Insecticidal activity of essential oils from six Moroccan plants against insect pests *Rhyzopertha dominica, Sitophilus oryzae* and *Tribolium castaneum*

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ABSTRACT

Six essential oils from Morrocan plants belonging to different botanical families (*Lamiaceae, Pinaceae* and *Verbenaceae*), were prepared by hydrodistillation. These essential oils from Morrocan plants, which have a long tradition in adjuvant therapy, were tested for insecticidal activity by the method of microcomputer-atmosphere against three major pests of stored products : *Rhyzopertha dominica (*F*), Sitophilus oryzae (*L*)* and *Tribolium castaneum* (herbst). The results obtained showed that the essential oils possessed highly significant insecticidal properties against the pests studied. R (+) pulegone, a monoterpene ketone and a major component of the essential oil of *Mentha pulegium* exhibited the highest insecticidal activity.

Keywords: GC-MS, Extraction, Hydrodistillation, Essential oil, Insecticidal activity, R (+) pulegone.

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INTRODUCTION

Insect pests cause significant losses in stored food products, particularly cereals in Morocco and other parts of the world. Currently, control of stored-product of cereals is generally achieved by fumigation and application of insecticides (El Arch, 2003; Arena, 2017). Nevertheless, the use of chemical agents is becoming less popular because of fears about potential harmful effects on man and because of the appearance of many resistant strains (Benhalima *et al*., 2004). Traditionally certain species of plants have been used to combat the insects damaging stored food products (Regnault-Roger and Hamraoui, 1997; Digilio, 2008). Previous research has shown certain plants to contain substances which are either repellent, antifeedant or toxic (Wang *et al*., 2006; Nerio *et al*. 2010; Ebadollahi, 2011; Stefanazzi *et al*. 2011). Antifeedant properties of *Sandoricum koetjape (*Merr) against the moth *Spodoptera frugiperda* (Powel *et al*., 1991), of *Eucalyptus globules* (Labill), *Lavandula stoechas* (L) and *Artemisia vulgaris* (L) against *Tribolium castaneum* (Herbst) (Wang *et al*., 2006; Ebadollahi, 2011) have been reported.

Also essential oils can show insecticidal activity, thus the essential oil extracted from six different Citrus species appeared toxic for *Sitophilus zeamais* (Motschulsky), *Prostephanus truncates* (Horn) and *T. castaneum* (Haubruge *et al.,* 1989; Abdelgaleil *et al*., 2016; Arena *et al*., 2017) and the oil of *Acorus calamus* (Linn.) (Araceae) was toxic to *Sitophilus granarius* (Linn.), S. *oryzae* and *Callosobruchus chinensis* (Linn.) (Schmidt, 1991; Shukla, 2016; Koutsaviti, 2017).

In this work, we present the study of the insecticidal properties of six essential oils of some Moroccan plants, which have a long tradition in adjuvant therapy (Eddouks *et al*., 2002; Tahraoui *et al*., 2007) against the adults of *R. dominica, S. oryzae* and *T. castaneum*.

METRIALS AND METHODS

The selected plants are: *Mentha pulegium* L. (Lamiaceae), *Thymus satureioide Coss.*

(Lamiaceae), *Mentha viridis* L. (Lamiacae), *Rosmarinus officinalis* L. (Lamiaceae), *Lippia citriodora* L. (Verbenaceae), *Cedrus atlantica Manetti* (Pinaceae).

Insects

The insects used were *R. dominica*, *S. oryzae* and *T. castaneum.* The breeding of the three species was carried out on grain of wheat in transparent plastic boxes of volume 1 liter. The boxes were placed in an enclosure with temperature maintained at 30° C and relative humidity at 70%.

Extraction of essential oil

The essential oils tested were extracted from six Moroccan plants by hydrodistillation using a distiller of the type Clevenger. R(+) pulegone of purity equal to 92% and density 1.478 was obtained from the Aldrich Company.

Chromatographic analyses

The analyses were carried out on a HP 5790 gas chromatograph coupled to a HP 5972 mass spectrometer, the apparatus functions in electronic impact. Fragmentation was carried out in an electronic field of 70 eV. The column used was a DB5 capillary tube containing molten silica 30 m in length and 0.25 mm in the internal diameter; the thickness of film was 0.25 mm. The conditions of the analysis were as follows: the temperature of the oven was programmed to heat from 50° C to 250°C at a rate of 5°C/min. Temperature (250°C) was maintained during 10 min, the carrier gas Helium was used with a flow of 1 mL/min, the temperature of the injector was 250°C and that of the detector was 280°C, the quantity injected of essential oil was 5 µL diluted in pentane and the spectrum of mass obtained was compared with computerized library spectra of masses of reference NBS 75K.

Bioassay

The procedure was similar to one described by Hamraoui and Regnaut-Roger (1997). The tests of toxicity of essential oils and (R+) pulegone was carried out on Whatman paper in an experimental box containing 20 adult insects. Doses corresponding to 0.053 mL/L, 0.11 ml/L and 0.21 ml/L, of each essential oil or quantities of (R+) pulegone ranging from $0.2x10^{-2}$ to $12x10^{-2}$ mL/L were deposited separately on to filter papers. Three tests were carried out for each concentration.

Treated papers were introduced into experimental boxes containing 10 g of grain of wheat and 20 adult insects (each species was tested separately). The tests were conducted in semi-aerated medium at 25° C and 10% relative humidity. Three replicates were employed for each sample and the mortality was recorded every 24 h for 4 days. When no leg or antennal movements were observed, insects were considered dead.

RESULTS AND DISCUSSION

In this study, the vapor toxicity of six essential oils, extracted from Moroccan plants was tested at various concentrations against *R. dominica*, *S. oryzae* and *T. castaneum.* The result showed that mortality was influenced by several factors including the plant species, the duration of exposure of the insect species and the concentration of essential oil.

Against *R. dominica* the essential oils of *Mentha pulegium* and *Thymus satureioide* proved to be the most toxic oils with 100% mortality after the first day of exposure at all tested doses (Table 1).

Table 1. Mean percent of mortality of *R. dominica* after exposure to essential oils

Doses (ml/L)	0.21				0.11				0.053			
Day	1	2	3	4	1	2	3	4	1	2	3	4
Plant												
M. pulegium	100	100	100	100	100	100	100	100	100	100	100	100
T. satureioide	100	100	100	100	100	100	100	100	100	100	100	100
M. viridis	100	100	100	100	100	100	100	100	35	36	41	45
L. citriodora	90	100	100	100	55	70	73	90	16	18	25	40
C. atlantica	66	75	83	85	13	20	30	36	16	26	33	33
R. officinalis	56	96	100	100	20	93	96	96	23	23	30	30

Control	0	0	0	8

The essential oil of *M. viridis* showed mortality of 45% at concentration of 0.053 ml/L. Essential oils from *L. citriodora*, *C. atlantica* showed a percentage of mortality of 40% and 33% respectively after the 4 days exposure. Other essential oils showed relatively low insecticidal activity of *R. officinalis* not exceeding 30% mortality at 0.053 mL/L.

Mortality of *S. oryzae* also showed that the essential oil of *M. pulegium* gives a very significant insecticidal activity with a mortality of 100% at all doses after one day exposure (Table 2).

Table 2. Mean percent of mortality of *S. oryzae* after exposure to essential oils

Doses (ml/L)	0.21				0.11				0.053			
Day	1	2	3	4	1	2	3	4	1	2	3	4
Plant												
M. pulegium	100	100	100	100	100	100	100	100	100	100	100	100
T. satureioide	3	15	61	90	5	11	33	85	1	11	20	71
M. viridis	100	100	100	100	70	93	100	100	75	91	98	98
L. citriodora	25	41	83	90	6	13	26	95	3	33	60	78
C. atlantica	15	16	95	100	15	73	98	100	21	78	93	98
R. officinalis	6	8	20	53	6	10	15	46	3	8	15	35
Control	5	12	12	12								

The table shows also that essential oils of *C. atlantica*, and *M. viridis* present similar insecticidal effects and very significant on *S. oryzae* with a percentage of mortality of 98% after 4 days at concentration of 0.053 ml/L.

The essential oil, from *L. citriodora* showed an appreciable toxicity to 78% and *T. satureioide* showed a percentage mortality of 71% at 0.053 ml/L after the fourth day of exposure.

Also high insecticidal activity of *M. pulegium* tested against *T. castaneum* was shown 100% mortality at the first day. Of all other essential oils tested, *M. viridis* shows 90% of mortality at 0.053 ml/L and the essential oil of *R. officinalis* showed a relatively weak insecticidal activity (30%) (Table 3).

Table 3. Mean percent of mortality of *T. castaneum* after exposure to essential oils.

Doses (ml/L)	0.21				0.11				0.053			
Day	1	2	3	4	1	2	3	4	1	2	3	4
Plant												
M. pulegium	100	100	100	100	100	100	100	100	100	100	100	100
T. satureioide	0	0	6	13	0	6	6	16	0	0	0	0
M. viridis	100	100	100	100	80	90	93	100	60	90	90	90
L. citriodora	6	6	10	13	0	3	3	6	0	0	0	0
C. atlantica	0	0	3	3	0	0	0	0	0	0	0	0
R. officinalis	96	96	96	96	33	43	43	43	20	23	30	30
Control	0	0	0	0								

According to the results obtained, it appears that at all doses the maximum mortality was obtained after the 4th day for *M. pulegium*.

Finally the percentage mortality of different species of insects varies clearly according to the concentration used for *R. dominica* and *S. oryzae*.

In literature similar results are reported by using the essential oil of sage against *S. oryzae*, essential oil of Bay-tree, *lavender* and *Rosemary* against *R. dominica* and essential oil of anise against *T. castaneum* but with amounts about three times less than those used in the present work (Shaaya *et al.*, 1991).

The essential oil of *M. pulegium* showed the highest insecticidal activity against the three pests with 100% mortality after the first day of

the treatment, was analyzed by GC-MS. The results showed that the essential oil of *M. pulegium* has a majority component at 52.56 % the (R+) pulegone, a monoterpene ketone. The monoterpenes, major components of aromatic essential oils, are known for their attractive effects (Pellemyr *et al.*, 1991; Gabel *et al*. 1992; Lamiri et *al*., 2001; Brahmi et *al*., 2016) and their antifeedant, repellent, toxic properties (Klocke *et al.*, 1989; Hamraoui, 1993; Pungitore *et al.*, 2005). The insecticidal tests showed that R (+) pulegone was strongly toxic against the three species tested (Table 4 and Fig.1: a, b, and c).

Table 4. Toxicity of R (+) pulegone on *R. dominica, S. oryzae* and *T. castaneum*

Insect	R. dominica		S. oryzae		T. castaneum	
Day	1	4	1	4	1	4
Doses (ml/L)						
$12x10^{-2}$	100	100	100	100	100	100
$6x10^{-2}$	100	100	100	100	100	100
$3x10^{-2}$	100	100	100	100	100	100
$2.6x10^{-2}$	100	100	100	100	100	100
$2x10^{-2}$	100	100	100	100	100	100
$1.5x10^{-2}$	98	100	100	100	87	98
$1x10^{-2}$	87	90	97	98	81	93
$0.8x10^{-2}$	40	43	93	98	33	47
$0.6x10^{-2}$	10	27	90	93	33	47
$0.4x10^{-2}$	10	12	81	87	33	40
$0.2x10^{-2}$	8	8	33	47	27	33
Control	0	0	0	0	0	0

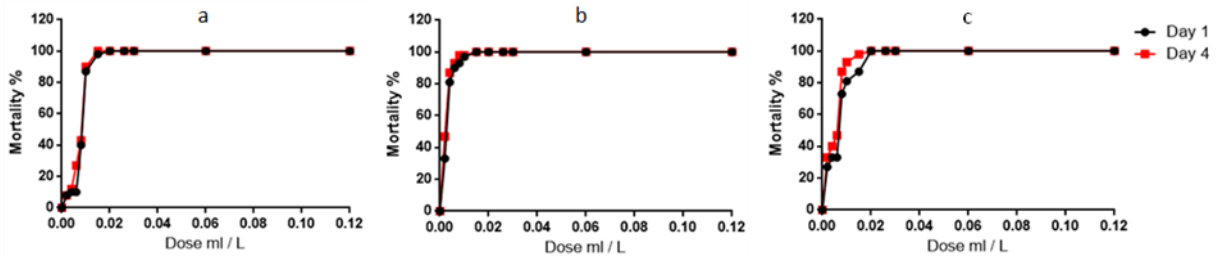

Figure 1. Toxicity of R (+) pulegone on a: *R. dominica* b: *S. oryzae* and c: *T. castaneum.*

The degree of activity changes with concentration and the species studied, with slightly increased mortality after 4 days exposure compared to the one day. Indeed after the first day and 4th day, R (+) pulegone at a concentration of $1.5x10^{-2}$ ml/L has gone with 100% mortality of *S. oryzae* and *R. dominica*. Against *T. castaneum* 100% of mortality was recorded at 2 $x10^{-2}$ ml/L (Table 4). The LD_{50} calculated of R(+) pulegone are 0.00846 (LC_{50} = 0.075 mM), 0.0024 (LC_{50} = 0.021 mM) and 0.0074 ml/L (LC_{50} = 0.064 mM) respectively for *R. dominica*, *S. oryzae* and *T. castaneum* and shown that *S. oryzae* the most sensible insect toward R (+) pulegone, (Table 5).

Table 5. LD_{50} and LC_{50} of R (+) pulegone toward *R. dominica, S. oryzae* and *T. castaneum*

Insect	*R. dominica*		*S. oryzae*		*T. castaneum*	
Day	1	4	1	4	1	4
LD_{50} (ml/L)	0.00846	0.00842	0.0024	0.0021	0.0074	0.0069
LC_{50} (mM)	0.075	0.075	0.021	0.018	0.064	0.061

These results are in agreement with those of Shaaya *et al*. (1991) and Lee *et al*. (2003) who studied the toxicity of R (+) pulegone towards the adult of *Oryzaephilus surinamensis, Tribolium castaneum* and *Rhyzoperta dominica* and showed that this compound involved a total mortality of the insects.

In this work, we extended our study to the search of the insecticidal activity of six essential oils of Moroccan plants with regard to three pests of stored cereals *R. dominica, S. oryzae* and *T. castaneum.* From the results obtained, the highest insecticidal activity toward the three pests studied was obtained with essential oil from *M. pulegium.* R (+) pulegone, a monoterpene ketone and major compound of essential oil of *M. pulegium,* showed a high toxic activity with respect to all species. R (+) pulegone and probably also other monoterpenes (ketonic) could be used in the protection of the stored food products.

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