JAZ INDIA

Journal of Advanced Zoology

ISSN: 0253-7214
Volume 44 Issue 05 Year 2023 Page 28:34

Chemical Composition and Toxic Effect of Plant Essential Oil Against *Sitophilus Oryzae*(L) (Insecta: Coleoptera:Curculionidae)

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Article History

Received: 04 January 2023
Revised: 16 August 2023
Accepted: 27 August 2023

Abstract

The aerial parts of Ruta montana were collected from two different locations in Algeria (Berrouaghia and HamamMelouane). A hydrodistillation was conducted and the resulting volatile oil was analyzed by Gas chromatography coupled with Mass Spectrometry (GC/MS). The results showed that the best yield in essential oil was obtained in full bloom (0.98%) from the region of Berrouaghia which was higher than the yield of the ecotype gathered in Hammam Melouane (0.82 %). The main predominant component of R.montana collected from Berroughia and HamamMelouane is 2-Undecanone ($C_{11}H_{22}O$); (59.34%) and (49.39%) respectively. The insecticidal test by in vitro contact assay showed a significant mortality of Sitophilus oryzae traited by R. Montana essential oil (EO). The results of this study showed that the toxicity of the R. montana oil vary with increasing concentration of applied doses on one hand, and a relatively gradual efficiency versus time, which results in improved efficiency on the other hand.

***Key words**: Contact toxicity, Essential oil, Rice weevl, Ruta montana, GC/MS*

1. Introduction

The genus *Ruta* (*Rutaceae*) is distributed throughout north regions of Algeria. It is represented by four species and subspecies in Algeria, *R. montana*, *R. chalepensis* subsp. *R.angustifolia*,

R. chalepensis subsp. *latifolia* and the Saharian species *R. tubercula* (Ozenda 1958&Quezel 1963). *Ruta montana* is used in digestive disordersand helminthiasis (Benítez et al. 2010). Traditionally, this species is known to be very harmful to humans especially for pregnant women. On the other hand, *Ruta's* species in several studies have highlighted that Essential oils of those species are believed to act as allelopathic agents that protect plants from predation by insects and infestation by parasites.Storage grain which is one of the safe ways to meet seed needs and ensure food security, is a problem that needs to be given special attention if damage caused by food pests is to be limited after harvest.These are responsible for worldwide loss of 10 - 40% in the stored grains annually (FAO, 2018). Among the beetles, the rice weevl (*Sitophilus oryzae* L.) (Coleoptera: Curculionidae) is universally recognized as one of the most devastating of stored cereal spest throughout the world, not only because of its own consumption, but also because it also opens the door to a whole range of detritivores, the most common of which is the red flour beetle (*Tribolium castaneum*Herbst) (Coleoptera: Tenebrionidae) which completes the damage (Markham et al., 1994).This pest has been reported to spend a significant part of their development inside the grain and are therefore only easily detectable in the adult stage, an emptied grain will then remain, perforated with an irregular hole, containing the droppings of larval development (Kavita, 2006).Their presence in stored foods directly affects both the quantity and quality of the commodity (Singh, 2017).

The conventional chemical control is a major challenge for underdeveloped agricultural countries. However, the use of these synthetic insecticides and fumigants to control storage insects generates adverse effects on human health and the environment (Sharp, 1986), they also have serious effects on increasing pest resistance to pesticides It should be noted also that natural and spontaneous degradation of chemical pesticides is extremely rare, the kinetics of biological disappearance of a pesticide in the soil always begin with a latency period, more or less long, during which degradation is practically absent. Environmental protection is increasingly becoming a major global concern. The Bio Insecticidal activity of many plants against several insect pests has been demonstrated as an alternative to the synthetic chemicals for being biodegradable, pest specific, non-hazardous to human health and can be used to develop environmental safe methods (Papachristos et al, 2004;Kavita, 2006). The essential oils are the complex phytochemical compounds produced as the secondary metabolites whose functions are other than the nutrition, defense and protection. Many botanical Species of those family: Meliaceae, Rutaceae, Asteraceae, Labiateae and Canellaceae are the most promising as sources of bio-insecticides (Jacobsen, 1989).

In the present study, we proceeded to the extraction of the essential oil of *R.montana* (traditional name: Fidjel, Family: Rutacaceae) collected from two locations in North Algeria by (i)hydrodistillation, (ii) analyzed its chemical composition by gas chromatography/mass spectrometry (GC/SM), and (iii) performed an insecticidal investigation against adults of *Sitophills oryzae* , different concentrations have been tested for their insecticidal activity as well as different durations of exposure.

2. Materials And Methods

Plant material

The aerial parts (leaves and flowers, at blossoming stage) of two ecotypes of *R. Montana* were collected from HammamMelouane (HEM) (Blida; 36° 29' N, 3° 03' E) and Berrouaghia(HEB) (Médéa;36° 40' N, 2° 55' E), (Fig. 1) which are both located in the southern margin of the Mitidja basin. The identification of the plants was made in the laboratory of Plant Biology, Department of Biology (University Blida 1, Blida) and confirmed to be *Ruta montana* by Quezel and Santa's (1963). The samples were dried separately at 30°C, and stored before use.

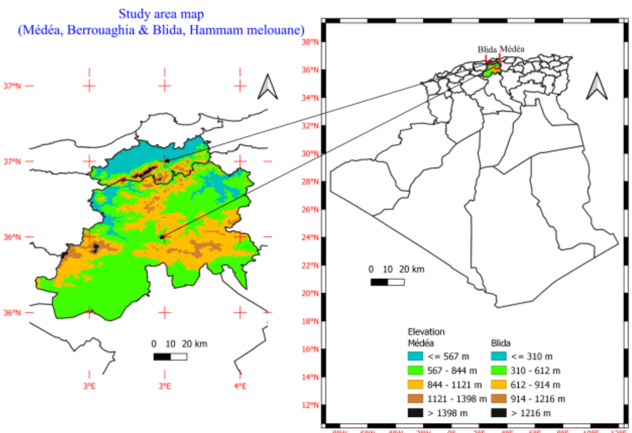

Fig.1: Study area map indicating the sampling site's location; Hammam Melouane (36° 29' N, 3° 03' E) and Berrouaghia (36° 40' N, 2° 55' E).

Essential oils Isolation and extraction

The dried samples of both plant species were ground with an electrical blender, and 100gofeach was dilutedin 500ml of distilled water and subjected to hydrodistillation (HD)using a Clevenger-typeapparatus.

Thehydro-solsproduced by HD, whichlasted3hr, were collected into flasks and covered with aluminum paper to protect them from light (Photo-oxidation) and stored in a refrigerator at a temperature of 4°C.Thevolatilescontained in the hydrosols were investigated as described by Petrakiset al. (2015) and the yield based on the dried weight of the samples was calculated.

Essential oils analysis

Gas chromatography mass spectroscopy analysis

Gas Chromatography (GC) analyses were performed using a Hewlett-Packard 5890 series II, apparatus equipped with flame ionization detection (FID) system. Afused silica capillary column 5% phenyl-poly-dimethyl-siloxane HP-5ms capillary column (30m×0.25mm, film thickness 0.25μm, J&W Scientific) was used. The column temperature was programmed as follows: 60 (3 min hold) to 240°C, at a rate of 4°C/min, and then held isothermally for 10 min. Diluted samples of 1 μl (1/100 in diethyl ether, v/v) were manually injected in split less mode (split ratio 1:30) at 270 °C, using helium as carrier gas (1 ml/min).Qualitative analysis of the volatile fractions was conducted under the same conditions with a mass spectrometer Hewlett-Packard 5972 Mass Selective Detector (MSD) series, in EI mode, with electron energy ionization at 70 eV, ion-source temperature 200°C and the interface temperature 270°C to separate the complex mixture of chemical compounds of the volatile oil lately identified and quantified(Sharp, 1986).

Components identification

Essential oil volatile compounds were identified based on their retention indices (RI) (determined with reference to a homologous series of (C_8- C_{20}) n-alkanes), by coelution and MS analysis and by comparison of their masses spectra, elution order and/or RI with those of the spectra present in the NIST/EPA/NIH Mass Spectral Library 2011 (NIST11) or in literature data. Data acquisition was performed using the software package G1701BA Hewlett Packard Enhanced ChemStation ver. B.01.00/1998 and processing of gas chromatography and mass spectrometry was performed using Hewlett Packard Enhanced Data Analysis program of the software package above (McLafferty and Stauffer, 1991, Adams, 2007).

Collection and identification of insects

Sitophylus oryzae species were used to examine the pesticidal activity of essential oil (EO) extract from *Rutamontan*. Adult and larval stages of insect were collected from **CCLS** (Coopérative des céréales et des légumes sec) Affroun(Blida, Algeria), and identified by a zoologist at the National School of Agricultural Sciences (ENSA), el Harrach, Algeria.

Insecticidal activity of the essential oil of *Ruta montana*

Insecticidal activity was determined by using an *in vitro* contact assay. The essential oil was dissolved in a mix of distilled water and tween 80 solution (97% and 3% respectively) and three doses of this essential oil were prepared (0.25, 0,50, 0.75%). A volume of 30µl of each dilution of the essential oil was applied on petri dishes (7 cm diameter) in such a way that it made a uniform film over the petri dishe (Inouye et al. 2006). After drying, 15 beetles (adult) were released in each petri dish contains 10g of wheat seed with three replications. The control samples consist of discs impregnated with the same volume of tween 80 solutions (3%). All the Petri dishes were immediately closed and sealed with parafilm to prevented evaporation of the oil.

Adult mortality

The petridishe treated and the control sample were placed in an incubator at the same temperature as reared in stock cultures (T° 25C° and 60% HR), the adult mortality rate was recorded during5 days of exposure to treatments. The estimation of death was carried for 120 hours from 24 h after treatment with the essential oil.

Statistical analyses

Hypothesis insecticidal efficacy of essential oil was tested by analysis of variance (GLM) and (ANOVA) bysystat software, ver.12, SPSS 2009.

3. Results and Discussion

The essential oil yield obtained by hydrodistillation of *R. montana* from Berroughia was 0.98 % which was higher than that of the ecotype harvested in Hammam Melouane (0.82%). This result was slightly higher than those obtained for *Ruta graveolens*, the most studied species of the genus *Ruta*. Indeed, Barbosa et al. (2012) and Faria et al. (2013) reported a yield of 0.50 to 0.90%.

Eighty compounds, representing 96.7% of the essential oil were identified; the chemical composition of our *R. montana* oil was dominated by predominance of 2-Undecanone compound. As seen in (Table 1), both ecotypes (Berrouaghia, HammamMelouane) were rich in 2-Undecanone: 59.4% and 49.4%, respectively. Moreover, 6-(3',5'-nzodioxyl)-2-hexanone and 2-Decanone were the major components in Berrouaghia ecotype (13.17% and 12.25%) respectively. 3-Decanone (11.75%.) and 6-(3',5'-Benzodioxyl)-2-hexanone (11.23 %) were the major constituents in sample from HammamMelouane ecotype. It was noted that the fractions (Z)-3-hexen-1-ol acetate (0.08%), and 3-tridecanone fraction (0.17%) were of low quantities in both samples. Undecan-2-one is the main constituent found in the literature of the essential oils of *Ruta* genus (Dob et al., 2008, Barbosa et al. 2012, Faria et al., 2013). Comparison with previous analysis of essential oil released in Algerian ecotypes showed contrasted results (Table 1). Indeed, most of these reports did not present essential yield. Moreover, authors have identified fewer components than observed in our study. In fact, three of the most important compounds identified in our study (6-(3',5'-Benzodioxyl)-2-hexanone, 3-Decanone ($C_{10}H_{20}O$), and 2-Decanone($C_{10}H_{20}O$)) were not observed in the other ecotypes investigated in different regions of Algeria (Table 1). In contrast, all results revealed that 2-Undecanone ($C_{11}H_{22}O$) was the most abundant component in *Ruta montana*. These differences in the oil composition and yield may be due to several factors such as, climatic, genetic, geographic conditions, time of collection and extraction methods (Bousbia 2004, Dobravalskyté et al. 2013, Ammad et al. 2014).

Table 1. Major chemical components of *Ruta Montana* essential oil (%), obtained by Gas chromatography coupled (GC / MS) harvested in two locations in North Algeria.

Comparison was made with previous report performed on this species.

Components	Retention Time (Min)	Berrouaghia (%)	Hammam Melouane (%)	Mila (%)	Constantine (%)	Tipaza (%)	Oran (%)
(Z)-3-hexen-1-ol-acetate ($C_8H_{14}O_2$)	9.86	0.08	0.45	-	**9.19**	-	-
p-Cymene ($C_{10}H_{14}$)	10.75	0.46	0.448	-	-	-	-
D-Limonene ($C_{10}H_{16}$)	11.96	0.10	0.379	-	1.08	-	-
2-Nonanone ($C_9H_{18}O$)	13.39	0.76	4.32	8.63	0.67	-	**29.5**
Nonanal ($C_9H_{18}O$)	13.77	1.64	1.61	**-**	-	-	**18.2**
2-Decanone($C_{10}H_{20}O$)	17.43	**12.25**	**9.06**	**-**	0.77	**-**	**-**
3-Decanone ($C_{10}H_{20}O$)	18.61	-	**11.57**	**-**	-	**-**	**-**
2-Undecanone ($C_{11}H_{22}O$)	21.38	**59.34**	**49.39**	**60.19**	**37.74**	**37.70**	**32.8**
3-Dodecanone ($C_{12}H_{24}O$)	23.50	1.32	1.53	0.27	-	-	-
2-Dodecanone ($C_{12}H_{24}O$)	24.45	4.21	4.45	1.43	0.21	-	-
3-Tridecanone ($C_{13}H_{26}O$)	26.17	0.12	0.17	-	2.27	-	-
2-Tridecanone ($C_{13}H_{26}O$)	27 .37	2.13	2.49	0.41	7.53	-	-
6-(3',5'-Benzodioxyl)-2-hexanone	37.47	13.17	11.23	-	-	-	-
8-(3',5'-Benzodioxyl)-2-octanone	42.22	0.59	0.65				
Ester	-	0.08	0.45	**10.25**	-	-	
Monoterpenshydrocarbons	-	0.56	0.83	0.62		--	
Ketones	-	**93.89**	**94.86**	**81.06**		**95**	
Aldehydes	-	1.64	1.61	**0.13**			
Essentiel oilYield	-	0.98	0,82	-	-	-	

Note: BER: Berrouaghia; HAM:Hamammelouane; Rt: Time retention of Berrouaghia and Hammammelouane

Rutamontana collected from some region of Algeria (North, East and West)

Rg1: Aerial parts of *Rutamontana*, collected from Mila [Belkassam et al,2011].

Rg2: Aerial parts of *RutaMontanaT*., collected from Constantine [Djarri et al,2013].

Rg3: Aerial parts of *Rutamontana*, collected from Tipaza [Boutoumi et al,2009].

Rg4: Aerial parts of *Ruta Montana*, collected from Oran [Kambocha,2008].

In the present investigation, the toxicity of the essential oil extracted from *R.montana*. Aerial parts was evaluated for insecticidal efficacy against an economically significant pest *S.oryzae*. The results showed that the essential oil significantly showed a mortality caused by this (EO) at different time and concentrations as shown in (Fig.3). The maximum dosage (D3: 0.75 %) and the median dose (D2: 0.5 %) of two essential oils from Berrouaghia (HEB) and HammamMelouane (HEM) displayed the most important mortality, compared to the lowest dose (D1: 0.25 %). whereas no mortality was registered, even after 96 h with the control test. D3 and D2 progressed from low toxicity at the start of treatment to high toxicity after up to 48 h. The dose D1 showed low toxicity at the beginning of its application and average toxicity at the end of treatment (Fig. 2). The effect of the essential oil of *R.montana* adults of *S.oryzae* exhibited a net efficiency. This tendency is verified by testing the variance or difference was highly significant ($p = 0.000$, $p < 0.001$). The concentrations and durations of treatment demonstrated a very significant difference in the suppression of the tested beetle.

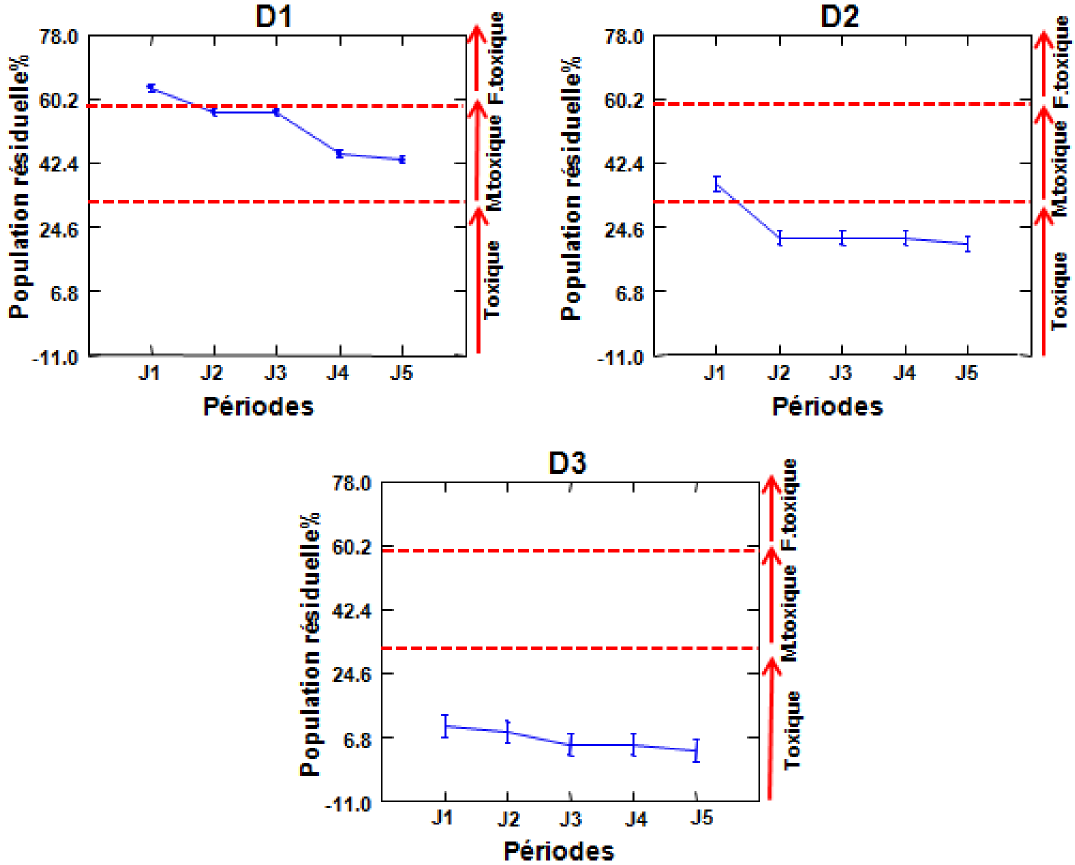

Fig.2.: ANOVA model applied to the period/dose interaction on *S. oryzae* by contact.

(**D1 :**1/4 dose (0,25g/l), **D2 :**1/2 dose (0,5g/l), **D3:** dose (075g/l).

- The results indicate that higher concentrations caused more efficiently than when been diluted. According to the statistical analysis, the volatile oils exhibited different degrees of mortalityof the tested pest. The same results showed that essential oil extracted from *Rutamontana* (Clus.) L. prevented the growth of the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples (Djarri et al., 2013). According to Jacobson (1989), *Meliaceae*, *Rutaceae*, *Asteraceae*, *Annonaceae*, *Abiateae*, and *Canellaceae* are the most promising medicinal plant families. Antimicrobial and insecticidal activities are known to exist in a broad range of essential oils, and in many instances, this activity is linked to the existence of active ingredients, primarily isoprenes such as monoterpenes, sesquiterpenes, and related alcohols, as well as various hydrocarbons and phenol. Chemical analysis revealed that *R. Montana* essential oils included a significant concentration of non-terpenic acyclic ketones, particularly 2-undecanone and 2-tridecanone.Undecan-2-one was reported to possess low activity towards bacteria, but it was more efficient against insect (Gibka et al., 2009). Several authors have recorded the activity of extracts and essential oils of the genus Ruta such as :the antimicrobial, antifungal (Ahmadi et al., 2010; Sadiki et al., 2014; Sharma et al., 2013), antioxidant (Zengin and Baysal, 2014),, insecticidal (K*hanam et al.*2015) ,anti-larval (Taran et al., 2013) and nematicidal properties (Abdel-Rahman et al., 2013).of some essential oils (EOs) that have been isolated from different species of the Ruta's family such as *Ruta graveolen* (i.e.,Active compound; Isogeijerene and *Ruta chalepensis* (rue) who exhibited an important effect on the mortality of *Aphis gossypii* and *Tetranychus urticae*(Traka et al., 2018; Zengin and Baysal, 2014).

Many authors showed that major components were not the only ones responsible for the activity, all of the chemical composition should be taken into account (Cosentino et al., 1999).Lahlou (2004)reported that the activity of essential oils is higher than that of their majority composition when tested separately. For these reasons, we can conclude that the insceticicdal activity of our essential oil is attributed to the synergism between different components, which play an important role. These earlier reported findings clearly support the result of the present study. The mode of action of these essential oils is yet to be confirmed but it appears that death of the adults, may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism (Mobolade Akinbuluma *et al.2017*).

4. Conclusion

The results show that the tested plant is a potential source of botanical insecticides against *S.oryzae* and the toxic effect depends on concentration and time. This may clearly support that exposure time play an important role in influencing susceptibility.

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

Acknowledgements

This study was conducted in the Department of Biotechnology, Laboratory of Zoology , university of Blida 1,Bilda,Algeria.

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