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Original Research Article

GC-MS Analysis of Essential Oil Composition and Insecticidal Activity of *Syzygium aromaticum* against *Callosobruchus Maculatus* of Chickpea

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ABSTRACT

The beetle *Callosobruchus maculatus* (Chrysomelidae: Bruchinae) is a destructive pest of stored chickpea seeds. The use of plant materials rather than conventional pesticides to control these pests is a promising alternative to chemical insecticides. The present study evaluated the insecticidal activity of clove essential oils by three different methods on *Callosobruchus maculatus*. The oils were obtained by hydro-distillation with a yield of 6.3 ± 0.23% and analyzed by GC-MS. In total, 65 compounds representing 99.92% of the oil were identified and the main components 80.26% Eugenol, 9.62% Eugenyl acetate, 6.74% β-Caryophyllene and 1.14% C-Humulene. The results of the insecticidal activity of *S. aromaticum* on *C. maculatus* performed by inhalation shows that a significant mortality was observed at a concentration of 20 µl/l against insects exposed at 24 hours (6.67%), 48 hours (36.67%) and 72 hours (100%).Contact test results are lower than those produced by inhalation. Essential oil decreased oviposition and fully suppressed adult growth at the highest dose of 20 µl/l of air per contact and inhalation test. The results of the present research have shown that EO had a high effectiveness against *Callosobruchus maculatus*, and in another hand these results can be used as a source of natural product to avoid the contamination of chickpea seeds, and also as an alternative to chemical products.

***Keywords*:** Insecticidal activity, Oviposition, Mortality, Contact, Inhalation

Introduction

Stored food can be invaded by insects, fungi and rodents.[1] Damage caused by insects is the most significant. Although the problem is global, it is very serious in developed countries and especially in the African continent because of the favorable climate conditions for their production.[2] In Morocco, legumes have always played an important role in various aspects, in food security, and also play an important role in the environment ecosystem. In addition to maintaining the equilibrium of human and animal nutrition, food legumes make a major contribution to the restoration of soil fertility and to ensuring the sustainability of production processes. On the economic and social level, small farmers cultivate food legumes to maintain their income and for rural households. Beetles from the Bruchidae family, whose larvae develop only in the seeds, are the most dangerous pests of legumes (Fabaceae)[3]. The chickpea seeds (*Cicer arietinum*) are heavily assaulted by *Callosobruchus maculatus* during storage. This species is a cosmopolitan food seed parasite in many parts of Africa, where a large part of the rural population lives on vegetable crops. This pest causes substantial harm and results in a loss of quality and quantity of stored legumes, in particular a degradation of its ability of germination.[4,5] Several control methods have been used to control these species, the first essays were conducted by chemical products.

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However, currently, these chemicals present significant health and environmental issues on a global scale, and the inappropriate use of this chemical cause many health threats[6–8] and several environnemental problems. [9–11] Essential oil (EO) from plants is an important resource of natural products and their components are mainly used as food flavors; however, they can be successfully used for various non-food applications such as antifungal, antimicrobial, antioxidant, and insecticidal activity.[12-16] Numerous studies have highlighted the biocidal effects of essential oils on plant pests (Bruchidae),[17,18] These compounds are acting as fumigants [19–21] Contact insecticides[22] or repellents.

In this study, we propose to investigate the chemical profile of essential oils extracted from *syzygium aromaticum* and their insecticidal activity in *Callosobruchus maculatus*.

Materials and Methods

Plant material

The cloves *Syzygium aromaticum* were purchased from the herbalists of the ancient city of Fez, known as (al aatarine) on 15th April, 2019 and were identified by professor Eloutassi Noureddine of the Regional Center for the Trades of Education and Training (CRMEF), Fez-Morocco where the Voucher specimen number: A01/10282. The chickpea seeds (*Cicer arietinum*) used, belong to the national variety 'Rizki', registered in the official catalog in 1992 of the agricultural domains of Douyet.

Method of extraction

The oils were extracted by hydrodistillation using a Clevenger-type from dry plant material. The distillation was performed by boiling 200 g of cloves for 2 hours with 1 L of distilled water in a 2 L flask. The temperature of the flask was maintained at 80°C during distillation. The oil-laden vapor passes through a condenser which condenses and

falls into a burette. The distillate contains hydrolate and essential oils. sodium sulfate and stored in an Eppendorf tube at 4°C and protected from light.[23]

Animal material
Adults of C. *Maculatus* were obtained from a mass-reared strain at the Biotechnology and Natural Resources Preservation Laboratory at a temperature of approximately 27 ± 1°C and a relative humidity of 70 ± 5 % and a photoperiod of 14 h (light)/10 h. (dark). The non-fogging form of insect with a higher reproductive capacity, was used.

GC-MS analysis
Chromatographic analyses were carried out by Perkin Elmer (Clarus 580) electronically pressure-controlled gas chromatography (GC). The capillary column type fused silica, 30m long, and 0.25 mm diameter. The helium is carrier gas with a flow rate of 1.0 ml/min. The temperature of the column was automatically programmed at 50°C for 10 min, then from 50 to 200°C for 75 min and finally at 200°C for 25 min. The injector and detector temperatures fixed at (200°C).

Assessment of essential oil toxicity by contact
Essential oil solutions are tested for fumigation. In order to facilitate their diffusion in the treatment medium, the oil charge is deposited on the Whatman No. 2 filter paper disc. The different concentrations of essential oils, defined in relation to the volume of air in the bocal enclosure, are expressed in microliter per liter (µl/l of air) [24]. The amount of oil depends on the volume of the glass jars; and four concentrations were added: 1, 5, 10 and 20 µL/L.
The mortality rate study was conducted on 100 grams of healthy chickpea seeds, with daily monitoring of all parameters such as adult longevity/mortality, female fertility (number of eggs laid) and the development of new individuals after the life cycle. Each glass jar received 100 g of chickpea seeds, a slice of filter paper soaked with the essential oil load to be studied, and 10 pairs of *C. maculatus*. Control was carried out using the same conditions as essential oil, with untreated filter paper. Thus, five repetitions were performed.
For mortality, the number of dead insects was counted every day after the completion of the experiment and the mortality rate is determined. Because of the importance of egg laying, the eggs laid on the walls of the jars and on the seeds were counted under a binocular magnifying glass. The calculation of their number was compared to the control. The rate of oviposition reduction was then determined.

Assessment of essential oil toxicity by Inhalation
In glass jars with a volume of one liter, small pieces of cotton were suspended with a thread attached to the inside of the lid. Doses of essential oils were stored in cotton with micropipettes 10 minutes before the insects were deposited in order to saturate the medium. Ten of C. *maculatus* (male and female) aged 24 hours were inserted in each jar, the closing of which was perfectly sealed. After 24 hours, dead insects were counted. Insects were considered dead when no movements were observed for 1 hour. Bioassays were carried out under the same constant rearing conditions, i.e. roughly 27 ± 1°C and with a relative humidity of 70 ± 5 percent and a photoperiod of 14h (light)/10h (dark). 5 µL/mL, 10 µL/mL and 20 µL/mL) to evaluate the difference in efficacy between the two tests [24]

Repellency test
In order to evaluate the repellent activity of the essential oil of cloves, a disk of Whatman filter paper, 11 cm in diameter, was cut into two equal parts to adapt to the size of the Petri dishes. Half of the filter paper was treated with 0.3 ml of acetone as a control and the other half with an acetone solution containing 1, 5, 10 and 20 µL/L of essential oils. Ten pairs of adult bruchids (less than 24 hours) were placed in the center of the box. The Petri dishes were then closed with Parafilm. This study was carried out in three replicates under the same conditions as insect rearing. After half an hour, we count the number of bruchids present on the part of the disk treated with essential oil and the number of individuals present on the part treated with acetone alone. [25]

Essential oils were collected by decantation, then dried on anhydrous

Calculation method
The observed mortality rate was performed by the Abbott formula.[26]

$$Pc = 100 \times \frac{Po - Pt}{100 - Pt}$$

Where
Pc = percent corrected mortality; Po = observed mortality in the trial and Pt = observed mortality in the control.
The egg-laying reduction rate is given by the following formula:

$$Tx = 100 \times \frac{Nt - Ne}{Nt}$$

Where
Tx = rate of reduction relative to the control; Nt = number of eggs in the control jar and Ne = number of eggs in the experimental trial.
The percentage reduction in adult emergence or inhibition rate (% IR) was determined by Tapondijou *et al.*[17] as follows: $\%\, IR = (Cn - Tn)\, 100/Cn$ or:
Cn is the number of newly emerged insects in the untreated (control) jar.
Tn is the number of insects newly emerged in the treatments
The percent repulsion (PR) is calculated according to the following formula [27]

$$PR\ (\%) = [(NC - NT) / (NC + NT)] \times 100$$

PR = percentage of repellency
NC = number of insects in the control area
NT = number of insects in the treatment area

Statistical analysis
The statistical analysis of essential oil yields was carried out using IBM SPSS Statistics version 21. Statistically significant differences were determined by a two-way ANOVA followed by a Fisher's LSD test at the 5% significance level.

Results and Discussion

Yield and chemical composition of essential oils
The essential oil obtained is light yellow in color, with a yield of 6.3 ± 0,23 percent. The findings of the analysis established 65 chemicals, comprising more than 99.92% of the chemical composition of the species' essential oil. Analysis of the chemical composition of *syzygium aromaticum* EO revealed the presence of 80.26% Eugenol, 9.62 % Eugenyl acetate, 6.74 % β-Caryophyllene and 1.14% C-Humulene.

Bioassay experiments
Inhalation test
This test has improved the potency of *Syzygium aromaticum* EO against *Callosobruchus maculatus*. Mortality was 53.33 ± 11.55% after 4 days at the dose of (1 µL HE/L air) and 100 % after 72 h at the dose of 20µl HE/L air (Table 2). For female fertility, we recorded a reduction of more than 95% at low doses (1 and 5 µL HE/L of air) and 100% at high doses (10 and 20 µL HE/ml of air). Similarly, no insect appeared in any of the treated batches (Table 3).

Contact test
At low doses (1 and 5µl/L) the findings are not satisfactory (Table 4 and Table 5), but at higher doses of 20µl/L, 96 ± 5.47 per cent of mortality was recorded after 3 days of treatment. This mortality increased gradually to 100 ± 0 per cent after 4 days of exposure. All oil concentrations showed a reduction in fecundity and a rate of emergence greater than 90 per cent with a total absence of onsets reported in batches treated at doses of 10 and 20 µL/L.

Repellency test:
The mean percentage of repellency of *S. aromaticum* essential oil against *C. maculatus* is shown in Table 6. According to the results, the

repellent effect of the essential oil increases with the concentrations after 2 and 4 hours and reaches its maximum at concentration 20 µL/L. At the concentration 20 µL/L, the percentage of repulsion of the essential oil against *C. maculatus* is 56.67 after 2 h, and 70 after 4 h. *S. aromaticum* is one of the major plant origins of phenolic compounds,[28-30] Eugenol is a compound mainly responsible for the aroma of cloves and accounts for between 72 and 90 per cent of the volatile oil of cloves.[29] Other common constituents of essential oil are eugenyl acetate, β-caryophyllene, methylsalicylate, pinene, vanillin[31], and α-humulène.[32] Variation in the components and composition of clove essential oil depends on the variety, agro-ecological factors, pre-treatment, refining and extraction methods.[31,23] The essential oil of *S. aromaticum* is effective against insects, the seed pest C. maculatus due to the high amount of Eugenol (80.26%), Eugenyl acetate (9.62%) and *β*-Caryophyllene (6.74%), and is non-toxic to humans and mammals. This hypothesis was also put forward by Lawal[33] to explain the efficacy of clove oils on various pests. These terpene compounds play a repellent role at low concentrations and a lethal role at high concentrations.[34] The study also showed that the essential oil of *S. aromaticum* tested resulted in a significant reduction in the amount of eggs laid by the females of C. maculatus. This reduction is observed from the lowest concentration. The oviposition reduction rate is 95.93 ± 0.97 per cent (inhalation test) and 93.46 ± 1.2% (contact test) respectively to 0 ± 0% in the control jar at a low dose of 1µl/l air. At a high dose of 20µl/l of air, a total reduction of 100% of egg laying and emergence was recorded. Similar results were reported by De Oliveira, Sasikala and Sahaf. [35–37] This reduction in oviposition is thought to be due to the early death of *C. maculatus* adults because of essential oil treatment, as shown by Schmidt *et al*.[38] and Mazibur and Gerhard [39] studying the effect of *Acorus calamus* oil on *Callosobruchus phaseoli*. The contact toxicity of volatile vegetable oils to pests of products stored as insecticides is due to their volatility and therefore their low persistence. These properties have forced researchers to repeat applications.

Table 1: Chemical composition of essential oils of *Syzygium aromaticum*

Compunds	Components	Relative percentage
1	Isovaleral	tr
2	Furfural	0.01
3	Benzaldehyde	tr
4	6-Methyl-5-hepten-2-one	tr
5	Limonene	tr
6	Benzyl alcohol	tr
7	(E)-@-Ocimene	tr
8	Linalool	0.01
9	(E)-4,8-Dimethylnona-1,3,7-triene	0.01
10	Ethyl benzoate	tr
11	Methyl salicylate	0.05
12	Chavicol	0.09
13	Eugenol	80.26
14	Dihydroeugenol	0.14
15	C-Copaene	0.14
16	β-Bourbonene	tr
17	1,5-diepi-β-Bourbonene	tr
18	Vanillin	0.01
19	Methyleugenol	0.03
20	β-Caryophyllene	6.74
21	Caryophylla-4(12),8(13)-diene	0.01
22	9-epi-Isocaryophyllene	0.01
23	C-Humulene	1.14
24	allo-Aromadendrene	0.01
25	trans-Cadina-1(6),4-diene	0.03
26	G-Muurolene	0.01
27	Germacrene D	0.01
28	*β*-Selinene	0.02
29	*α*-Selinene	0.02
30	*α*-Muurolene	0.02
31	*γ*-Cadinene	0.07
32	Cubebol [0,07]	0.01
33	trans-Calamenene	0.04

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34	*β*-Sesquiphellandrene	0.14
35	*γ*-Cadinene	0.14
36	Eugenyl acetate	9.62
37	C-Calacorene	0.02
38	Unknown	0.08
39	Unknown	0.02
40	(E)-Nerolidol	0.03
41	Caryophyllene oxide	0.31
42	Caryophyllene oxide isomer	0.26
43	Unknown	0.02
44	Unknown	tr
45	Humulene epoxide I	0.01
46	Widdrol	0.01
47	Humulene epoxide II	0.04
48	(E)-Isoeugenyl acetate	tr
49	1-epi-Cubenol	0.02
50	Caryophylladienol I	0.02
51	Caryophylladienol II	0.03
52	K-Cadinol	0.01
53	K-Muurolol	0.01
54	C-Muurolol	tr
55	Unknown	0.01
56	14-Hydroxy-(Z)-caryophyllene	0.04
57	14-Hydroxy-9-epi-(E)-caryophyllene	0.01
58	14-Hydroxy-(E)-caryophyllene	0.04
59	Germacra-4(15),5,10 (14)-trien-1C-ol	0.03
60	(E)-Coniferyl alcohol	0.04
61	Benzyl benzoate	0.01
62	Unknown	tr
63	Unknown	0.03
64	Unknown	0.01
65	Squalene	0.02
Total identified		99.92

Table 2: Effects of essential oils of *Syzygium aromaticum* tested by inhalation on adult mortality of *C. maculatus*

Dosage (µ/L)	**Percentage of mortality at hours post-treatment**			
	24h	**48h**	**72h**	**96h**
Control	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
1	0 ± 0^{a}	16.67 ± 5.77^{b}	40 ± 0^{b}	53.33 ± 11.55^{b}
5	3.33 ± 4.4^{a}	20 ± 0^{b}	56.67 ± 5.77^{c}	63.33 ± 15.27^{b}
10	3.33 ± 4.4^{a}	33.33 ± 5.77^{c}	80 ± 10^{d}	100 ± 0^{c}
20	6.67 ± 4.4^{a}	36.67 ± 5.77^{c}	100 ± 0^{e}	100 ± 0^{c}

Each value is a mean ± the standard error of three repetitions. The means in the same column followed by the same letter(s) are not significantly different ($p > 0.05$) using the LSD test.

Table 3: Effects of essential oils of *Syzygium aromaticum* on oviposition and emergence of adults of *C. maculates*

Dosage (µm/L)	**Egg-laying reduction rate**	**Adult inhibition rate (% IR)**
control	0 ± 0^{a}	0^{a}
1	95.93 ± 0.97^{b}	100^{b}
5	97.83 ± 0.57^{c}	100^{b}
10	100^{d}	100^{b}
20	100 ± 0^{d}	100^{b}

Each value is a mean ± the standard error of three repetitions. The means in the same column followed by the same letter(s) are not significantly different ($p > 0.05$) using the LSD test.

Table 4: Effects of essential oils of *Syzygium aromaticum* tested by contact on the mortality of adules of C. *maculatus*

Dosage (µm/L)	Percentage of mortality at hours post- treatment			
	24h	48h	72h	96h
Control	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
1	2 ± 4.47^{a}	10 ± 7.07^{b}	38 ± 4.47^{b}	48 ± 8.36^{b}
5	6 ± 5.47^{b}	14 ± 8.94^{b}	54 ± 5.47^{c}	62 ± 8.36^{c}
10	4 ± 5.47^{ab}	36 ± 5.47^{c}	84 ± 8.94^{d}	78 ± 7.07^{d}
20	18 ± 4.47^{c}	40 ± 7.07^{c}	96 ± 5.47^{e}	100 ± 0^{e}

Each value is a mean ± the standard error of five repetitions. The means in the same column followed by the same letter(s) are not significantly different ($p > 0.05$) using the LSD test.

Table 5: Effects of essential oils of *Syzygium aromaticum* tested by contact on oviposition and emergence of adults of *C. maculatus*

Dosage (µm/L)	Egg-laying reduction rate	Adult inhibition rate (% IR)
Control	0 ± 0a	0 ± 0^{a}
1	93.46 ± 1.2b	95.33 ± 2.64^{b}
5	95.98 ± 1.3b	97.75 ± 2.08^{c}
10	97.9 ± 1.24b	100 ± 0^{c}
20	99.44 ± 0.67b	100 ± 0^{d}

Each value is a mean ± the standard error of five repetitions. The means in the same column followed by the same letter(s) are not significantly different ($p > 0.05$) using the LSD test.

Table 6: Repellent activity of different concentrations of *S. aromaticum* essential oil against *Callosobruchus maculates*

Insect	Conc. (µl/L)	Repellency (%) ± Standard error	
		2h	4h
Callosbruchus maculatus	1	30 ± 10^{a} (II)	46.66 ± 5.77 (III)
	5	33.33 ± 5.77^{a} (II)	50 ± 10 (III)
	10	43.33 ± 11.55^{a} (III	53.33 ± 11.54 (III)
	20	56.67 ± 5.77^{ab} (III)	70 ± 10 (IV)

Each value is a mean ± the standard error of five repetitions. The means in the same column followed by the same letter(s) are not significantly different ($p > 0.05$) using the LSD test. Repellency class: Class 0 – 0-0.1%, Class I – 0.1 – 20%, Class II – 20.1-40%, Class III – 40.1-60%, Class IV – 60.1-80%, Class V – 80.1-100%

In the present study, certain volatile oils displayed remarkable insecticidal properties against stored seed parasites, mainly *C. maculatus*. Our conclusions are in agreement with the results of several previous researchers who reported that the insecticidal activity of essential oils against a broad spectrum of insects in stored products with an influence on all parameters of the life cycle of pests that may be slightly or profoundly affected by treatments. Indeed, bioactive molecules may not even kill insects directly, as in the case of our study, but may lead to a significant reduction in oviposition, as well as a number of developmental disorders, which may completely inhibit the development of individuals[36]. The inhalation test totally inhibits the C *maculatus* population. This is probably due to the test's ability to evenly distribute the volatile oils throughout the jar as opposed to the contact test.

Conclusion

The results of this study revealed that the essential oil *S. aromaticum* is effective for the mortality, reproduction, growth and repulsion of *C. maculatus.* In view of the above results and the low risk of plant compounds to humans and living beings, as well as their biodegradability in nature and lower environmental impact compared to synthetic pesticides, this essential oil may be an appropriate alternative to chemical insecticides in the fight against *C. maculatus*, at least in small warehouses. Further study is also required to determine the rate of absorption of essential oils by stored seeds and to achieve an appropriate formulation for their use as protectors of stored products.

Conflict of Interests

The authors declare no conflict of interest.

Author's declaration

The authors declare that the work in this article is original and that any liability for claims relating to this article content will be borne by them.

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