CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF *CRITHMUM MARITIMUM* L. ESSENTIAL OIL AGAINST STORED-PRODUCT BEETLE *TRIBOLIUM CASTANEUM* (HERBEST)

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Several plant essential oils have been used against diverse insect pests since, unlike conventional pesticides, they pose almost no risk to humans and the environment. For this reason, the essential oil (EO) isolated from the fresh leaves of *Crithmum maritimum* L. and its fractions (F_{1-5}) obtained by chromatographic simplification were investigated for their chemical profile, as well as for their toxicity and repellency effects against *Tribolium castaneum* (Herbst) adults. The analysis by GC/MS allowed the identification of 92.8-99.1% of the compositions the total oil (EO) and of its fractions (F_{1-5}). The EO and its fractions F_{3-5} were characterized by the presence of a high amount of phenylpropanoids (94.4, 94.8, 93.6 and 88.7%, respectively): in all the samples, dill apiole was the most abundant component (EO: 94.1%, F_3, 94.6%, F_4: 93.4% and F_5: 83.3%). In addition, the repellency assay results showed that the volatile fraction F_5 and the complete EO exhibited a higher repellency towards *T. castaneum* (97% and 93%, respectively) after 2 h of exposure at the dose of 0.04 $\mu L/cm^2$. The median lethal dose of the topical application of the EO was 9%. Furthermore, the fraction F_1 possessed interesting contact toxicity against *T. castaneum* (80% of mortality) at the concentration of 10%. These results suggested that the essential oil of *C. maritimum* leaves might be used as an alternative to synthetic insecticides in order to prevent insects from damaging the stored products.

Key words: *Crithmum maritimum*, essential oil, fractionation, chemical composition, insecticidal activity.

Introduction

Stored insect pests present a serious menace to stored food commodities, leading to qualitative and quantitative losses throughout the world.[1-4] The widespread scavenger known as the red flour beetle (*Tribolium castaneum*) is considered one of the most destructive pests of stored products.[5-9] This insect feeds particularly on cereals, oil seeds, flour, spices, nuts, milled rice and wheat based products. [10-12] During the consummation of the product, this pest releases an unpleasant liquid secretion with a pronounced smell: this liquid makes the remaining stored product non-proper for further use, with consequent financial losses for facilities like flour mills and grocery stores.[13] In order to tackle this issue, classical solutions, based on the use of synthetic insecticides such as organophosphates and carbamates compounds, are still employed.[14-16] However, we can not neglect the negative impact that these chemicals have on human health and on the environment.[17-19] Recently, Zhou et al.[20] reported that the organophosphate chlopyrifos, a pesticide used in agriculture, can cause cytotoxicity to human cell lines. Therfore researchers were trying to replace these dangerous substances with more environmentally friendly alternatives.[21,22] In this context, several options have been suggested as a replacement of toxic insecticides, such as those based on plants.[23,24] Indeed, many aromatic plants are a rich sources of essential oils (EOs) having active properties.[25,26] EOs are complex combination of volatile secondary metabolites, molecular weight and lipophilic compounds.[27] These complex mixtures and their major constituents have gained great attention due to their promising biological activities.[28] The ability of some EOs to repel insects makes them one of the best green insecticides alternative, as there are generally considered safe for the protection of stored products.[29-33] Apiaceae is one of the largest plant families: its species produce EOs which can be used in agriculture and other industries for several purposes.[34,35] In this framework, the insecticidal activity of EOs of species belonging to the Apiaceae family has been investigated on a wide diversity of insects.[36-40] *Crithmum maritimum* L., also known as sea fennel or rock samphire, is an aromatic halophyte that belongs to the Apiaceae family.[41] It is widely distributed in coastal areas of Europe and Mediterranean Sea, especially on rocks, walls and sands.[41] The sea fennel has many claimed medicinal properties such as antiscorbutic, diuretic, digestive and carminative.[42,43] The chemical composition of the essential oil isolated from *C. maritimum* has been studied by several researchers from all over the world.[44-52] Various chemotypes have been identified in the chemical profile of *C. maritimum*, namely monoterpene hydrocarbon-types (*α*-pinene/limonene, sabinene/*γ*-terpinene/limonene, *γ*-terpinene/sabinene, *γ*-terpinene/sabinene/*β*-phellandrene, *β*-phellandrene/(*Z*)-*β*-ocimene/*p*-cymene), aromatic monoterpene-types (thymol methylether/carvacrol methylether) and phenylpropanoid-types (dill apiole, dill apiole/methylchavicole).[53] Numerous biological activities have been previously ascribed to this EO, such as antioxidant, antiacetylcholinesterase,[50] antibacterial,[54] antifungal,[49] and insecticidal against *Culex quinquefasciatus* Say and *Spodoptera littoralis*.[53]

The aim of this study was the valorization of *C. maritimum*, an abundant and perennial species, and the evaluation of its use as a natural resource to replace hazardous synthetic insecticides. In this context, we isolated, fractionated on silica gel column chromatography and studied the chemical composition of the EO of the leaves of this species and its fractions. Then, the extracted EO and its fractions (F_{1-5}) were evaluated for their insecticidal activity against *Tribolium castaneum*, one of the most common insects of the stored products.

Results and Discussion

Chemical composition of the essential oil and its volatile fractions (F_{1-5})

The fresh leaves of *C. maritimum* were extracted by hydrodistillation giving a pale yellow colored EO in a 0.19% (w/w) yield. The chemical composition of the EO and its fractions (F_{1-5}) obtained by chromatographic simplification was determined by GC-FID and GC/MS and the components were identified by comparison of their linear retention indices values and mass spectra with those reported in the literature (Figures 1-6). Analysis of the EO and its volatile fractions (F_{1-5}) led to the identification and quantification of 8, 35, 6, 4 and 16 compounds, representing 100, 99.5, 99.1, 100, 100 and 96.8%, respectively belonging to different chemical classes (Table 1). We noted that the major chemical class of the EO of *C.*

maritimum and its fractions F_{3-5} was that of phenylpropanoids (88.7-94.8%) mainly represented by dill apiole **39** (83.3-94.6%). The high amounts of non-terpene derivatives were detected in fraction F_1 (65.0%), (*Z*)-11-hexadecen-1-ol **47** (40.1%) was identified as the most abundant component of this group. The fraction F_2 chiefly consisted of sesquiterpene hydrocarbons (90.4%), among which *γ*-muurolene **18** (25.9%), *β*-sesquiphellandrene **28** (23.8%), *α*-zingiberene **21** (15.6%) and *β*-bisabolene **25** (9.1%) were the most abundant ones. Thymol methyl ether **5**, quantified in the EO and its fractions F_{3}-$_{5}$ at a relatively low concentrations (1.2-6.3%), was the main compound of the oxygenated monoterpenes class. The chemical structures of some of the most abundant compounds are shown in Figure 7. The chromatographic simplification was particularely carried out to confirm the identification of the EO constituents, and to locate the insecticidal activity of EO in one or a few fractions and therefore try to understand the origin of this activity. The fractionation of EO allowed us to concentrate certain constituents in some fractions such as *γ*-Terpinene **1** (in F_1) and *γ*-Muurolene **18** (in F_2) and to identify some undetected components on the chromatogram of the EO, in particular *trans*-*α*-Bergamotene **13** (in F_1 and F_2), *α*-Zingiberene **21** (in F_2), *β*-Sesquiphellandrene **28** (in F_2) and (*Z*)-11-Hexadecen-1-ol **47** (in F_1).

According to the literature, the composition of *C. maritimum* volatiles from the Mediterranean area has been reported. The leaves EO from Medenine (South of Tunisia) was characterized by the presence of dill apiole (41.35 %), thymol methyl ether (27.75 %) and *γ*-terpinene (22.54 %);[55] in the aerial parts oil from Monastir (Center-East of Tunisia) were mainly composed by *γ*-terpinene (39.3%), methylcarvacrol (21.6%) and dill apiole (19.7%); in the roots oil, the main components were terpinolene (36.9%), dill apiole (26.8%) and *γ*-terpinene (21.9%).[50] The aerial parts EO of *C. maritimum* from different locations of Italy have been found to be rich in *γ*-terpinene (37%), methyl thymol (29%), *p*-cymene (10%) (Campania); in thymol methylether (25.5%), limonene (22.3%), *γ*-terpinene (22.9%) (Sicily) and in dill apiole

(41.0%), γ-terpinene (29.8%), β-phellandrene (13.3%) in a sample (Sardinia).[52] On the other hand, the major components of the EO of *C. maritimum* from different locations of Turkey were reported as γ-terpinene (24%) and dill apiole (21%) in the aerial parts EO from Mersin;[54] as methylthymol (29.8–8.1%), γ-terpinene (29.8%) (24.5–8.2%), dill apiole (21.5–1.9%), terpinen-4-ol (21.2–2.7%) and sabinene (20.5–13.0%) in the stems and leaves EO from Silifke;[56] as γ-terpinene (39.3%), β-phellandrene (22.6%), carvacrol methylether (10.5%) and (*Z*)-ocimene (8.2%) in the leaves EO from Lapta-Kyrenia coasts.[52] By comparison with these literature data, our essential oil did not contain limonene, methylcarvacrol and terpinolene, except γ-terpinene which was identified as a minor constituent (0.3-0.9%). On the other hand, a similarity can be noted between our EO and those isolated from the aerial parts and seeds from France, which contained dill apiole (55.7 and 39.9%, respectively) as a major compound.[53] Our results showed a relevant variability in the EO composition of *C. maritimum* from one region to another. This can be explained by the difference in the microclimatic zones affected by the influence of altitude, the cultivation zone, the origin and the stage of the material collected. [57,58]

Repellent activity

The average repellency values for the essential oil of *C. maritimum* leaves and its fractions ($F_{1\text{-}5}$) towards *T. castaneum*, recorded after 15, 30, 60 and 120 min, were given in Table 2. The EO repelled this insect very quickly and strongly at all tested experiment times. This EO repelled 93% of insects, after 2 h of treatment, at the concentration of 0.04 µL/cm^2. Such a repellent activity ascribed this EO to the repulsive class V. No appreciable differences were observed for the repellent activity of this oil at all tested exposure times.

This finding can be explained by the high content of dill apiole **39** (94.1%) in the tested EO, known for its insecticidal activity.[59-63] Gomes et al.[64] found that combination of dill apiole/pyrethroids exhibited a synergistic effect in controlling *Aedes aegypti* and *Anopheles* *albitarsis* mosquitos. In addition, dill apiole **39** synergistically interacted with several insecticides, against the mosquito larvae *Aedes atropalpus* and the flour beetle *Tribolium castaneum*.[65] However, a synergistic effect with the minor compounds should be taken into account. On the other hand, with the exception of F_1 and F_2, all the other fractions (F_{3-5}) exhibited a strong repellent activity against *T. castaneum*. It was also found that F_4 was more repellent than F_3 (PR = 90%) against adults after 2 h of exposure. This result may be related to the relatively high content of dill apiole **39** and thymol methyl ether **5** in F_4 compared to F_3. Thymol methyl ether was assessed as repellent agent against *Aedes aegupti*.[66] The strong activity of fraction F_5 (97% after 2 h of exposure), compared to the other fractions, may be explained by a synergistic effect between dill apiole **39** and the oxygenated sesquiterpenes and diterpenes, both detected only in this fraction (1.9 and 1.4%, respectively). Previous works have demonstrated the insecticidal property of spathulenol **34** and viridiflorol **36**, two oxygenated sesquiterpenes detected in F_5.[67-69]

Contact toxicity

Another test to evaluate the insecticidal activity is based on contact toxicity. The results, noted after 24 h of exposure, are depicted in Table 3: the mortality percentage increased proportionally with the concentration of the EO. However, the highest dose (10%) caused 50% mortality when applied topically on the the abdomen of insects. Statistical analyses showed that *C. maritimum* EO was toxic to *T. castaneum* adults, with LD_{50} value of 9%. Dill apiole **39** (94.1%), could be responsible for this insecticidal activity. Almeida et al.[70] showed the toxic effect of dill apiole **39** on larvae and adults of *Anopheles marajoara* and *Aedes aegypti*. Another study performed by Passreiter et al.[71] claimed the toxic effect of dill apiole **39** against 3rd instar armyworms *Pseudaletia unipuncta*, with a LD_{50} value of 5.8 μg/larva. These findings substantially reinforced the strong contribution of this compound to the toxicity of *T. castaneum* EO by contact.

On the other hand, the comparison of the activity between the different fractions, illustrated in Table 4, showed that fraction F_1 was more toxic (80% after 24 h of treatment) than the other ones. The presence of a high content of non-terpene derivatives (65%) in this fraction could be partly responsible for its activity towards the stored product beetles. Furthermore, our results showed that fractions F_3 and F_4 displayed a mortality percentage of 60% and 70%, respectively, against *T. castaneum*. This slight difference may be due to the variable proportion of some common compounds in their compositions, such as the thymol methyl ether **5** (2.5 and 6.3%, respectively). Previous studies indicated that this compound caused 80% and 60% of mortality of *A. aegypti* larvae at the doses of 62.50 and 31.25 ppm, respectively.[66]

Conclusion

This work constitutes a contribution to the study of the chemical composition of the essential oil of *Crithmum maritimum* leaves through its fractionation into five fractions by column chromatography and their valorization as potential insecticidal agents. Their chemical profiles consisted mainly of the phenylpropanoid dill apiole 39. Furthermore, the EO and its fractions (F_{1-5}) were assessed for their insecticidal activity against the ubiquitous stored product beetle *Tribolium castaneum.* The EO exhibited a strong repellent activity at short time of exposure, which seems ascribable to its large relative content of dill apiole **39** (94.1%). This essential oil was also found to be moderately toxic on this insect when applied topically. Moreover, the tested fractions have shown significant insecticidal effects. These findings allow us to conclude that *C. maritimum* essential oil could be used as primary material in the formulation of a biopesticide, as an effective alternative to chemical synthetic insecticides, to protect stored foodstuffs against this pest.

Experimental Section

Plant material

Leaves of *Crithmum maritimum* L. were collected in the region of Monastir (Tunisia) in March 2018 and identified by Professor Fethia Harzallah-Skhiri, in the Laboratory of Genetic, Biodiversity and Valorisation of Bioresources (LR11ES41), Higher Institute of Biotechnology of Monastir. A voucher specimen (*C.m*-L.18) has been deposited in our laboratory.

Chromatographic analysis

The essential oil and its fractions (F_{1-5}) were analyzed by GC using a flame ionisation detector (FID), equipped with a HP-5 capillary column (30 m × 0.25 mm ID, 0.52 mm film thickness). The column temperature was programmed at 50 °C for 1 min then increased to 280 °C at 5 °C/min where it was held at isothermal for 1 min. The injector and detector temperatures were 250 °C and 280 °C, respectively using the nitrogen as the carrier gas at a flow rate of 1.2 ml/min. The injection volume was 0.1 ml of 1% solution in *n*-hexane.

GC-EI-MS analyses were performed with a CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with an HP-5 capillary column (30 m × 0.25 mm; film thickness 0.25 µm) and a Varian Saturn (Varian Inc., Palo Alto, CA) 2000 ion-trap mass detector. Oven temperature was programed from 60 to 240 °C at 3 °C/min. The injector and transfer line temperatures were 220 and 240 °C respectively using the helium as carrier gas at a flow rate of 1 ml/min. The injection volume was 0.2 µl of a 1% hexane solution, with a split ratio of 1:30. The acquisition parameters were in this way: full scan; scan time: 1.0 sec; scan range: 35-300 m/z; threshold: 1 count. The identification of the constituents was performed by the comparison of the retention times with those of pure authentic samples, comparing their LRI relative to the series of *n*-hydrocarbons, and on a computer matching them against commercial and a home-made libraries of mass spectra, built from pure substances and components of known oils, and the MS literature data.[72-77]

Isolation and Fractionation of the essential oil

Fresh leaves of *C. maritimum* (5.2 kg) were cut into little pieces and were subjected to hydrodistillation during 3 h using a Clevenger-type system. The obtained essential oil (EO) was decanted, dried over anhydrous sodium sulfate and stored in sealed glass vials at 4-5 °C until chemical and biological analysis.

A sample of essential oil of *C. maritimum* (4 g) was fractionated on a silica gel 60 (0,063-0,200 μm) column (L = 70 cm, ID = 3.5 cm) using hexane – ethyl acetate mixture (95: 5; 90: 10; 80: 20; 70: 30) to afford five fractions (F_{1-5}): fraction F_1 (119.5 mg, 3.0% of oil); fraction F_2 (157 mg, 3.9% of oil); fraction F_3 (497 mg, 12.4% of oil); fraction F_4 (2.8 g, 70.0% of oil); fraction F_5 (94 mg, 2.4% of oil) based on an analytical study on a TLC plate. These fractions (F_1-F_5) were also submitted to gas chromatography.

Insecticidal activity

Insect rearing

Adults of *Tribolium castaneum* used were taken from laboratory rearing (Laboratory of Entomolgy of the Regional Center of Research of Horticulture and Organic Agriculture of Chott- Mariem, Sousse, Tunisia). These insects were cultured on food medium based on wheat flour with 5% yeast extract. The rearing conditions were as follows: darknes, 26 °C and 60% humidity. Adults were transferd weekly on new medium plastic boxes to have same stage generation insects.

Repellent activity bioassay

The insecticidal activity of the essential oil and its fractions (F_{1-5}) against *T. confusum* was determined by a repellency test.[78] A repellant is a substance capable of repelling insects from treated surfaces to an untreated surface, this can ensure the reduction of damage from the insect pest.

Briefly, *C. maritimum* EO and its fractions (F_{1-5}) were put on a 9 cm Whatman filter paper no. 1 circular disks sheared into semicircles. Tested samples were adjusted by a dilution of 4 µL in 1 mL of acetone providing a corresponding concentration of 0.12 $\mu L/cm^2$. A measure of 0.5 mL of each solution was evenly distributed on the first half filter paper, while the other half was steeped with 0.5 mL of acetone as a control using a 1000 µL micropipette (single-channel mechanical micropipette; DG1120 model; Labo moderne, France). After drying for 10 min, treated and control half disks were taped together (Figure 8). Then, 20 insects were introduced in the center of the filter paper and the Petri dishes were covered and kept in the dark. After 15 min, 30 min, 60 min and 120 min of exposure, we registered the number of insects present on the control (C) and treated (T) areas. All bioassays were performed in three repetitions. Moreover, the percentage repellency (PR) was calculated as follows:

$$PR= [(Nc-Nt)/ (Nc+Nt)]*100$$

Where Nc and Nt were the number of insects in the negative control half and in the treated half, respectively. The mean PR values were used to classify the essential oil and its volatile fractions in different repellent classes suggested by McDonald (1970)[79] from 0 to V as follows: Class 0 (PR < 0.1%), Class I (PR = 0.1 to 20%), Class II (PR = 20-40%), Class III (PR = 40- 60%), Class IV (PR = 60-80%) and Class V (PR = 80-100%).[79]

Contact toxicity bioassay

Contact toxicity is a method consisting of contacting a quantity of the sample dissolved in a solution with the body of the insect and measuring its toxicity by counting mortalities. Contact toxicity assays were assessed testing *C. maritimum* leaves essential oil on *T. castaneum* adult. Aliquots of 1 µL of EO at different concentrations (1, 5, 10% of EO diluted with acetone) or fractions (10% of each fraction diluted with acetone) were applied topically to the dorsum of *T. castaneum* adults using a micro-syringe (ten insects per replicate, five replicates per dose for EO and three replicates for each fraction) (Figure 9). After evaporating the solvent, ten adults were separately introduced on each Petri dish (9 cm diameter). Insects treated with acetone were used as negative controls. The experiment was carried out in five repetitions. Mortality of insects was recorded after 24 h of treatment. *T. castaneum*, without any movement in legs and and antennae, were considered dead.[80]

Statistical analysis

Data were performed by using the software from Statistical Package of Social Sciences (SPSS).[81] Duncan's multiranage experiment was used to estimate the difference between the means at $p < 0.05$. The correction employing Abott's formula was applied to correct mortality data for control response.[82] LD_{50} value (representing the lethal dose in percentage that produced 50% mortality of insects) for *C. maritimum* essential oil was determined by probit analysis based on 24 h mortality with five replicates of 3 doses ranged from 1% to 10% with ten insect adults.[83]

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Author Contribution Statement

Mayssa Ben Mustapha did all the phytochemical work. Afifa Zardi-Bergaoui contributed to the discussion of the results and completed the redaction of the manuscript. Ikbel Chaieb guided the insecticidal experiments and performed the statistical analysis. Guido Flamini and Roberta Ascrizzi performed the gas chromatographic analyses and elaborated the relative results. Hichem Ben Jannet was the supervisor of the present work, he also completed the redaction of the manuscript.

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Table 1. Chemical composition of essential oil (EO) and its volatile fractions F_{1-5} from fresh leaves of *Crithmum maritimum*.

			Composition (%)[b]						
N°	**Compounds**	**LRI[a]**	**EO**	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**Identification**
1	*γ*-Terpinene	1062	0.3	6.3	0.9	-	-	-	GC/MS, RI
2	4-Terpineol	1178	-	-	-	-	-	1.0	GC/MS, RI
3	*p*-Cymen-8-ol	1183	-	-	-	-	-	0.1	GC/MS, RI
4	*α*-Terpineol	1191	-	-	-	-	-	0.2	GC/MS, RI
5	Thymol methyl ether	1235	4.5	-	-	2.5	6.3	1.2	GC/MS, RI
6	Carvacrol	1298	-	-	-	-	-	0.7	GC/MS, RI
7	*α*-Copaene	1376	-	1.4	-	-	-	-	GC/MS, RI
8	*β*-Bourbonene	1384	-	1.6	-	-	-	-	GC/MS, RI
9	*β*-Ylangene	1414	-	1.8	-	-	-	-	GC/MS, RI
10	*β*-Caryophyllene	1420	-	-	1.8	-	-	-	GC/MS, RI
11	*β*-Copaene	1429	-	1.4	0.1	-	-	-	GC/MS, RI
12	*γ*-Elemene	1433	-	0.3	0.6	0.3	-	-	GC/MS, RI
13	*trans*-*α*-Bergamotene	1438	-	**13.4**	0.4	-	-	-	GC/MS, RI
14	Aromadendrene	1441	-	-	0.5	-	-	-	GC/MS, RI
15	α-Humulene	1456	-	-	0.1	-	-	-	GC/MS, RI
16	(*E*)-β-Farnesene	1460	-	-	0.6	-	-	-	GC/MS, RI
17	*cis*-Muurola-4(14),5-diene	1462	-	-	0.3	-	-	-	GC/MS, RI
18	*γ*-Muurolene	1477	0.3	0.7	**25.9**	-	-	-	GC/MS, RI
19	*α*-Selinene	1494	-	1.0	-	-	-	-	GC/MS, RI
20	Bicyclogermacrene	1495	0.2	-	-	0.4	-	-	GC/MS, RI
21	*α*-Zingiberene	1496	-	-	**15.6**	-	-	-	GC/MS, RI
22	*α*-Muurolene	1498	-	1.1	-	-	-	-	GC/MS, RI
23	*trans*-*β*-Guaiene	1499	-	0.5	-	-	-	-	GC/MS, RI

24	*γ*-Patchoulene	1502	-	0.9	-	-	-	-	GC/MS, RI
25	*β*-Bisabolene	1509	-	-	**9.1**	-	-	-	GC/MS, RI
26	*trans*-*γ*-Cadinene	1513	-	-	4.6	-	-	-	GC/MS, RI
27	Myristicin	1522	0.2	-	-	0.2	0.2	0.2	GC/MS, RI
28	*β*-Sesquiphellandrene	1523	-	-	**23.8**	-	-	-	GC/MS, RI
29	*δ*-Cadinene	1524	-	1.2	-	-	-	-	GC/MS, RI
30	*trans*-Cadina-1(2),4-diene	1534	-	0.4	4.3	-	-	-	GC/MS, RI
31	Selina-3,7(11)-diene	1542	-	0.4	1.9	-	-	-	GC/MS, RI
32	Germacrene B	1554	0.4	-	1.0	2.1	0.1	-	GC/MS, RI
33	Elemicin	1556	0.1	-	-	-	-	5.0	GC/MS, RI
34	Spathulenol	1576	-	-	-	-	-	0.7	GC/MS, RI
35	Globulol	1583	-	-	-	-	-	0.5	GC/MS, RI
36	Viridiflorol	1590	-	-	-	-	-	0.3	GC/MS, RI
37	1-Hexadecene	1592	-	0.5	-	-	-	-	GC/MS, RI
38	*n*-Hexadecane	1600	-	0.6	-	-	-	-	GC/MS, RI
39	Dill apiole	1622	**94.1**	0.5	-	**94.6**	**93.4**	**83.3**	GC/MS, RI
40	*α*-Cadinol	1654	-	-	-	-	-	0.2	GC/MS, RI
41	Apiole	1684	-	-	-	-	-	0.1	GCMS, RI
42	Juniper camphor	1692	-	-	-	-	-	0.1	GC/MS, RI
43	*α*-Vetivol	1756	-	-	-	-	-	0.2	GC/MS, RI
44	1-Octadecene	1786	-	0.5	-	-	-	-	GC/MS, RI
45	*n*-Octadecane	1800	-	0.8	-	-	-	-	GC/MS, RI
46	Neophytadiene I	1841	-	1.0	-	-	-	-	GC/MS, RI
47	(*Z*)-11-Hexadecen-1-ol	1867	-	**40.1**	-	-	-	-	GC/MS, RI
48	*n*-Nonadecane	1900	-	0.5	-	-	-	-	GC/MS, RI
49	*n*-Eicosene	1990	-	0.6	-	-	-	-	GC/MS, RI

50	*n*-Eicosane	2000	-	1.2	-	-	-	-	GC/MS, RI
51	(*Z*)-Falcarinol	2040	-	-	-	-	-	1.7	GC/MS, RI
52	Kaurene	2043	-	0.8	-	-	-	-	GC/MS, RI
53	(10 *E*)-10-Heneicosene	2060	-	0.4	-	-	-	-	GC/MS, RI
54	*n*-Heneicosane	2100	-	2.1	-	-	-	-	GC/MS, RI
55	(*Z*)-Phytol	2116	-	-	-	-	-	1.4	GC/MS, RI
56	1-Docosene	2190	-	0.5	-	-	-	-	GC/MS, RI
57	*n*-Docosane	2200	-	2.8	-	-	-	-	GC/MS, RI
58	1-Eicosanol	2281	-	0.3	-	-	-	-	GC/MS, RI
59	*n*-Tricosane	2300	-	7.7	-	-	-	-	GC/MS, RI
60	1-Tetracosene	2394	-	1.2	-	-	-	-	GC/MS, RI
61	*n*-Tetracosane	2400	-	4.2	7.8	-	-	-	GC/MS, RI
62	*n*-Pentacosane	2500	-	1.2	-	-	-	-	GC/MS, RI
Monoterpene hydrocarbons			0.3	6.3	0.9	-	-	-	
Oxygenated monoterpenes			4.5	-	-	2.5	6.3	3.1	
Sesquiterpene hydrocarbons			0.8	26.0	90.4	2.7	0.1	-	
Oxygenated sesquiterpenes			-	-	-	-	-	1.9	
Diterpene hydrocarbons			-	1.7	-	-	-	-	
Oxygenated diterpenes			-	-	-	-	-	1.4	
Phenylpropanoids			94.4	0.5	-	94.8	93.6	88.7	
Other non-terpene derivatives			-	65.0	7.8	-	-	1.7	
Total (%)			100.0	99.5	99.1	100.0	100.0	96.8	

[a]LRI, linear retention indices (HP-5 column).

[b]%, percentage calculated by GC-FID on non-polar capillary column HP-5.

Bold type indicates major component.

Table 2. Repellent activity of *C. maritimum* essential oil and its volatile fractions F_{1-5} on *Tribolium castaneum* after different exposure times.

Echantillon	**t (exposure) (min)**	**Percentage repellency (mean ± SE)**	**Class**
Essential oil	15 min	83 ± 6^{a}	V
	30 min	87 ± 6^{a}	V
	60 min	87 ± 6^{a}	V
	120 min	93 ± 6^{a}	V
F_1	15 min	-10 ± 0^{a}	0
	30 min	3 ± 35^{a}	I
	60 min	17 ± 6^{a}	I
	120 min	27 ± 15^{a}	II
F_2	15 min	57 ± 6^{b}	III
	30 min	50 ± 17^{b}	III
	60 min	57 ± 21^{b}	III
	120 min	37 ± 25^{a}	II
F_3	15 min	53 ± 6^{b}	III
	30 min	67 ± 6^{b}	IV
	60 min	73 ± 21^{b}	IV
	120 min	83 ± 6^{b}	V
F_4	15 min	60 ± 10^{b}	III
	30 min	70 ± 10^{b}	IV
	60 min	70 ± 0^{b}	IV
	120 min	90 ± 10^{b}	V
F_5	15 min	77 ± 15^{c}	IV
	30 min	73 ± 15^{b}	IV
	60 min	73 ± 6^{b}	IV
	120 min	97 ± 6^{b}	V

Values are means ± SE of 3 replications

No appreciable differences were observed for the PR of the essential oil at all tested exposure times.

Significant differences were evident for the PR between fractions at all tested exposure times.

Table 3. Percentage of mortality of *T. castaneum* after 24 h of exposure with *C. maritimum* essential oil.

Concentration (%)	**Mean % adults mortality ± SE**	**LD_{50} (%)**
1	$10 \pm 8^{a*}$	
5	$30 \pm 19^{b*}$	9
10	50 ± 18^{b}	

Values are presented as mean ± SE (n = 5)

Means in column followed by different letter are significantly different at $P < 0.05$

Table 4. Percentage of mortality of *T. castaneum* after 24 h of exposure with different fractions at concentration of 10%.

	Mean % adults mortality ± SE
$\mathbf{F_1}$	80 ± 0[b]
$\mathbf{F_2}$	0 ± 0[a]
$\mathbf{F_3}$	60 ± 0.2[b]
$\mathbf{F_4}$	70 ± 0.2[b]
$\mathbf{F_5}$	57 ± 0.1[b]

Values are presented as mean ± SE (n = 5)

Means in column followed by the same letter are not significantly different at $P < 0.05$

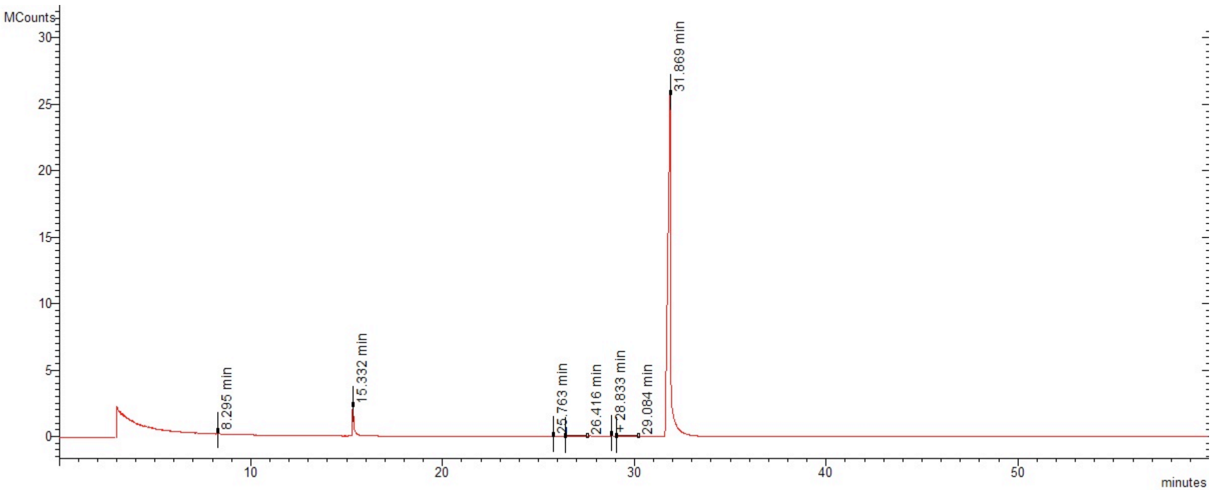


Fig 1. Chromatogram of the leaves essential oil (EO) of *Crithmum maritimum*

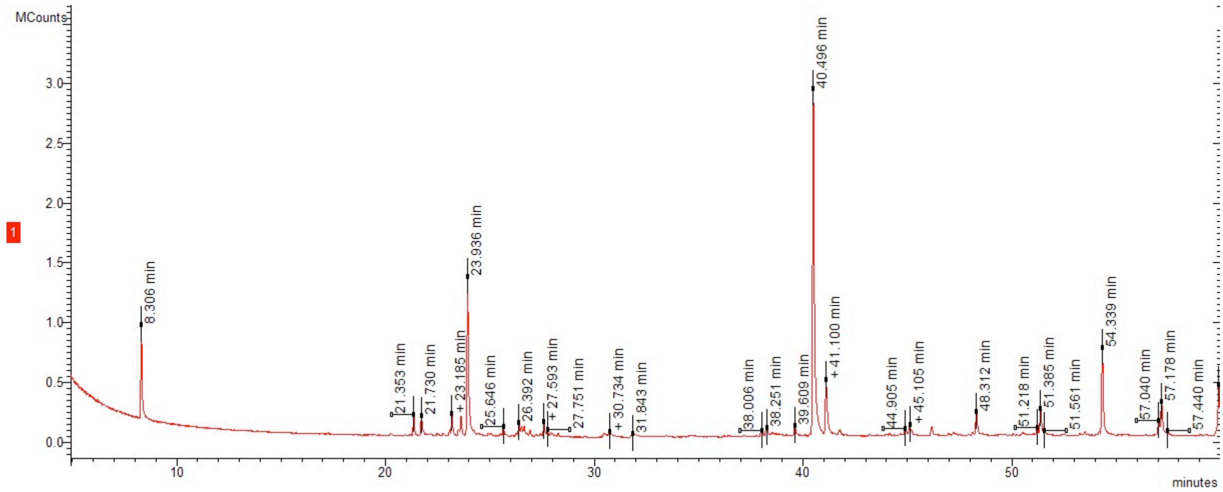


Fig 2. Chromatogram of the fraction F_1 from the leaves essential oil of *Crithmum maritimum*

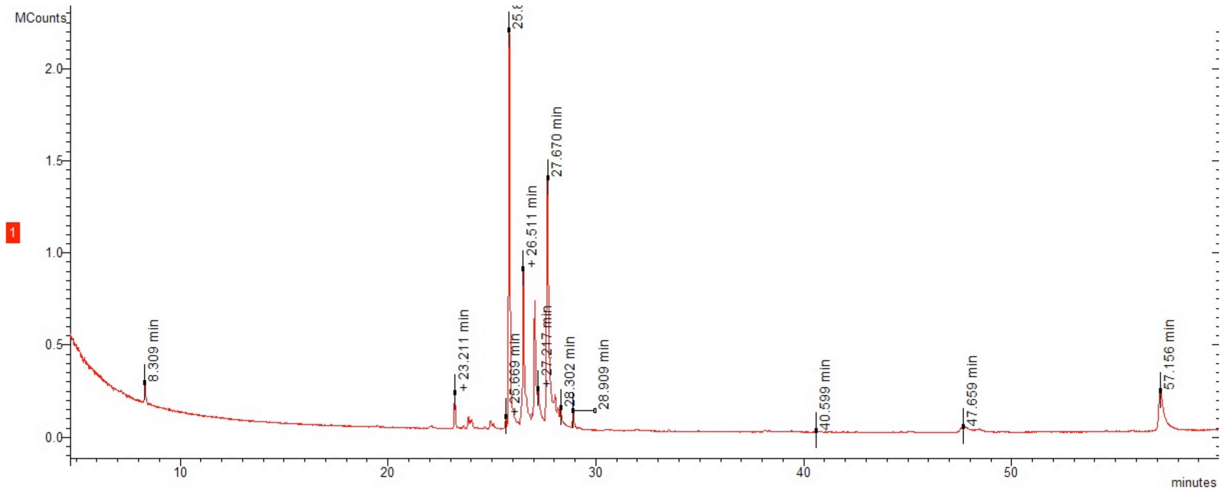


Fig 3. Chromatogram of the fraction F_2 from the leaves essential oil of *Crithmum maritimum*

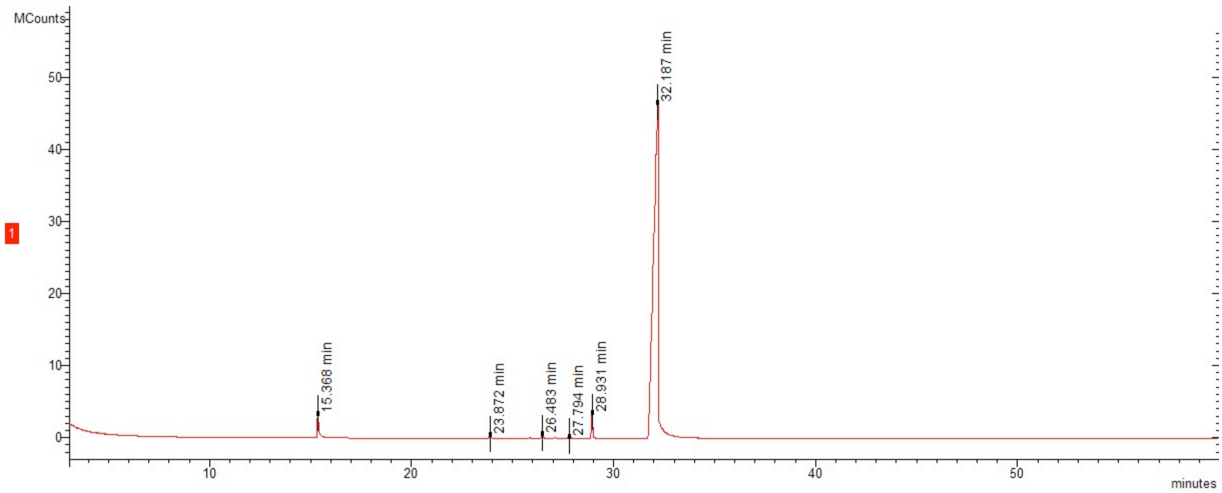


Fig 4. Chromatogram of the fraction F_3 from the leaves essential oil of *Crithmum maritimum*

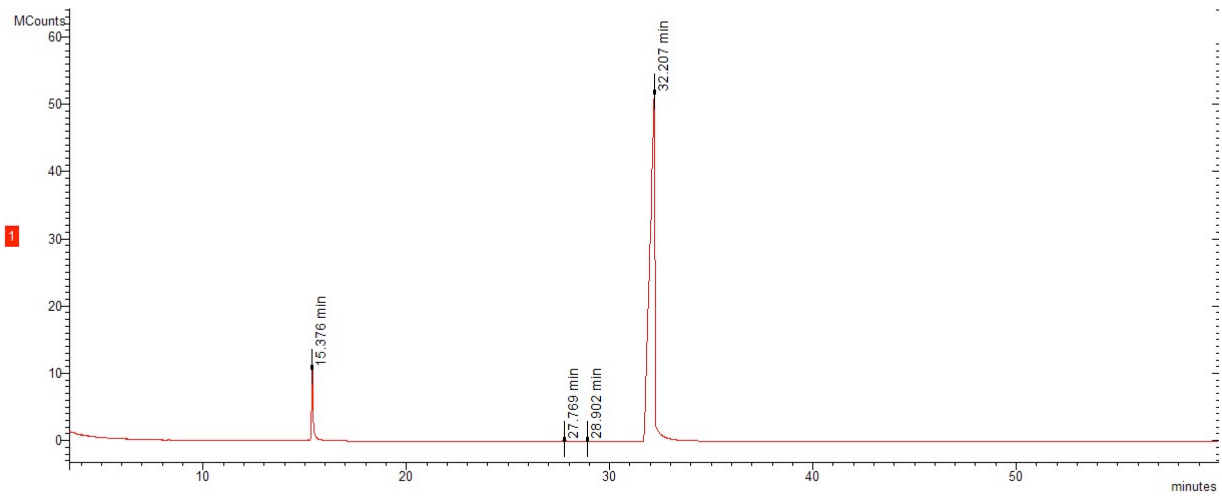


Fig 5. Chromatogram of the fraction F_4 from the leaves essential oil of *Crithmum maritimum*

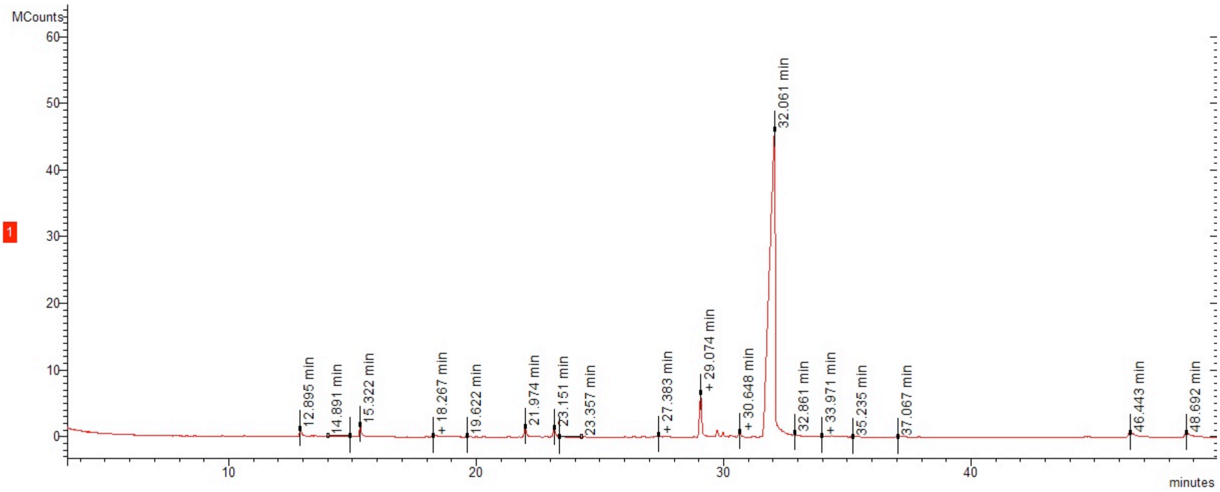
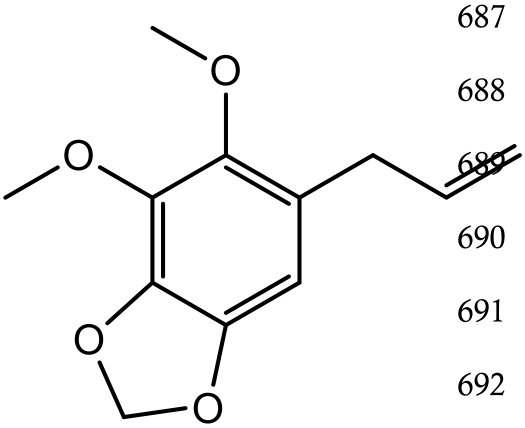
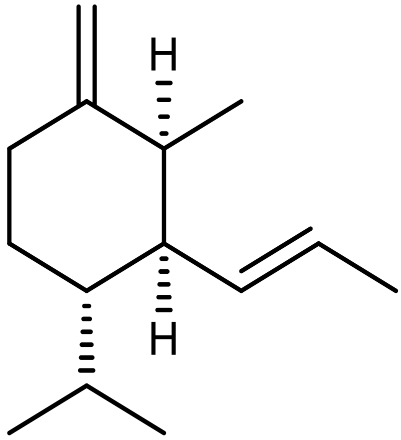


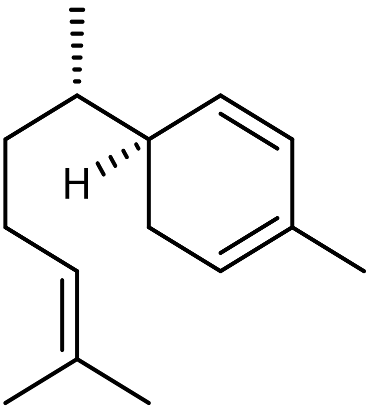
Fig 6. Chromatogram of the fraction F_5 from the leaves essential oil of *Crithmum maritimum*

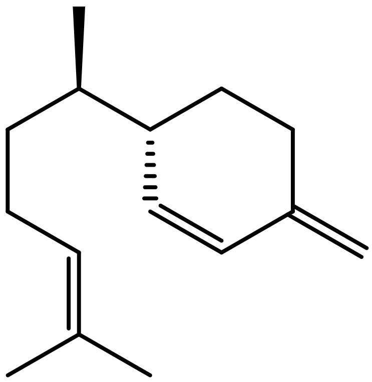




Dill apiole

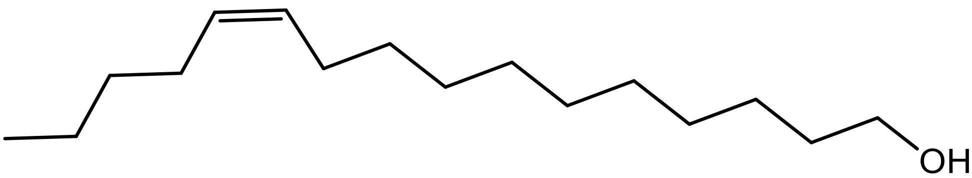
γ-Muurolene





α-Zingiberene

β-Sesquiphellandrene



(Z)-11-Hexadecen-1-ol

Fig 7. Chemical structures of some major compounds identified in the essential oil (EO) and its volatile fractions F_{1-5} from fresh leaves of *Crithmum maritimum*.

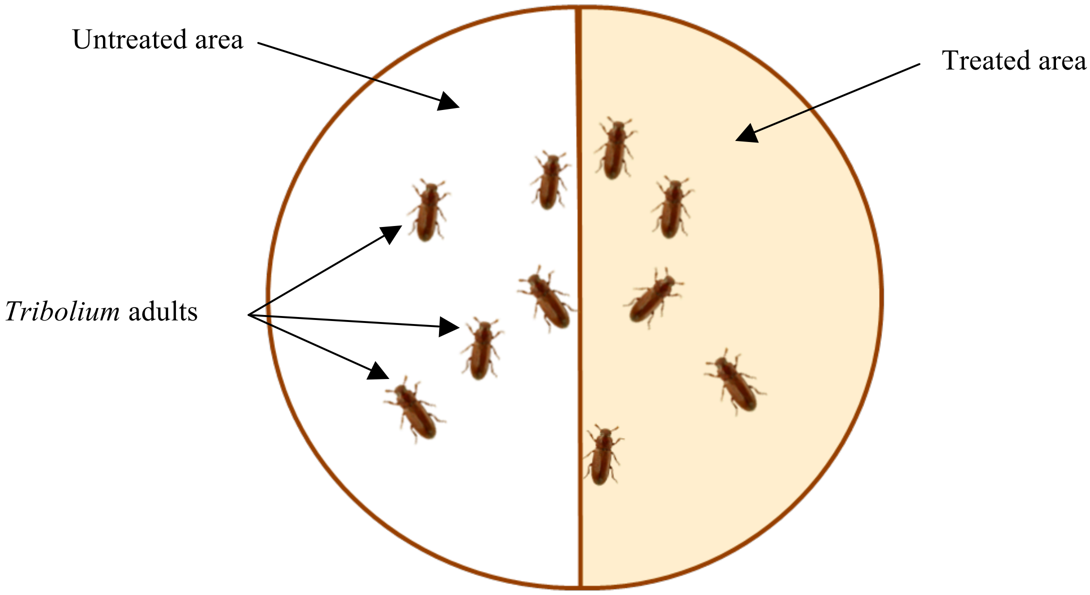


Fig 8. Repellent activity test.

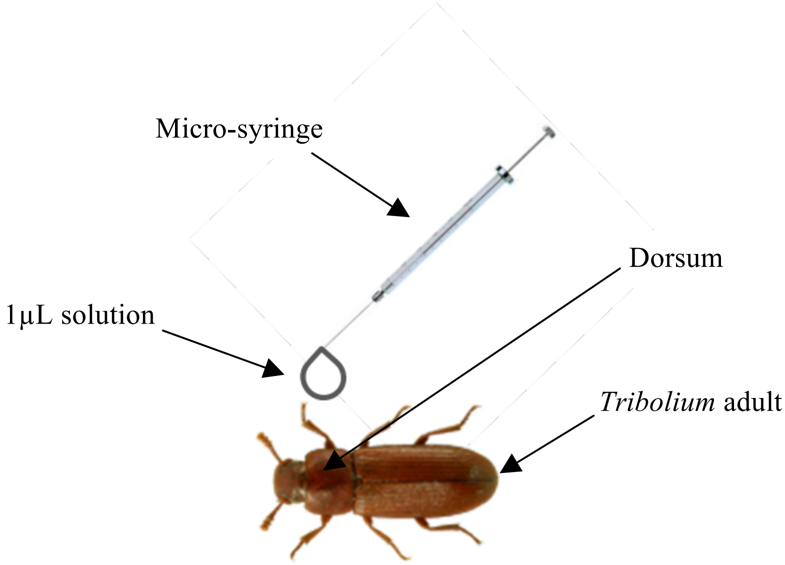


Fig 9. Contact toxicity bioassay