

Chemical Composition of *Ruta chalepensis* Essential Oils and their Insecticidal Activity against *Tribolium castaneum*

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ABSTRACT

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Essential oils are secondary plant metabolites well known for their defensive role in plants. Many essential oils were described as having potent insecticidal activity. In the present work, the chemical composition and the insecticidal activity of *Ruta chalepensis* essential oils against stored product pest *Tribolium castaneum* (adults and larvae) were investigated. The determination of their chemical composition was carried out by using GC-MS technique. Twenty compounds were identified and results showed that essential oil of *R. chalepensis* are rich on 2-undecanon (48.28%) and 2-nonanon (27%). The insecticidal activity of the indicated volatile fractions was screened against adults and larvae of *T. castaneum*. *R. chalepensis* essential oil was found to be more active against adults ($LC_{50} = 176.075 \mu\text{l/l}$ air and $LC_{90} = 291.9 \mu\text{l/l}$ air) than larvae ($LC_{50} = 415.348 \mu\text{l/l}$ air and $LC_{90} = 685.907 \mu\text{l/l}$ air). After 24 h of exposure at the dose of 200 $\mu\text{l/l}$ air, 14% and 60% of mortality were recorded for larvae and adults, respectively. These preliminary findings may be useful for further studies of *R. chalepensis* essential oil use against other food storage pests and for deeper investigations on their mode of action.

Keywords: Chemical composition, essential oils, insecticidal activity, LC_{50} , LC_{90} , *Ruta chalepensis*, *Tribolium castaneum*.

The major cause of post-harvest losses in storage is due to insect pests. These pests are able to destroy a whole stock in a very short period of time during

their development (13). Synthetic insecticides are currently the primary means used to protect stored grains from insects. However, chemical control has been associated with dangers like environmental pollution, human toxicity, development of insecticide resistance and adverse effects on non target organisms (23, 22, 17). The use of natural plant compounds, having insecticidal activity

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and little environmental effect, is one of promising alternative to protect post-harvest products (7, 19). Among botanicals, the Rutaceae species have attracted a lot of attention due to the range of biological activities induced by their secondary metabolites including antifungal, antioxidant, and anti-inflammatory properties (5, 6, 8, 9, 12, 15). One of the widely diffused species in the Mediterranean area is *Ruta chalepensis* commonly known as fringed rue (6). This perennial herb is characterized by oval, large, pinnate and blue-green leaves that have many oblong lanceolate lobes. The inflorescence of this species is in the form of cyme (3).

The main purpose of this work is to determine the chemical composition and to study, under laboratory conditions, the insecticidal effects of the essential oils from *R. chalepensis* leaves against an important pest of stored grains *Tribolium castaneum*.

MATERIALS AND METHODS

Insect rearing.

Adults and larvae of *T. castaneum* used for testing were obtained from laboratory rearing, maintained in growth chamber in total obscurity at $28 \pm 1^\circ\text{C}$ and 60% RH in the Laboratory of Entomology of the Regional Center of Research on Horticulture and Organic Agriculture of Chott-Mariem (CRRHAB ChM), Tunisia.

Plant material.

R. chalepensis leaves were collected from the southern Tunisia (Mednine) where it grows spontaneously.

Essential oil extraction.

Essential oils were extracted from fresh leaves by hydrodistillation using a

Clevenger apparatus for 4 h at 350°C . The extracted oils were weighed in order to calculate their extraction yield and stored at 4°C prior to analysis.

Chemical analysis.

The essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) using a HP 6890N GC Mass spectrometer with electron impact ionization (HP 5975B). A HP-5MS capillary column (30 m \times 250 μm , 0.25 μm film thickness) was used. Oven temperature was programmed to rise from 60 to 220°C at a rate of $40^\circ\text{C}/\text{min}$; transfer line temperature was 230°C . The carrier gas was helium with a flow rate of 0.8 ml/min and a split ratio of 50:1. Scan time and mass range were 1 sec and 50-550 m/z, respectively. The essential oils constituents were identified by comparing their relative retention times. The determination of the percentage composition was based on peak area normalization without using correction factors.

Fumigant test.

This bioassay was determined by using closer container method, which consists in isolating groups of ten insects, and then, fixing paper discs, treated by different doses of oil (25, 50, 100 and 200 $\mu\text{l}/\text{l}$ air), on the top of containers. Five replicates were used for tested oils and control. The mortality of insects (%) was observed and determined after 3, 6, and 24 h of exposure.

Data analysis.

The estimation of LD_{50} and LD_{90} values of *R. chalepensis* leaf essential oils against adults and larvae of *T. castaneum* were determined by probit analysis. Mean mortality separation were done using

Duncan's multirange test at the 5% level through SPSS (Version 19) (18).

RESULTS

Essential oil composition.

Table 1 summarizes the composition of *R. chalepensis* leaf essential oils. In total, 22 compounds

were identified. The 2-undecanone is the most predominant compound (48.28%) followed by the 2-nonanone (27.15%). The terpenic compounds are present in low proportion in comparison to the aromatic ones. In fact, the 1,8-cineole (1.87%) and the viridiflorol (1.74%) are the most frequent compounds in this oil.

Table 1. Chemical composition of *Ruta chalepensis* leaf essential oils

N°	Compound	Peak area (%)	Retention time (min)
1	α -pipene	0.71	6.38
2	Camphene	0.65	6.70
3	β -Pinene	0.22	7.33
4	2-Octanone	0.47	7.64
5	Limonene	0.24	8.49
6	1,8-Cineole	1.63	8.56
7	γ -terpinene	0.17	9.16
8	2-Nonanone	23.70	10.10
9	2-Nonanol	1.71	10.17
10	Nonanal	0.24	10.23
11	Geyrene	1.33	11.02
12	Camphor	0.85	11.11
13	Borneol	0.30	11.58
14	2-Decanone	2.58	12.07
15	Octyl acetate	0.11	12.41
16	1-Nonene	14.20	13.04
17	Pregeijerene	0.12	13.31
18	2-Undecanone	42.15	14.14
19	2-Dodecanone	1.34	15.47
20	2-Tridecanone	0.49	17.83
21	Viridiflorol	1.52	19.52
22	α -eudesmol	0.19	20.65

Fumigant activity.

Mortality. Fumigant toxicity of essential oils of *R. chalepensis* on adults and larvae of *T. castaneum* noted after 3, 6 and 24 h of exposure is shown in Table 2. Statistical analyses showed that mortality rate is proportional to the dose and time of exposure. Results also indicated that adults were more susceptible to *R. chalepensis* essential oils

than larvae with 60 and 14% of mortality, respectively, noted after 24 h post-treatment.

Determination of LC_{50} and LC_{90} values. Calculated LC_{50} and LC_{90} values makes out that fumigant toxicity of *R. chalepensis* leaf oil was more active against adults than larvae of *T. castaneum* (Table 3).

Table 2. Mortality rate of adults and larvae of *Tribolium castaneum* noted depending on doses of *Ruta chalepensis* essential oils and exposure time.

Insect instar	Dose ($\mu\text{l/l air}$)	Mortality per exposure time (%)		
		3 h	6 h	24 h
Adult	0	0 a	0 a	0 a
	25	2 \pm 4.47 a	2 \pm 4.47 ab	2 \pm 4.47 ab
	50	4 \pm 5.48 a	18 \pm 10.95 c	18 \pm 10.95 b
	100	4 \pm 5.48 a	12 \pm 10.95 bc	16 \pm 13.42 ab
	200	12 \pm 8.37 b	18 \pm 8.37 c	60 \pm 18.71 c
Larva	0	0 a	0 a	0 a
	25	0 a	4 \pm 5.48 a	4 \pm 5.48 a
	50	0 a	4 \pm 5.48 a	6 \pm 5.48 ab
	100	0 a	6 \pm 5.48 a	8 \pm 4.47 ab
	200	2 \pm 4.47 a	8 \pm 4.47 a	14 \pm 5.48 b

For each developmental stage et exposure time, values followed by the same letter are not significantly different based on Duncan's multirange test at $P < 0.05$.

Table 3. Determination of LC_{50} and LC_{90} values of *Ruta chalepensis* leaf oil against *Tribolium castaneum* depending on target instars.

<i>T. castaneum</i>	C_{50} ($\mu\text{l/l air}$)	C_{90} ($\mu\text{l/l air}$)
Adult	176.075	291.9
Larva	415.348	685.907

DISCUSSION

R. chalepensis essential oil was obtained by hydrodistillation. The obtained essential oil yield (0.34%) is comparable to those reported in the literature (4, 10). Moreover, the abundance of 2-undecanone in oils is also observed in the works done on *R. chalepensis* essential oils but with some variation of the percentage mentioned as those from Algeria (68.95%)(11), Argentina (38.1%) (20), Turkey (66.5%) (2), Iran (52.5%) (16) and Tunisia (77.18%) (10). The nature and proportions of other compounds present in the oils investigated in the current study are not always the same in comparison with those reported in previous studies. These variations can be attributed to climatic, seasonal and geographic conditions, harvest period,

chemotypes and/or extraction procedures (14). Concerning the fumigant toxicity of the obtained essential oil, insecticidal activity was highest against adults of *T. castaneum* than larvae. This result can be explained by the relationship that may exist between the insect physiology and the mode of action of essential oils. In fact, these volatile compounds act directly on the nervous system of the insect which is primitive for larvae and explains their resistance to essential oils. Unfortunately, in the literature we did not found studies that highlight the potential bio-insecticide of *R. chalepensis* essential oils against stored products pests. However, several biological activities of *R. chalepensis* essential oils were reported such as antifungal, antimicrobial, antioxidant, anti-inflammatory, phytotoxic activities (3, 5, 6, 8, 9, 12, 15). In addition, LC_{50}

and LC₉₀ values were lower for adults than for larvae. Similar effect was observed by Wang *et al.* (21) where *Artemisia vulgaris* oil had strong fumigant activity against *T. castaneum* adults and larvae but adults were much more susceptible than larvae. Moreover, Bachrouch *et al.* (1) highlighted that the essential oil of *Pistacia lentiscus* have fumigant toxicity against the red flour beetle *T. castaneum*. This toxicity potential also depended on developmental stages, oil concentrations and exposure times. In fact, the fumigant toxicity of *P.*

lentiscus was higher against adults (LC₅₀ = 28.03 µl/l, LC₉₅ = 63.46 µl/l) than against the third instar larvae (LC₅₀ = 112.12 µl/l, LC₉₅ = 253.53 µl/l).

The results from study revealed an interesting potential of *R. chalepensis* essential oils in controlling the food storage pest *T. castaneum*. Further studies are needed to better explain the observed phenomenon and to determine a relationship between the chemical composition of the essential oils and their mode of action against the target insect pests.

RESUME

Majdoub O., Dhen N., Souguir S., Haouas D., Baouandi M., Laarif A. et Chaieb I. 2014. Composition chimique des huiles essentielles de *Ruta chalepensis* et leur activité insecticide contre *Tribolium castaneum*. Tunisian Journal of Plant Protection 9: 83-90.

Les huiles essentielles sont des métabolites secondaires des plantes, connues pour leur rôle défensif chez les plantes. De nombreuses huiles essentielles ayant une activité insecticide puissante ont été décrites. Dans le présent travail, la composition chimique et l'activité insecticide des huiles essentielles de *Ruta chalepensis* contre un ravageur des denrées stockées, *Tribolium castaneum* (adultes et larves) ont été étudiées. La détermination de leur composition chimique a été réalisée moyennant la technique GC-MS. Vingt composés ont été identifiés et les résultats ont montré que les huiles essentielles de *R. chalepensis* sont riches en 2-undécanone (48,28%) et en 2-nonanone (27%). L'activité insecticide de ces huiles a été criblée contre des adultes et des larves de *T. castaneum*. Les huiles essentielles de *R. chalepensis* se sont montrées plus actives contre les adultes (CL₅₀ = 176,075 µl/l air et CL₉₀ = 291,9 µl/l d'air) que les larves (CL₅₀ = 415,348 µl/l air et CL₉₀ = 685,907 µl/l d'air). Après 24 h d'exposition à la dose de 200 µl/l air, 14% et 60% de mortalité ont été enregistrés chez les larves et les adultes, respectivement. Ces résultats préliminaires peuvent être utiles pour mener des études ultérieures sur l'utilisation des huiles essentielles de *R. chalepensis* contre d'autres ravageurs des denrées stockées et des investigations plus profondes sur leur mode d'action.

Mots clés: Activité insecticide, composition chimique, huile essentielle, LC₅₀, LC₉₀, *Ruta chalepensis*, *Tribolium castaneum*

ملخص

مجدوب، أنس ونجلاء دهان وصلاح الدين سوقيير ودلييلة حواس ومريم باوندي وأسماء العريف وإقبال الشايب. 2014. المكونات الكيميائية للزيوت الروحية لنباتة *Ruta chalepensis* ونجاعتها المبيدة ضد حشرة خنفساء الدقيق *Tribolium castaneum*. Tunisian Journal of Plant Protection 9: 83-90.

تعرف الزيوت الروحية على أنها مركبات ثانوية تنتجها النباتات، وتقوم بدور دفاعي عند النباتات. وقد تم تشخيص العديد من الزيوت الروحية بقوة نشاطها المبيد للحشرات. ضمن هذا العمل، تمت دراسة التركيبة الكيميائية والنشاط المبيد الحشري للزيوت الروحية لنباتة *Ruta chalepensis* ضد آفة المنتجات المخزنة، خنفساء الدقيق *Tribolium castaneum* (البالغات واليرقات). تم تحديد التركيبة الكيميائية باعتماد تقنية GC-MS. وتم التعرف على عشرين مركبا

كيميائيا، كما أظهرت النتائج أن الزيوت الروحية لنبته *R. chalepensis* غنية بـ 2-undecanone (48.28%) و 2-nonanone (27%). تم تتبع النشاط المبيد الحشري لدى هذه الزيوت ضد طور البالغات واليرقات لخنفساء الدقيق. أظهرت الزيوت الروحية لـ *R. chalepensis* أكثر سمية ضد البالغات ($LC_{50} = 176.075$ مكل/ل هواء و $291.9 = LC_{90}$ مكل/ل هواء) منها ضد اليرقات ($LC_{50} = 415.348$ مكل/ل هواء و $685.907 = LC_{90}$ مكل/ل هواء). بعد تعرض البالغات واليرقات لمدة 24 ساعة إلى جرعة مقدارها 200 مكل/ل هواء، لوحظ تسهم 14% و 60% من اليرقات والبالغات، على التوالي. تفتح هذه النتائج الأولية الأفاق لمزيد دراسة الزيوت الروحية لنبته *R. chalepensis* ضد آفات أخرى للمنتجات المخزنة والبحث في طرق عملها.

كلمات مفتاحية: تركيبة كيميائية، زيت روية، نشاط المبيد الحشري، LC_{90} ، LC_{50} ، *Ruta chalepensis*، *Tribolium castaneum*

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