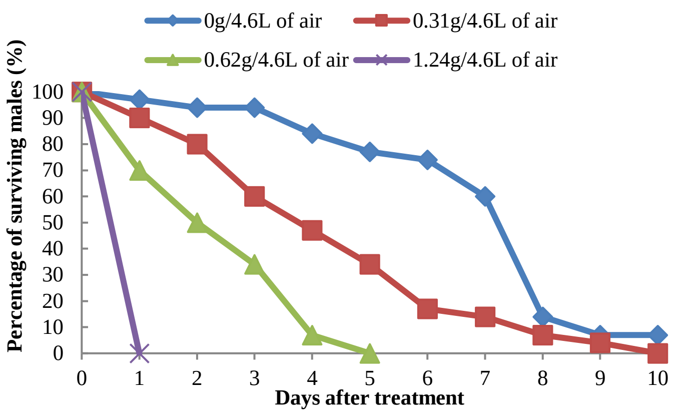

Figure 2 Survival curves of *Callosobruchus maculatus* males in contact with chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oils

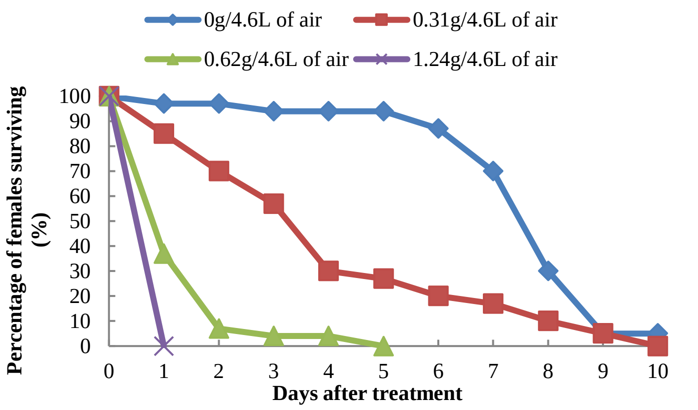

Figure 3 Survival curves of *Callosobruchus maculatus* females in contact with chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oils

Effects on fecundity

The fecundity of bruchids on fumigated chickpea seeds is strongly affected by the different concentrations of *Artemisia herba alba* Asso essential oil, whatever the concentration considered. Indeed, the average number of eggs laid varies from 27 to 116 eggs on the essential oil of *Artimisia herba alba* against 402 to 579 on the control lot. The fecundity of bruchids is variable with coefficients of variation ranging from 16.52% to 55.03% (Table 4).

Table 4 Fecundity of *Callosobruchus maculatus* found on chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oils

Concentration (g/4.6L of air)	Fecundity/10 females ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0.00	491.00±88.50	402	579	18.03
0.31	84.00±46.23	31	116	55.03
0.62	42.67±14.19	30	58	33.26
1.24	33.33±5.51	27	37	16.52

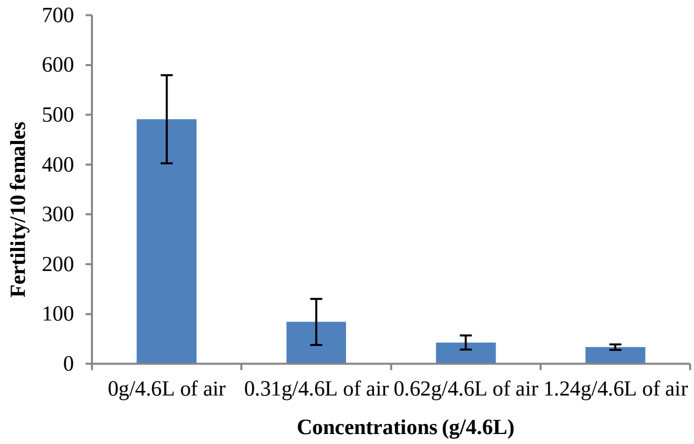

Figure 4 Fecundity of *Callosobruchus maculatus* on chickpea seeds treated with essential oils of *Artemisia herba alba* Asso

Effect on fertility

The essential oil of *Artemisia herba alba* Asso strongly affects the fertility of *Callosobruchus maculatus* bruches. At a concentration of 1.24g/4.6L, the essential oil of *Artemisia herba alba* Asso induces a total absence of hatched eggs. In this trial, and the light of the results presented, it was found that the essential oil of *Artemisia herba alba* Asso strongly affects the number of eggs hatching on the fumigated seeds. The individual variability is very high and the coefficient of variation varies from 5.42% to 14.08% (Table 5).

Table 5 Fertility of *Callosobruchus maculatus* found on chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oils

Concentration (g/4.6L of air)	Average fertility ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0.00	77.75±6.28	70.64	82.52	8.08
0.31	77.83±10.96	69.83	90.32	14.08
0.62	94.37±5.12	90.00	100.00	5.42
1.24	0.00	0.00	0.00	0.00

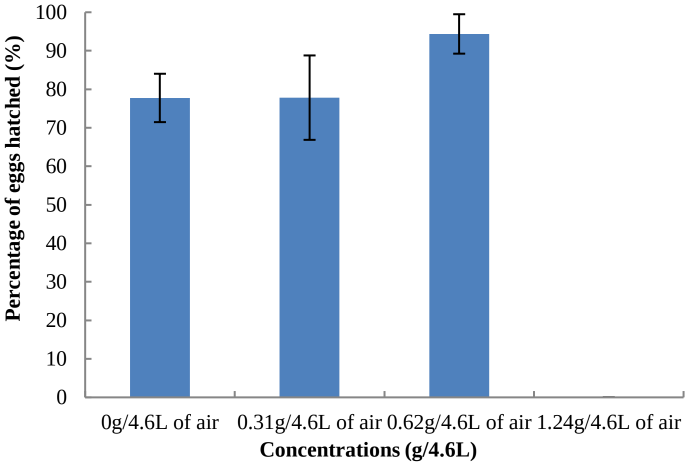

Figure 5 Fertility of *Callosobruchus maculatus* found on chickpea seeds treated with essential oils of *Artemisia herba alba* Asso

Effect on success rate

From chickpea seeds fumigated with the essential oil of *Artemisia herba alba* Asso, only the high concentration does not allow the emergence of any adult of the insect, the other concentrations have no detectable effect, the success rates obtained are comparable to those from control seeds. Also, besides, table 6 gives the number of seeds varying from 0 to 61 in the treated lots against 314 to 415 in the control lot with coefficients of variation ranging from 14.48% to 52.40% (Table 6).

Table 6 Numbers of adult *Callosobruchus maculatus* progeny from chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oils

Concentration (g/4.6L of air)	Average workforce ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0.00	376.33±54.50	314	415	14.48
0.31	45.33±23.76	18	61	52.40
0.62	32.33±6.81	27	40	21.05
1.24	0.00	0	0	0.00

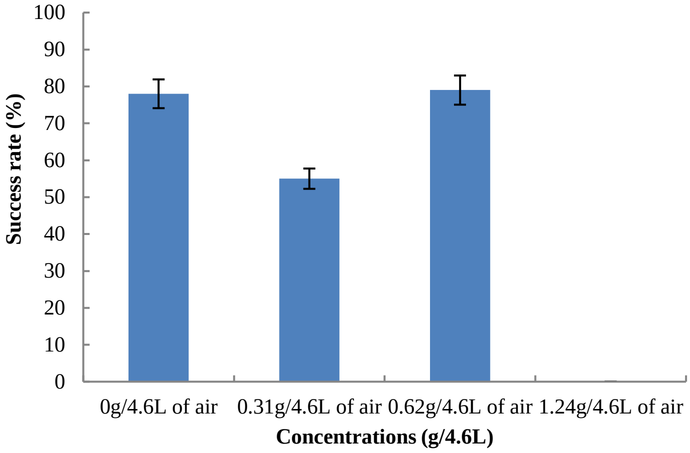

Figure 6 Success rate of *Callosobruchus maculatus* on chickpea seeds treated with the essential oils of *Artemisia herba alba* Asso

The net reproductive rate of *Callosobruchus maculatus* decreases as the concentration increases on seeds treated with the essential oil of *Artemisia herba alba* Asso (Table 7).

Table 7 Net reproduction rate (Ro) of *Callosobruchus maculatus* on chickpea seeds treated with different concentrations of *Artemisia herba alba* Asso essential oil

Concentration (g/4,6L of air)	Net reproduction rate (Ro)
0.31	2.27
0.62	1.62
1.24	0.00

In our trial, the essential oil of *Artemisia herba alba* Asso affected the longevity, fecundity, and emergence of *Callosobruchus maculatus*. The significant effects of the essential oils differed according to the concentration and sometimes sex of *Callosobruchus maculatus* bruche.

Several works have proven the action of essential oils on all these parameters. Thus, Papachristos and Stamopoulos (2002) showed that the effect of the essential oil of *Mentha viridis* on fertility, fertility and the rate of emergence of *Callosobruchus maculatus*. Others found that the essential oil of *Ocimum basilicum* did not allow *Callosobruchus maculatus* to emerge from fumigated seeds (Kéîta *et al.*, 2000). However, Ketoha *et al.* (2005) stated that *O. basilicum* essential oil did not affect the reproductive rate of adults of Indian brook trout (Ketoha *et al.*, 2005). Kellouche and Soltani (2004) also noted negative effects on the success rate of *Callosobruchus maculatus* developed at the expense of chickpea seeds treated with essential oils extracted from *Melaleuca quinquenervia*, or *Ocimum gratissimum*. Also, Tripathi *et al.* (2000) found that essential oils of the genus *Mentha*, containing piperitenone oxide, have toxic and repellent effects on the bristle of *Callosobruchus maculatus*.

The mechanisms of action of essential oils are poorly understood and relatively few studies have been carried out on this subject (Isman, 2000). Recent work shows that monoterpenes act at the level of acetylcholinesterase receptors of neuromuscular junctions (Ngamo and Hance, 2007). Indeed, according to the work of Obeng-Ofori *et al.* (1997), 1,8-Cineole in contact with insects acts by blocking the synthesis of the juvenile hormone, it inhibits acetyl-cholinesterase by occupying the hydrophobic site of this enzyme which is very active. In general, essential oils are nowadays known as neurotoxins (Ngamo and Hance, 2007).

CONCLUSION

In this work, the effectiveness and chemical composition of the essential oil of *Artemisia herba alba* Asso was demonstrated. The analysis of this essential oil by gas chromatography coupled with mass spectrometry (GC/MS) showed the existence of terpene hydrocarbons, oxygenated terpenes, oxygenated sesquiterpenes, and sesquiterpenes and a majority product which is camphor with 96.15% of the global constituents.

L'huile essentielle d' *Artemisia herba alba* Asso obtenue par hydrodistillation testée par fumigation à différentes concentrations contre *Callosobruchus maculatus*, provoque la mortalité de 100% de la population des brunes étudiées à la concentration 1,24g/4,6L d'air en 24 heures. Elle entraîne également une réduction très importante du pont de fertilité, du taux de réussite et du taux de reproduction des brunes. Ces effets peuvent être dus aux mono terpènes qui sont responsables de la toxicité et de l'inhibition de la reproduction chez d'autres insectes.

The essential oil of *Artemisia herba alba* Asso, has a definite action in the control of *Callosobruchus maculatus*, it has proved to be more toxic to the insect studied with a very high mortality rate. The essential oil can already be used in the fumigation of legume seeds against *Callosobruchus maculatus*, the main pest of these commodities during storage.

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