Bio-insecticidal activity of three essential oils against *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae)

S. Ncibi[1*], S. Attia[1], S.M'B. Diop[2], M. Ammar[1] & T. Hance[3]

[1]*Laboratoire de Bio-Agresseurs et Protection Intégrée en Agriculture, Institut National Agronomique de Tunisie, Université de Carthage, 43 Avenue Charles Nicolle 1082, Tunis, Tunisia*
[2]*Département de Chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, BP 5005 Dakar, Senegal*
[3]*Laboratoire Earth and Life Institute, Biodiversity Research Centre, Université Catholique de Louvain, Croix du Sud, 4–5 Bte L7.07.04, B-1348 Louvain-la-Neuve, Belgium*

Natural plant extracts, especially essential oils, are used largely against plant pests. The aim of this study was to investigate the bio-insecticidal effect of chemical extracts of three plants, *Mentha pulegium* L. (Lamiales: Lamiaceae), *Lavandula stoechas* L. (Lamiales: Lamiaceae) and *Ruta chalepensis* L. (Sapindales: Rutaceae) collected from Tunisia, on the lesser grain borer *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae), which is a primary pest of stored wheat. To reach this goal, we firstly did bio-assays to test the toxicity of these plant extracts on *R. dominica* adults using different concentrations of plant extracts on wheat seeds. Secondly, we analysed the chemical compounds of these plant extracts using GC-MS and GC-FID. Our results demonstrated that *R. dominica* mortality was significantly higher with increasing plant essential oils concentrations (CL_{95} values 45.68, 113.12 and 170.52 $\mu l/l_{(air)}$) for *M. pulegium*, *R. chalepensis* and *L. stoechas*, respectively. The main chemical compounds identified of *M. pulegium* oils were: pulegone (76.9 %), isomenthone (12.0 %) and limonene (1.73 %), whereas those from *L. stoechas* were: camphor (23.8 %), 1,8-cineole (17.8 %) and camphene (7.69 %). *Ruta chalepensis* oil revealed high contents of undecanone (51.2 %), 2-nonanone (39.16 %) and 2-decanone (2.32 %). Our results also showed that *M. pulegium* essential oil has a significant effect against *R. dominica* with LC_{50} value of 13.51 $\mu l/l_{(air)}$ compared to *R. chalepensis* and *L. stoechas* corresponding to 39.11 and 42.9 $\mu l/l_{(air)}$, respectively.

Key words: insecticidal activity, fumigant toxicity, GC-MS, fast GC-FID.

INTRODUCTION

The lesser grain borer *Rhyzopertha dominica* is one of the major primary pests of stored grains. It has a high capacity of adaptation and development (Pires & Nogueira 2018; Wijayaratne & Rajapakse 2018). It mainly attacks stored cereals causing a lot of damage. It causes major qualitative and quantitative losses (Banga *et al*. 2018) reaching more than 20 % (Phillips & Throne 2010). Synthetic pesticides such as phosphine and methyl bromide have been widely used to manage this pest (Suthisut *et al*. 2011; Wijayaratne & Rajapakse 2018). However, methyl bromide, a broad-spectrum fumigant, was phased out following the Montreal protocol because it is an ozone-depleting substance (MBTOC 2002; Gareau 2015). Due to the excessive use of chemical products and the associated problems of development of resistance (Suthisut *et al*. 2011; Opit *et al*. 2012; Khan *et al*. 2019) and environmental pollution (Grewal *et al*. 2017), there is also an increasing demand for healthy control methods such as biological control of stored grains using natural chemical plant extracts (*e.g*. essential oils), which have been successfully used as an alternative method to chemical pesticides. Because of their rapid degradation and low environmental and mammalian toxicity, plant essential oils were found to be an efficient and safe method for controlling stored grain pests (Shaaya *et al*. 1997; Wong *et al*. 2005; Ogendo *et al*. 2008; Rajendran & Sriranjini 2008; Suthisut *et al*. 2011; Tampe *et al*. 2016).

Their efficiency as insecticides is due to their behavioural and physiological outcomes such as antifeedant activity, cuticle disruption and growth

*Author for correspondence. E-mail: s.ncibi@gmail.com

Received 26 October 2019. Accepted 4 May 2020

ISSN 1021-3589 [Print]; 2224-8854 [Online]
DOI: https://doi.org/10.4001/003.028.0339

African Entomology **28**(2): 339–348 (2020)

(Ortu *et al*. 2016). Many essential oils have been tested to control insect pests of stored grain and most of them seem to be effective insecticides (Abdelli *et al*. 2016; Said-Al Ahl *et al*. 2017; Ben Chaaban *et al*. 2019).

Ebadollahi (2011) showed that *E. globulus* Labill (Myrtaceae) and *Lavandula stoechas* L. (Lamiaceae) essential oils had significant antifeedant activities against adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) at lower doses corresponding to 12.161 $\mu l/l_{(air)}$ and 13.61 $\mu l/l_{(air)}$, respectively. Other studies showed that *L. stoechas* essential oil exhibited a strong toxicity against adults of *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Ptinidae), *R. dominica* (Coleoptera: Bruchidae) and *T. castaneum* with LC_{50} values of 3.835 $\mu l/l_{(air)}$, 5.66 $\mu l/l_{(air)}$, 39.685 $\mu l/l_{(air)}$ after 24 h of treatment, respectively (Ebadollahi *et al*. 2010).

Many plants such as *Ocimum basilicum*, *L. stoechas*, *Mentha pulegium*, *Rosmarinus officinalis*, *Melissa officinalis*, *Colebrookea oppositifolia*, *Thymus vulgaris* (Lamiaceae), *Citrus hystrix*, *Ruta chalepensis* (Rutaceae), *Artemisia maritima* (Asteraceae) were tested for their toxicity against insect pests of stored grains such as *T. castaneum*, *Trogoderma granarium* (Everts, 1898) (Coleoptera: Dermestidae), *Plodia interpunctella (H*übner, 1813) (Lepidoptera: Pyralidae), *L. serricorne*, *R. dominica* (Ebadollahi et al. 2010; Brari & Thakur 2018; Panezai *et al*. 2019). In fact, many plants belonging to Lamiaceae family such as *L. stoechas* and *M. pulegium* essential oils showed both fumigant toxicity against *T. castaneum* and *L. serricorne* (Ebadollahi *et al*. 2010; Salem *et a*l. 2017). *Mentha pulegium* essential oil recorded LC_{50} values of 11.57 and 8.46 $\mu l/l_{(air)}$ against *T. castaneum* and *L. serricorne*, respectively (Salem *et al*. 2017). However, *L. stoechas* oil showed LC_{50} values of 39.685 and 3.835 $\mu l/l_{(air)}$ on *T. castaneum* and *L. serricorne,* respectively, after 24 h of exposure to essential oil concentrations (Ebadollahi *et al*. 2010).

Syzygium aromaticum (Myrtaceae) and *L. officinalis* (Lamiaceae) essential oils had fumigant toxicity against the pest stored grain *Sitophilus oryzae* with LD_{50} values of 0.04 and 0.07 mg/cm^2, respectively (El-Bakry *et al*. 2016 in Campolo *et al*. 2018). Germinara *et al*. (2017) also showed that *Lavandula angustifolia* Miller (Lamiaceae) essential oil exhibited fumigant toxicity against the adults of *S. granarius* (L.). The essential oil from *M. pulegium* recorded LD_{50} value of 6.95 μl/ml in contact toxicity bioassays and showed fumigant toxicity with 39.2 % mortality after 96 h of treatment and repellent effect with 46.03 % after 30 min of exposure at 2 μl/ml against *R. dominica* (Brahmi *et al*. 2016a).

There is a lack of information about the use of essential oils against the stored grain pest *R. dominica*. In this context, the aim of this study was to evaluate fumigant toxicity of three essential oils extracted from three wild plants traditionally used in medicine in Tunisia: *R. chalepensis* L., *L. stoechas* L. and *M. pulegium* L. against *R. dominica* the lesser grain borer a major insect pest of stored grains in Tunisia and North Africa.

MATERIAL AND METHODS

Insect material

Rhyzopertha dominica adults were collected from infested wheat brought from two storage silos of Cereals Office from Bir El Kasâa and Manouba and the farm of INAT in Morneg, Tunisia. Insects were reared on wheat seeds in a climate room at 28 °C and 40 % RH under complete darkness in the laboratory of the Biodiversity Research Center, UCL (Louvain-la-Neuve, Belgium). Unsexed adults 1–4 days old were used for bioassays tests.

Plant material

Fresh aerial parts of the three spontaneous plants of *R. chalepensis*, *L. stoechas* and *M. pulegium*, used in this study, were collected during their flowering stage in the three northwest regions of Tunisia (Bouaouene, Nefza and Rimmel) and were free of any pre-harvest chemical treatment.

Essential oils extraction and analysis

Essential oils were obtained from 1 kg of fresh aerial parts by steam distillation for 3 h using a Clevenger-type apparatus. Samples were freshly harvested, sorted for uniformity and inspected to ensure the absence of defects, before being stored at –2 °C until analysis.

GC-MS analyses were conducted in the Department of Analytical Chemistry from Gembloux Agro-Bio Tech (University of Liège, Belgium). For quantitative analyses (percentage determination), we used fast GC, which proved to be powerful for analysis of essential oil constituents (Bicchi *et al*. 2004).

Essential oils solutions (10 mg/10 ml (EOs/n-hexane)) were prepared and diluted four times before analysis. One μl of these solutions were injected for analysis. In GC-FID as in GC-MS, the following chromatographic conditions were found to be optimal for good peak resolution. The temperature programme was as follows: isotherm at 40 °C for 5 min then increased to 280 °C by 8 °C/min and final held at 280 °C for 5 min. Injector (splitless mode) and detector temperatures were of 280 °C and 290 °C, respectively. Helium was the carrier gas at a constant flow rate fixed at 1.5 ml/min. The column used was a fused silica capillary column (Optima-5-MS-Accent (Macherey-Nagel, Düren-Germany), 5 % phenyl and 95 % methylsiloxane (30 m × 0.25 mm i.d., film thickness 0.25 μm). GC-FID a Trace Ultra GC (Thermo Electron Corporation, 5 Interscience, Louvain-la-Neuve, Belgium) fitted with a flame ionisation detector was used for determination of the proportions (%) of essential constituents. The percentage of each component was calculated as the ratio of peak area on the total of GC peak areas. Air and hydrogen flows were 350 ml/min and 35 ml/min, respectively. GC-MS identification of components was carried out on a mass spectrometer (Agilent 5973 Network Mass Selective Detector Quadrupole, coupled to a gas chromatograph Agilent 6890N (G1530N), U.S.A). Mass spectra were recorded at 70 eV and the mass scanned range was from 50 to 550 amu. The mass spectra were compared to those from Wiley 275 L, a computerised database and those given in the literature (Joulain & König 1998; Adams 2001). Pure compounds confirmed the identification of the major constituents. Identification of the major components was confirmed by comparing their GC data and mass spectra with those from pure compounds whenever commercially available. For that purpose, stock solutions (10 mg/10 ml *n*-hexane) were prepared and diluted enough to obtain symmetrical peaks when using the mentioned chromatographic conditions. The retention data of pure compounds were compared with those of essential oils constituents. The tolerance limit was ± 0.1 min.

Fumigant toxicity

Essential oils extracted from *R. chalepensis*, *L. stoechas* and *M. pulegium* were tested by fumigation for their insecticidal activities on the adults of *R. dominica*. Tests were conducted in 31 ml glass jars. Different concentrations were used corresponding to 6.45, 11.29, 16.13, 32.26, 64.51 and 129.03 μl/l$_{(air)}$. To determine the natural mortality of insects, other treatments were carried out without essential oils. For this purpose, filter paper (Whatman No.1 disks of 1.7 cm diameter) impregnated with essential oils at the mentioned concentrations, were attached to the lids of glass vials. Ten unsexed adults of *R. dominica* were added into a vial and tightly sealed and kept under the same conditions as rearing (28 ± 1 °C and 40 ± 10 % RH) at continuous darkness. Insect mortality was recorded daily starting from the first day after treatment until the seventh day. Individuals were considered dead when neither leg nor antenna showed a movement even when probed. Each treatment with and without essential oils (control treatment) were replicated seven times.

Data analysis

Mortality was corrected using Abbot's formula (Abbot 1925). Lethal concentrations 50 % (LC_{50}) were calculated using Probit analysis (Finney 1971). All data were subjected to the Statistical Analysis System (SAS 9.0) and the PDMix procedure to detect the difference between essential oils, concentrations and time at the 5 % probability level.

RESULTS

Oil yields

The oil yields by steam distillation of the aerial parts of *R. chalepensis*, *L. stoechas* and *M. pulegium* were 0.39, 0.15 and 0.68 %, respectively.

Fumigant toxicity

Results of the three essential oils toxicity against *R. dominica* are presented in Fig. 1, which shows the effect of essential oils on the mortality of *R. dominica* adults. After 24 h, all insects in the control group were still alive. The mortality of insects increased with increasing concentrations of essential oils. In addition at the higher concentration (129.03 μl/l$_{(air)}$), mortalities reached 100 % after 24 h after treatments with the three essential oils used in this study. *Mentha pulegium* essential oil caused 36.93, 73.87 and 81.83 % of mortality for 6.45, 11.29 and 16.13 μl/l$_{(air)}$, respectively. Mortalities reached 100 % for higher concentrations which correspond to 32.26, 64.51 and 129.03 μl/l$_{(air)}$ after 24 h, respectively. For *R. chalepensis* oil, low mortalities with 8.36, 11.22 and 15.96 % were noted at 6.45, 11.29 and 16.13 μl/l$_{(air)}$, after 24 h, respectively. These rates increased strongly at 32.26 μl/l$_{(air)}$ with 48.98 % mortality and reached 100 % at the higher concentration of 129.03 μl/l$_{(air)}$. After 24 h, *L. stoechas* essen-

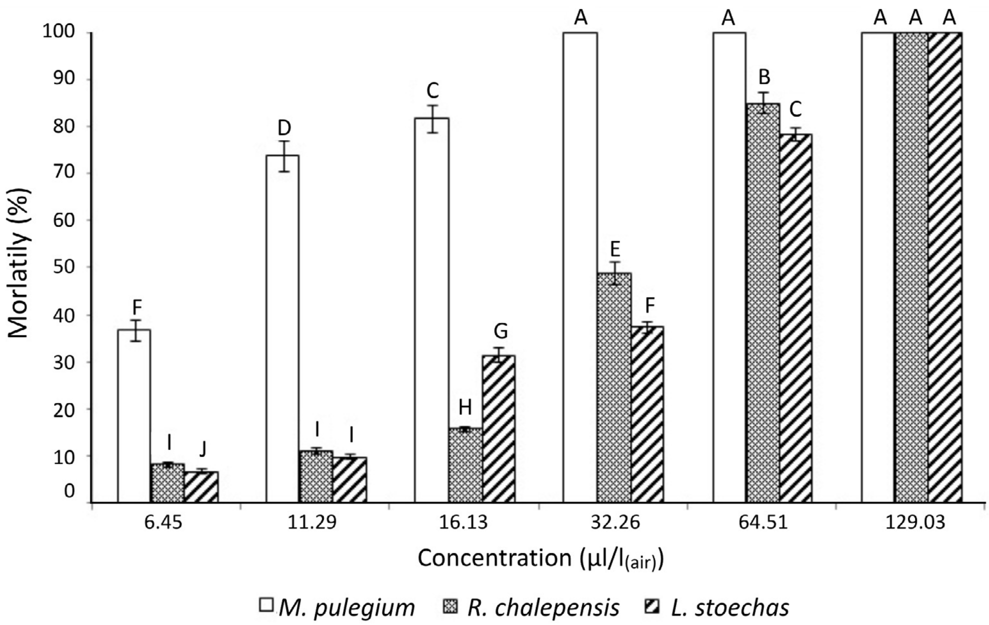

Fig. 1. Mortality caused by *Mentha pulegium*, *Ruta chalepensis* and *Lavandula stoechas* essential oils against *R. dominica adults.* Error bars represent the standard error of the mean of seven replicates, each replicate contained 10 individuals. Means corresponding to each treatment with different letters are significantly different from each other (Tukey-Test, $P < 5$ %).

tial oil caused also low mortalities at 6.45 and 11.29 μl/l$_{(air)}$, which corresponds to 6.9 and 10 %, respectively. Better mortality rates of 34 and 38 % were obtained after 24 h at 16.13 and 32.26 μl/l$_{(air)}$ against *R. dominica*. LC_{50} values of *M. pulegium*, *R. chalepensis* and *L. stoechas* essential oils were 13.52, 39.12 and 42.90 μl/l$_{(air)}$, respectively (Table 1).

Essential oils composition

The determination and quantification by GC-MS and Fast GC-FID of the three essential oils extracted from *R. chalepensis*, *L. stoechas* and *M. pulegium* are presented in Tables 2, 3 and 4.

Twelve, 37 and 22 compounds were identified from essential oils of *R. chalepensis*, *L. stoechas* and *M. pulegium*, respectively representing 98.54 %, 95.01 % and 99.51 % of the total essential oil constituents. In *M. pulegium* essential oil, two

Table 1. Lethal concentrations LC_{50} and LC_{95} of *Mentha pulegium*, *Ruta chalepensis and Lavandula stoechas* essential oils against *Rhyzopertha dominica*

	LC_{50} µl/l$_{(air)}$	LC_{95} µl/l$_{(air)}$
M. pulegium oil	13.516	45.682
R. chalepensis oil	39.115	113.126
L. stoechas oil	42.898	170.529

Table 2. GC-MS and GC-FID analysis of chemical extracts (in relative %) of pure *Mentha pulegium* essential oil

No.	Compound	Retention index (measured)	%
1	α-Pipene	937	0.51
2	Sabinene	976	0.15
3	β-Pinene	982	0.42
4	Myrcene	989	0.26
5	Octan-3-ol	995	1.69
6	Limonene	1034	1.73
7	1,8-Cineole	1038	0.44
8	Linalool	1100	0.13
9	Menthone	1162	0.49
10	Isomenthone	1172	11.95
11	Isopulegone	1181	1.35
12	Menthol	1187	0.06
13	(1)-Isomenthol	1195	0.76
14	Pulegone	1250	76.85
15	Iso-menthylacetate	1311	0.30
16	Piperitenone	1349	0.61
17	β-Elemene	1400	0.14
18	β-Caryophyllene	1437	0.48
19	Aromadendrene	1460	0.05
20	α-Humulene	1473	1.00
21	Germacrene D	1496	0.10
22	γ-Eudesmol	1645	0.04

Table 3. GC-MS and GC-FID analysis of chemical extracts (in relative %) of pure *Lavandula stoechas* essential oil.

No.	Compound	Retention index (measured)	%
1	α-Pipene	937	6.61
2	Camphene	955	7.69
3	Sabinene	976	0.35
4	β-Pinene	982	0.85
5	Myrcene	990	0.35
6	Para-cymene	1028	0.38
7	Limonene	1034	1.19
8	1,8-Cineole	1038	17.57
9	Fenchone	1095	2.79
10	Linalool	1100	3.58
11	α-Thujone	1112	0.22
12	Neo,allo-ocimene	1150	0.38
13	Trans-pinocarveol	1153	0.51
14	Camphor	1158	23.75
15	Iso-pinocamphone	1176	0.45
16	Borneol	1181	0.31
17	Terpinen-4-ol	1187	0.30
18	Myrtenol	1203	1.61
19	Bornylacetate	1291	1.51
20	Myrtenylactate	1329	3.88
21	α-Copaene	1386	0.67
22	Geranylacetate	1390	0.94
23	β-Cubebene	1400	0.44
24	β-Caryophyllene	1437	1.11
25	Allo-aromadendrene	1473	0.26
26	Epi-bicyclosesqui-phellandrene	1478	0.61
27	α-Amorphene	1485	0.38
28	β-Selinene	1506	1.31
29	Trans-α-bisabolene	1509	2.30
30	δ-Cadinene	1530	3.21
31	Cadina-1,4-diene	1536	0.50
32	γ-Selinene	1547	0.93
33	Selina-3,7(11)-diene	1561	3.48
34	Cis-α-Copaene-8-ol	1579	1.47
35	Viridiflorol	1616	2.57
36	Tau-muurolol	1654	0.37
37	Oxo-α-ylangene	1692	0.18

monoterpenes which were pulegone (76.85 %) and isomenthone (11.95 %) were identified as the major constituents of the oil. Octan-3-ol (1.69 %), limonene (1.73 %) and isopulegone (1.35 %) were also identified in important amounts (Table 2).

The prominent compounds of *L. stoechas* essential oil were camphor (23.75 %), 1, 8-cineole (17.57 %), camphene (7.69 %) and α-pipene (6.61 %). Other important constituents identified in *L. stoechas* essential oil were myrtenyl actate (3.88 %), linalool (3.58 %) and fenchone (2.79 %). The main sesquiterpenes of the oil were β-caryophyllene (1.11 %), β-selinene (1.31 %), *trans*-α-bisabolene (2.30 %), δ-cadinene (3.21 %), selina-3, 7(11)-diene (3.48 %), *cis*-α-copaene-8-ol (1.47 %) and viridiflorol (2.57 %) (Table 3).

Table 4. Relative amounts (area %) of identified chemical extracts of pure *Ruta chalepensis* essential oil using GC/MS and GC-FID analysis.

No.	Compound	Retention index (measured)	%
1	Sabinene	976	0.21
2	2-Octanone	989	0.31
3	Limonene	1034	1.75
4	2-Nonanone	1092	39.16
5	2-Nonanol	1100	0.15
6	Geyrene	1150	1.05
7	2-Decanone	1190	2.32
8	*n*-Decanal	1206	0.14
9	1-Octanol acetate	1229	0.46
10	2-Undecanone	1294	51.23
11	2-Dodecanone	1363	0.62
12	2-Tridecanone	1493	1.14

Table 4 shows the chemical composition of *R. chalepensis* oil and their relative proportions in the pure oil identified by GC-MS and quantified by Fast GC-FID. Both 2-undecanone (51.23 %), 2-nonanone (39.16 %) were the major compounds of *R. chalepensis* essential oil (Table 4). Other compounds obtained in important proportions were 2-decanone (2.32 %), limonene (1.75 %), 2-tridecanone (1.14 %) and geyrene (1.05 %).

Results showed that limonene and sabinene were identified in the three essential oils in various amounts (Tables 2, 3 and 4). Linalool, α-pipene, β-pinene, 1,8-cineole, β-caryophyllene and myrcene were the common compounds obtained in essential oils extracted from *L. stoechas* and *M. pulegium* aerial parts.

DISCUSSION

Essential oil yields

In this study, the yield of essential oils depends considerably upon plant species. Our results showed that the oil yields of *R. chalepensis*, *L. stoechas* and *M. pulegium* were 0.39, 0.15 and 0.68 %, respectively. Several authors studied the yield of essential oils extracted from *M. pulegium* (Franzios *et al*. 1997; Attia *et al*. 2012; Brahmi *et al*. 2016a; Chraibi *et al*. 2016, Ben Chaaban *et al*. 2019), *R. chalepensis* (Mejri *et al*. 2010; Akkari *et al*. 2015; Tampe *et al*. 2016) and *L. stoechas* (Hassiotis 2010; Carrasco *et al*. 2015). They reported some relative variations in the essential oil yields.

Steam distillation of *L. stoechas* yielded 0.15 % of essential oil, whereas Carrasco *et al*. (2015) recorded yields from 0.3 to 1 % from *L. stoechas* growing in southeast Spain. A study by Chekoual *et al*. (2018) showed that essential oil yields varied from 0.5 % to 1.6 % depending upon collecting region and length of time of distillation of Algerian *L. stoechas*.

The yield of *R. chalepensis* (0.4 %) was higher in comparison with those recorded by Tampe *et al*. (2016) which was 0.3 % collected from the commune of Puerto Montt. While Mejri *et al*. (2010) showed higher yield of essential oil from *R. chalepensis* from region of El Fahs reaching 5.51 %.

The yield of *M. puleguim* essential oil was lower (0.68 %) compared to other studies carried out by Kokkini *et al*. (2002), Abdelli *et al*. (2016), Brahmi *et al*. (2016a) and Ben Chaaban *et al*. (2019) which reached 2.6 %, 1.45 %, 1.41 % and 1.56 %, respectively. However, it was higher than the yield reported by Attia *et al*. (2012) which was 0.1 %.

These differences depend on many factors such as the origin of plants, the method or extraction time, the plant parts used and the collecting period (Wong *et al*. 2005; Mejri *et al*. 2010). However, essential oil extraction required a high quantity of plants; consequently they are expensive and available in limited quantities.

Fumigant toxicity of essential oils against *R. dominica*

In this study we assessed the toxicity of three essential oils from three plants *R. chalepensis*, *L. stoechas* and *M. pulegium* against adults of *R. dominica*. Results showed that fumigant toxicity against *R. dominica* differs greatly among plant species used for oil extraction reflecting their differences in composition. Indeed, LC_{50} values of *M. pulegium* essential oil reached 13.51 $\mu l/l_{(air)}$ after 24 h of exposure, whereas a study by Brahmi *et al*. (2016a) recorded the LC_{50} of *M. pulegium* and *M. rotundifolia* as 6.9 and 3.39 $\mu l/ml_{(air)}$, respectively, after 24 h of exposure. *Mentha pulegium* used in our experiments reflected better efficiency against *R. dominica* compared to literature data even if the two species of *Mentha* presented pulegone as a major compound with similar percentages. This suggested that secondary compounds probably also play a role in the toxicity. The difference in results with the literature data may be also explained by differences in the experimental conditions. In our case, jars were hermetically sealed so volatile compounds remained in contact with *R. dominica* individuals whereas we assumed it was not always the same with other studies (Brahmi *et al*. 2016a). *Ruta chalepensis* oil used in the current study seems more effective (LC_{50} = 39.11 $\mu l/l_{(air)}$) compared to previous research with other insects, *e.g.* Majdoub *et al*. (2018) showed that the essential oil of *R. chalepensis* has an insecticidal effect against adults and larvae of *T. castaneum* and recorded LC_{50} values of 176.075 and 415.348 $\mu l/l_{(air)}$ after 24 h of exposure, respectively. However, the toxicity of this essential oil may be due to the amounts of 2-undecanone (51.23 %) and 2-nonanone (39.16 %). A recent study of Abdellaoui *et al*. (2018) reported that the main components of *R. chalepensis* are: 2-octanol, acetate (30.98 %), 2-undecanone (25.94 %) and 2-nonanone (16.28 %). The same authors showed that *R. chalepensis* essential oil exhibited toxicity against pupae and adults of *T. confusum* with LC_{50} values of 0.05 and 0.08 $\mu l/cm^2$. Another research by Abbad *et al*. (2014) showed that *R. chalepensis* essential oil possessed toxicity against both *T. castaneum* and *T. confusum*. The susceptibility of these two pests was not significantly different. The major component of this oil was 2-undecanone with 93.1 %.

Essential oil extracted from *L. stoechas* was less effective against *R. dominica* with LC_{50} value of 42.89 $\mu l/l_{(air)}$ in comparison with literature with LC_{50} value of 5.66 $\mu l/l_{(air)}$) after 24 h of treatment (Ebadollahi *et al*. 2010).

The differences in toxicity between different species of plants are probably related to the proportion of main components present in each essential oil (Attia *et al*. 2012). In that context, the toxicity of each constituent should be studied separately. In the literature, there are many studies that reported differences in chemical composition of the same species of plant. In this study, the major components of *L. stoechas* essential oil are camphor (23.75 %), 1,8-cineole (17.57 %), camphene (7.69 %) and α-pipene (6.61 %). However, those in the same plant grown in Iran were 1,8-cineole (7.02 %), γ-cadinene (5.33 %), T-cadinol

(5.07 %), p-mentha-1-en-8-ol (5.02 %) and caryophyllene (5.01 %).

Suthisut *et al.* (2011) showed that *Alpinia conchigera* oil was toxic against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 85 ml/$l_{(air)}$ and 73 ml/$l_{(air)}$ after 48 h of exposure, respectively.

Other reports investigated the use of essential oils against *R. dominica*. In 2008, Ogendo *et al.* reported that the oil from *Ocimum gratissimum* L. caused 98 %, 99 % and 100 % mortality of *R. dominica*, *Oryzaephilus surinamensis* L. (Coleoptera: *Silvanidae)* and *Callosobruchus chinensis* L. (Coleoptera: *Chrysomelidae)*, respectively, at 1 μl/$l_{(air)}$ after 24 h of treatment. The oil from *O. gratissimum* seems to be more effective on *R. dominica* than the oils tested in the present study.

In comparison, a study by Ebadollahi (2018), the three essential oils tested in this study possess more toxicity against *R. dominica* than *Apium graveolens* L. essential oil which its LC_{50} corresponded to 53.50 μl/$l_{(air)}$ after 24 h of exposure compared to *M. pulegium*, *R. chalepensis* and *L. stoechas* essential oils with LC_{50} values of 13.52, 39.12 and 42.90 μl/$l_{(air)}$, respectively. Essential oils showed differences in their activities depending upon plant species, geographic origin, chemical composition, concentration, exposure time, insect tolerance and its stage of development. In the same way, Bond (2011) showed that the level of fumigant used to kill stored grain pests depended on stage, species, duration, temperature and commodity. Essential oils are used to manage insects; they are without appreciable mammalian toxicity (Edris 2007; Vigan 2010). Some of their components are toxic such as pulegone, α-thujone (Isman & Machial 2006), their use must be recommended with caution.

Chemical composition of essential oils

Qualitative and quantitative compositions of essential oils from *M. pulegium*, *R. chalepensis* and *L. stoechas* in this study were different. These differences depend considerably upon plant species, method of extraction and origin and period of collecting plant. Qualitative and quantitative compositions of essential oils from *L. stoechas* in this study were different compared to other studies. Indeed, chemical composition of *L. stoechas* showed that camphor is considered to be the major component with 23.75 % followed by 1,8-cineole with 17.57 % presenting about 50 % from all components whereas a study carried out by Hassiotis (2010) showed that fenchone is the major component present 45.19 % followed by 1,8-cineole with 16.30 %. The composition of *L. stoechas* essential oil was reported in another paper by Cherrat *et al.* (2014) who showed that 10s,11s-himachala-3(12),4-diene and cubenol were the major compounds with 23.62 % and 16.19 %, respectively.

Chemical composition of essential oil extracted from *R. chalepensis* present two main compounds: 2-undecanone (51.23 %) and 2-nonanone (39.16 %). This result confirmed previous findings by Tampe *et al.* (2016) and Ortu *et al.* (2016) who identified the two compounds levels reaching 40.11 %, 41.71 % of 2-undecanone and the 2-nonanone and 24.01 %, 25.31 %, respectively. However, Tounsi *et al.* (2011) reported that the 2-nonanol and the 2-undecanone are the predominant compounds found in essential oils obtained from the leaves, flowers, stems and fruits of *R. chalepensis*. Whereas Mejri *et al.* (2010), reported that the 2-decanone and the 2-undecanone are obtained from dried and fresh leaves with highest level in fresh leaves. This study demonstrated also that quantitative and qualitative composition of essential oil extracted from *R. chalepensis* depended on the plant origin, the duration of the extraction, the distilled plant parts and the drying process.

In our case, pulegone was the main compound and represented 76.85 % of *M. pulegium* essential oil. This was confirmed by Franzios *et al.* (1997), Abdelli *et al.* (2016), Brahmi *et al.* (2016a) and Chraibi *et al.* (2017). Whereas 1,8-cineole (31.9 %) (Salhi *et al.* 2017), from *M. pulegium* collected in Morocco and menthone with amounts of 53.4 % and 49.6 % (Kokkini *et al.* 2002) found in *M. pulegium* collected from the island of Crete and island of Amorgos, respectively are the major components of this essential oil. Another study reported by Ben Chaaban *et al.* (2019) demonstrated that 1,8-cineole and carene (14.6 % and 10.19 %) from the same plant collected from Tozeur in the south of Tunisia, were the major constituents identified in *M. pulegium* essential oil. If the insecticidal activity of pulegone is now well established, its use must be recommended with caution because of its toxicity to humans, and pulegone is not recommended to be used at very low doses.

Mentha spicata essential oil exhibited high toxicity against *R. dominica* with LD_{50} values of 6.1 μl/$ml_{(air)}$ with its major components being carvone (48.5 %), limonene (20.7 %), 1,8-cineole (5.36 %), β-

caryophyllene (3.4 %), and germacrene-D (3.4 %). This oil caused a mortality of 43 % on the adults of *R. dominica* at the dose of 2 μl/ml after 96 h of treatment (Brahmi *et al*. 2016b). It is less effective than *Mentha* essential oil tested in this study.

In spite of being the subject of considerable scientific research, few products made of essential oils for pest control are commercialised.

CONCLUSION

This study was carried out to determine the insecticidal activity of three essential oils extracted from *Lavandula stoechas*, *Mentha pulegium* and *Ruta chalepensis* collected in Tunisia against *R. dominica*. It also concerned their chemical composition characteristics. As a result, it revealed that all essential oils presented fumigant toxicity against *R. dominica*. *M. pulegium* recorded the best insecticidal activity compared to the two other essential oils. Future research efforts should be to investigate the toxicity of major components on humans and mammals and determine if synergistic or antagonistic phenomena exist between different components.

ACKNOWLEDGEMENTS

We are grateful to G. Lognay for his collaboration with chemical analyses of essential oils in the Department of Analytical Chemistry from Gembloux Agro-Bio Tech, University of Liège, Belgium.

CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

REFERENCES

ABBAD, A., KASRATI, A., JAMALI, C.A., ZEROUAL, S., M'HAMED, T.B., SPOONER-HART, R. & LEACH, D. 2014. Insecticidal properties and chemical composition of essential oils of some aromatic herbs from Morocco. *Natural Product Research* **28**(24): 2338–2341.

ABDELLAOUI, K., ACHEUK, F., MILADI, M., BOUGHATTAS, I. & OMRI, G. 2018. Phytochemistry, biochemical and insecticidal activities of *Ruta chalepensis* essential oils on *Tribolium confusum*. *Agriculture & Forestry* **64**(3): 31–45.

ABDELLI, M., MOGHRANI, H., ABOUN, A. & MAACHI, R. 2016. Algerian *Mentha pulegium* L. leaves essential oil chemical composition, antimicrobial, insecticidal and antioxidant activities. *Industrial Crops and Products* **94**: 197–205.

ABBOTT, W.S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265–267.

ADAMS, R.P. 2001. *Identification of Essential Oils Components by Gas Chromatography Mass Spectrometry*. Allured Publishing Corp., Carol Stream, IL, U.S.A.

AKKARI, H., EZZINE, O., DHAHRI, S., B'CHIR, F., REKIK, M., HAJAJI, S., DARGHOUTH, M.A., BEN JAMÂA, M.L. & GHARBI, M. 2015. Chemical composition, insecticidal and in vitro anthelmintic activities of *Ruta chalepensis* (Rutaceae) essential oil. *Industrial Crops and Products* **74**: 745–751.

ATTIA, S., GRISSA, K.L., GHRABI, Z.G., MAILLEUX, A.C., LOGNAY, G. & HANCE, T. 2012. Acaricidal activity of 31 essential oils extracted from plants collected in Tunisia. *The Journal of Essential Oil Research* **24**(3): 279–288.

BANGA, K.S., KOTWALIWALE, N., MOHAPATRA, D. & GIRI, S.K. 2018. Techniques for insect detection in stored food grains: an overview. *Food Control* **94**: 167–176.

BEN CHAABAN, S., HAMDI, S.H., MAHJOUB, K. & BEN JEMÂA, J.M. 2019. Composition and insecticidal activity of essential oil from *Ruta graveolens*, *Mentha pulegium* and *Ocimum basilicum* against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Plant Diseases and Protection* **126**(3): 237–246.

BICCHI, C., CORDERO, C., LIBERTO, E., RUBIOLO, P. & SGORBINI, B. 2004. Automated headspace solid-phase dynamic extraction to analyze the volatile fraction of food matrices. *Journal of Chromatography A* **1024**: 217–226.

BOND, E.J. 2011: *Manual of Fumigation for Insect Control*. *FAO Plant Production and Protection Paper 54*. Food and Agricultural Organization of the United Nations, Rome, Italy.

BRAHMI, F., ABDENOUR, A., BRUNO, M., SILVIA, P., ALESSANDRA, P., DANILO, F., DRIFA, Y-G., FAHMI, E.M., KHODIR, M. & MOHAMED, C. 2016a. Chemical composition and in vitro antimicrobial, insecticidal and antioxidant activities of the essential oils of *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds growing in Algeria. *Industrial Crops and Products* **88**: 96–105.

BRAHMI, F., ADJAOUD, A., MARONGIU, B., FALCONIERI, D., YALAOUI-GUELLAL, D., MADANI, K., CHIBANE, M. 2016b. Chemical and biological profiles of essential oils from *Mentha spicata* L. leaf from Bejaia in Algeria. *The Journal of Essential Oil Research* **28**(3): 211–220.

BRARI, J. & THAKUR, D.R. 2018. Larvicidal effects of eight essential oils against *Plodia interpunctella* and *Tribolium castaneum*, serious pests of stored products worldwide. *Journal of Entomology and Zoology Studies* **6**(5): 738–742.

BRITO, J.P., OLIVEIRA, J.E. DE M. & DE BORTOLI, S.A. 2006. Toxicidade de óleos essenciais de *Eucalyptus* spp. sobre *Callosobruchus maculatus* (Fabr., 1775) (Coleoptera: Bruchidae). *Revista de Biologia e Ciências da Terra* **6**(1): 96–103.

CAMPOLO, O., GIUNTI, G., RUSSO, A., PALMERI, V. & ZAPPALÀ, L. 2018. Essential oils in stored product insect pest control. *Journal of Food Quality*. https://doi.org/10.1155/2018/6906105

CARRASCO, A., ORTIZ-RUIZ, V., MARTINEZ-GUTIERREZ, R., TOMAS V. & TUDELA, J. 2015. *Lavandula stoechas* essential oil from Spain: aromatic profile determined by gas chromatography-mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. *Industrial Crops and Products* **73**: 16–27.

CHEKOUAL, L., AISSAT, A., AIT-KACIAOURAHOUN, A. & BENABDELKADER, T. 2018. Comparative study of yield, chemical composition and antioxidant activity of wild Algerian *Lavandula stoechas* L. obtained by ultrasound pre-treatment and by conventional hydrodistillation. *Phytothérapie* **16**: S109–S118. https://doi.org/10.3166/phyto-2018-0105

CHERRAT, L., ESPINA, L., BAKKALI, M., PAGÁN, R. & LAGLAOUI, A. 2014. Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Saturejaca lamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes. *Innovative Food Science and Emerging Technologies* **22**: 221–229.

CHRAIBI, M., FARAH, A., LEBRAZI, S., EL AMINE, O., HOUSSAINI, M.I. & FIKRI-BENBRAHIM, K. 2016. Anti-mycobacterial natural products from Moroccan medicinal plants: chemical composition, bacteriostatic and bactericidal profile of *Thymus satureioides* and *Mentha pulegium* essential oils. *Asian Pacific Journal of Tropical Biomedicine* **6**(10): 836–840.

CHRAIBI, M., BENBRAHIM, K.F., ELMSELLEM, H., FARAH, A., ABDEL-RAHMAN, I., EL MAHI, B., BABA, Y.F., RODI, Y.K. & HLIMI, F. 2017. Antibacterial activity and corrosion inhibition of mild steel in 1.0 M hydrochloric acid solution by *M. piperita* and *M. pulegium* essential oils. *Journal of Materials and Environmental Sciences* **8**(3): 972–981.

COLLINS, P.J., DAGLISH, G.J., PAVIC, H. & KOPITTKE, R.A. 2005. Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. *Journal of Stored Products Research* **41**: 373–385.

EBADOLLAHI, A. 2011. Antifeedant activity of essential oils from *Eucalyptus globulus* Labill and *Lavandula stoechas* L. on *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Biharean Biologist* **5**(1): 8–10.

EBADOLLAHI, A. 2013. Essential oils isolated from Myrtaceae family as natural insecticides. *Annual Review & Research in Biology* **3**(3): 148–175.

EBADOLLAHI, A., SAFARALIZADEH, M.H. & POURMIRZA, A.A. 2010. Fumigant toxicity of *Lavandula stoechas* L. oil against three insect pests attacking stored products. *Journal of Plant Protection Research* **50**: 56–60.

EBADOLLAHI, A. 2018. Fumigant toxicity and repellent effect of seed essential oil of celery against lesser grain borer, *Rhyzopertha dominica* F. *Journal of Essential Oil Bearing Plants* **21**(1): 146–154.

EDRIS, A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytotherapy Research* **21**: 308–323.

FINNEY, D.J. 1971. *Probit Analysis*. 3rd Edition. Cambridge University Press, Cambridge U.K.

FRANZIOS, G., MIROTSOU, M., HATZIAPOSTOLOU, E., KRAL, J., SCOURAS, Z.G. & MAVRAGANI-TSIPIDOU, P. 1997. Insecticidal and genotoxic activities of mint essential oils. *Journal of Agricultutal and Food Chemistry* **45**: 2690–2694.

GAREAU, B.J. 2015. Lessons from the Montreal Protocol delay in phasing out methyl bromide. *Journal of Environmental Studies and Sciences* **5**(2): 163–168.

GERMINARA, G.S., DI STEFANO, M.G., DE ACUTIS, L., PATI, S., DELFINE, S., DE CRISTOFARO, A. & ROTUNDO, G. 2017. Bioactivities of *Lavandula angustifolia* essential oil against the stored grain pest *Sitophilus granarius*. *Bulletin of Insectology* **70**(1): 129–138.

GREWAL, A.S., SINGLA, A., KAMBOJ, P. & DUA, J.S. 2017. Pesticide residues in food grains, vegetables and fruits: a hazard to human health. *Journal of Medicinal Chemistry and Toxicology* **2**(1): 40–46.

HASSIOTIS, C.N. 2010. Chemical compounds and essential oil release through decomposition process from *Lavandula stoechas* in Mediterranean region. *Biochemical Systematics and Ecology* **38**: 493–501.

ISMAN, M.B. & MACHIAL, C.M. 2006: Pesticides based on plant essential oils: from traditional practice to commercialization. In: Rai, M. & Carpinella, M.C. (Eds) *Naturally Occurring Bioactive Compounds*. 29–44. Elsevier, New York, U.S.A.

JOULAIN, D. & KÖNIG, W.A. 1998. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. E.B.-Verlag Hamburg, Germany.

KHAN, S.A., RANJHA, M.H., KHAN, A.A., SAGHEER, M., ABBAS, A. & HASSAN, Z. 2019. Insecticidal efficacy of wild medicinal plants, *Dhutura alba* and *Calotropis procera*, against *Trogoderma granarium* (Everts) in wheat store grains. *Pakistan Journal of Zoology* **51**(1): 289–294.

KOKKINI, S., HANLIDOU, E. & KAROUSOU, R. 2002. Variation of pulegone content in pennyroyal (*Mentha pulegium* L.) plants growing wild in Greece. *Journal of Essential Oil Research* **14**: 224–227.

LEE, B-H., ANNIS, P.C., TUMAALII, F. & CHOI, W-S. 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *Journal of Stored Products Research* **40**: 553–564.

MAJDOUB, O., DHEN, N., SOUGUIR, S., HAOUAS, D., BAOUANDI, M., LAARIF, A. & CHAIEB, I. 2014. Chemical composition of *Ruta chalepensis* essential oils and their insecticidal activity against *Tribolium castaneum*. *Tunisian Journal of Plant Protection* **9**: 83–90.

MBTOC. 2002. *2002 Report of the Methyl Bromide Technical Options Committee* (MBTOC). United Nations Environment Programme (UNEP), Nairobi, Kenya.

MEJRI, J., ABDERRABBA, M. & MEJRI, M. 2010. Chemical composition of essential oil of *Ruta chalepensis* L: inflluence of drying, hydro-distillation duration and plant parts. *Industrial Crop Production* **32**: 671–673.

OGENDO, J.O., KOSTYUKOVSKY, M., RAVID, U., MATASYOH, J.C., DENG, A.L., OMOLO, E.O., KARIUKI, S.T. & SHAAYA, E. 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. *Journal of Stored Products Research* **44**: 328–334.

OPIT, G.P., PHILLIPS, T.W., AIKINS M.J. & HASAN, M.M. 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. *Journal of Economic Entomology* **105**: 1107–1114.

ORTU, E., SANNA, G., SCALA, A., PULINA, G., CABONI, P. & BATTACONE, G. 2016. In vitro anthelmintic activity of active compounds of the fringed rue *Ruta chalepensis* against dairy ewe gastrointestinal nematodes. *Journal of Helminthology* **91**(4): 447–453.

PANEZAI, G.M., JAVAID, M., SHAHID, S., NOOR, W., BIBI, Z. & EJAZ, A. 2019. Effect of four plant extracts against *Trogoderma granarium* and *Tribolium castaneum*, *Pakistan Journal of Botany* **51**(3): 1149–1153.

PHILLIPS, T.W. & THRONE, J.E. 2010. Biorational approaches to managing stored-product insects. *Annual Review of Entomology* **55**: 375–397.

PIRES, E.M. & NOGUEIRA, R.M. 2018. Damage caused by *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) in stored Brazil nuts. *Scientific Electronic Archives* **11**(1): 57–61.

RAFIE, S., NAMJOYAN, F., GOLFAKHRABADI, F., YOUSEFBEYK, F. & HASSANZADEH, A. 2016. Effect of lavender essential oil as a prophylactic therapy for migraine: a randomized controlled clinical trial. *Journal of Herbal Medicine* **6**: 18–23.

RAJENDRAN, S. & SRIRANJINI, V. 2008. Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research* **44**: 126–135.

SAID-AL AHL, H.A., HIKAL, W.M. & TKACHENKO, K.G. 2017. Essential oils with potential as insecticidal agents: a review. *International Journal of Environmental Planning and Management* **34**: 23–33.

SALEM, N., BACHROUCH, O., SRITI, J., MSAADA, K., KHAMMASI, S., HAMMAMI, M., SELMI, S., BOUSHIH, E., KOORANI, S., ABDERRABA, M., MARZOUK, B., LIMAM, F. & MEDIOUNI BEN JEMAA, J. 2017. Fumigant and repellent potentials of *Ricinus communis* and *Mentha pulegium* essential oils against *Tribolium castaneum* and *Lasioderma serricorne*. *Journal of Food Properties* **20** (suppl. 3): S2899–S2913. DOI: 10.1080/10942912.2017.1382508

SALHI, A., BOUYANZER, A., CHETOUANI, A., EL BARKANY, S., AMHAMDI, H., HAMDANI, I., ZARROUK, A., HAMMOUTI, B., DESJOBERT, J. M. & COSTA, J. 2017. Chemical composition of essential oil and antioxidant and anti-corrosion activity of extract and essential oil of pennyroyal mint (*Mentha pulegium*). *Moroccan Journal of Chemistry* **5**(1): 59–71.

SHAAYA, E., KOSTJUKOVSKI, M., EILBERG J. & SUKPRAKARN, C. 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research* **33(**1): 7–15.

SUTHISUT, D., FIELDS P.G. & CHANDRAPATYA, A. 2011. Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. *Journal of Stored Products Research* **47**: 222–230

TAMPE, J., PARRA, K., HUAIQUIL, K. & QUIROZ, A. 2016. Potential repellent activity of the essential oil of *Ruta chalepensis* (Linnaeus) from Chile against *Aegorhinus superciliosus* (Guérin) (Coleoptera: Curculionidae). *Journal of Soil Science and Plant Nutrition* **16**(1): 48–59.

TOUNSI, M.S., WANNES, W.A., OUERGHEMMI, I., MSAADA, K., SMAOUI, A. & MARZOUK, B. 2011. Variation in essential oil and fatty acid composition in different organs of cultivated and growing wild *Ruta chalepensis* L.. *Industrial Crops and Products* **33**: 617–623.

VIGAN, M. 2010. Essential oils: renewal of interest and toxicity. *European Journal of Dermatology* **20**(6): 685–692

WIJAYARATNE, L.K.W. & RAJAPAKSE, R.H.S. 2018. Effects of spinosad on the heat tolerance and cold tolerance of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae). *Journal of Stored Products Research* **77**: 84–88.

WONG, K.C., LEE, B.C., LAM, N.F. & IBRAHIM, P. 2005. Essential oils of the rhizomes of *Alpinia conchigera* Griff. and *Alpinia latilabris* Ridl. *Flavour and Fragrance Journal* **20**: 431–433.