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Insecticidal activity of several Tunisian essential oils against two major pests of stored grain *Rhyzopertha dominica* (Fabricius, 1792) and *Tribolium castaneum* (Herbest 1797)

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Abstract - Essential oils (EOs) extracted by hydrodistilation from fifteen Tunisian plant species namely *Pistacia lentiscus, Artemisia arborescens, Artemisia herba-alba, Cupressus sempervirens, Juniperus communis, Pelargonium graveolens, Lavandula angustifolia, Mentha pulegium, Rosmarinus officinalis, Salvia officinalis, Thymbra capitata, Laurus nobilis, Myrtus communis, Citrus aurantium, Ruta chalepensis,* are tested for their insecticidal activities on adults of both pests of stored grains *Rhyzopertha dominica* (Bostrichidae) and *Tribolium castaneum* (Tenebrionidea).

Fumigant toxicity bioassays showed that *R. dominica* is more sensitive towards these EOs than *T. castaneum*. *L. angustifolia*is the most effective essential oil followed by *R. chalepensis* essential oil with LC_{50} values of 11.14 and 14.82µl/l air,respectively. Moreover, *M. pulegium* and *R. officinalis* oils also exibited significant fumigant toxicity with LC_{50} values of ~ 16.6µl/l air. Besides, *T. castaneum* was more tolerant to these EO except those from *R. chalepensis*(LC_{50} = 21.03 µl /l air) and *M. pulegium*(LC_{50} = 49.84µl /l air).

Repellent activity against both insects showed th at*C. sempervirens* EO was the most effective against *T. castaneum*compared with other treatments; it caused 100% repellency after 6 hours of exposure to the dose 0.15µl /cm² while *M. communis* EO was the most effective against*R. dominica* after 24 hours of exposure at the dose of 0.076µl /cm².

The ingestion toxicity of *R. chalepensis* and *M. pulegium* EOs showed the most important activity against the two insects withLC$_{50}$values of 131.86µl / l and 55.5µl / L for*R. dominica* respectively and with LC_{50}values of 121.8µl / l and 178.46µl / l for *T. castaneum* respectively.

These results pointed out that among EO tested, those extracted from *R. chalpensis, M. pulegium* could be the target of further research to demonstrate their efficacy as biopesticides against stored grain insects.

Keywords: bioinsecticide, essential oils, *Ruta chalepensis, Mentha pulegium, Rhyzopertha dominican, Tribolium castaneum.*

1. Introduction

Considerable losseson stored grains during the storage period in developing countries may reach more than 20% and are mainly caused by insect pests affecting the quantity and quality of grain (Jood et al. 1993; Tripathi 2018).

The grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and red flour beetle, *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae) are among the most important insect pests of stored grain in Tunisia and North Africa (Balachowsky and Pierre 1962; Jerraya 2003). *R. dominica*, a primary pest of stored-products, is able to infect healthy grains easily, while *T. castaneum* is considered a secondary colonizer because it grows easily on broken grains, flour or grains already infested by a primary insect (Vayias et al. 2010). Adults and larvae of both species are serious

economic pests causing serious quantitative and qualitative losses (Banga et al. 2018)and these require effective solutions to protect cereal stocks (Pires and Nogueira, 2018).

To control insect pests of stored grains, synthetic products were used mainly fumigants such as phosphine (Daghlish et al. 2018; Wijayaratne and Rajapakse2018). However, excessive use of synthetic insecticides has resulted in many negative consequences such as the loss of efficiency for the resurgence of pests developing resistance, human and environmental toxicity (Daglish 2004; Lorini et al. 2007; Okonkwo and Okoye. 1996; Sousa et al. 2009).

Furthermore, interest augmented to look for natural products such as plant extracts including essential oils to control insect pest in stored-grains because they have the advantage of rapid degradation and have a low environmental and mammalian toxicity (Campolo et al. 2014; Gonzalez-Coloma et al. 2013;Mediouni-Ben Jemâa et al. 2012b; Ogendo et al. 2008; Rajendran and Sriranjini 2008; Suthisut et al. 2011;Tampe et al. 2016;Wong et al. 2005).

Essential oils have various insecticidal activities. They may act by fumigation, have repellent and antifeedant activities, or may affect biological parameters such as growth rate, life cycle and fecondity (Isman 2006; Shayaa et al. 1997; Stamopoulos 1991). The bioactivity of essential oils is related to their chemical composition, part of plant from which oil was extracted, the environmental conditions and the extraction method(Angioni et al. 2006; Isman 2000;Nerio et al. 2010;Zapata and Smagghe 2010).

This study aimed to evaluate the insecticidal activities of essential oils extracted from fifteen plants species collected from different regions of Tunisia. The biological tests of EO were done on two major insect pests of stored grains: *R. dominica* and *T. castaneum.*

2. Materials and Methods

2.1 Plant Material

Fifteen plant species belonging to eight different botanical families, were collected from different regions in Tunisia except essential oil of *C. aurantium* (Neroli) that was purchased from Tunisia (Table 1). The plant collection was carried out during their flowering period in 2015.

Table 1: List of plant species tested for their insecticidal and repellent activities, plant part used, site of collecting and yield in 2015

Plant Family	Scientific name	Simpled site	Plant organ	Yields(%)
Anacardiaceae	***Pistacia lentiscus***	Tabarka	Leaves, fruits	0.16
Asteraceae	***Artemisia arborescens L.***	Bousalem	Leaves, fruits	0.13
	Artemisia herba-alba	Zaghouen	Aerial parts	0.17
Cupressaceae	***Cupressus sempervirens***	INAT (Tunisia)	Leafy stems and berries	0.12
	Juniperus communis	Tbourba	Leafy stems and berries	0.22
Geraniaceae	***Pelargonium graveolens***	Monastir	Leaves, flowers	0.09
Lamiaceae	***Lavandula angustifolia***	Kef	Flowers	0.67
	Mentha pulegium	Bizerte	Aerial parts	0.685
	Rosmarinus officinalis	Monastir	Aerial parts	0.096
	Salvia officinalis	INAT (Tunisia)	Leaves, flowers	0.03
	Thymbra capitata (L.) Cav.	Monastir	Aerial parts	0.346
Lauraceae	***Laurus nobilis***	Dar Chaaben	Leaves	0.1
Myrtaceae	***Myrtus communis***	Tabarka	Leaves	0.18
Rutaceae	***Citrus aurantium***	—	—	—
	Ruta chalepensis L.	Bousalem	Leaves, flowers	0.2

2.2 Extraction of essential oils

Essential oils were extracted by steam distillation of fresh aerial parts of plant species using a Clevenger-type apparatus. Essential oils were kept in tinted glass vials tightly closed at 4 ° C until used in the bioassays.

2.3 Insect material

*Rhyzopertha dominica*and *Tribolium castaneum* were collected from infested storage wheat in Tunisia. Adults of both insects were reared under constant conditions of temperature (28 ± 1 ° C) and relative humidity (60% ± 5%) at complete darkness in the laboratory of Zoology at National Agronomic Institute of Tunisia (INAT). The rearing of *R. dominica* was done on whole wheat, whereas of *T. castaneum* rearing was done on wheat flour. Unsexed adults of both insects were used for bioassays tests.

2.4 Repellent effect of essential oils

To evaluate the repellent activities of essential oils, we used the method of the preferred zone at 25 ° C ± 1 ° C and 65% ± 5% RH.

This method consist to use filter paper discs Whatman n°1 (diameter 8 cm) placed in Petri dishes glass (diameter 9 cm). The filter paper discs are cut into two equal parts. Five doses of EO (1, 2, 4, 8 and 10 µl) were prepared by dissolving in acetone to have 0.5 ml of each concentration. Solutions are homogeneously applied to half a filter paper disc using a micropipette, while the other half of the disk is treated only with 0.5 ml of acetone and is considered as a control. After complete evaporation of the solvent, the treated and untreated half discs were attached with adhesive tape in the Petri dishes. Ten unsexed adults were placed in the center of each filter paper disc. The Petri dishes were covered and sealed with Parafilm. Five replications were performed for each essential oil dose. Observations were done after 3, 6 and 24h of the beginning of the treatment to count the number of adults present on each half filter paper disc. Percentage repellency (PR) were calculated according toCosimi et al. (2009) andNerio et al. (2009) et formula as follow:

$PR = [(N_c-N_t) / (N_c+N_t)]*100$

N_c: The number of insects on the untreated half filter paper disc

N_t: The number of insects on the treated half filter paper disc with essential oil

2.5 Fumigant toxicity bioassays

To evaluate the fumigant activity of essential oils at the concentrations: 23.58; 47.17; 94.34; 188.68 and 235.85 µl/l air, filter paper discs (Whatman No. 1) 2 cm diameter, were impregnated with essential oils and air-dried. Filter paper discs were then attached to the lids Plexiglas spittoon of 42.4 ml volume. The spittoon is then closed hermetically. Ten unsexed adults of each insect species were added to the Plexiglas spittoon and tightly sealed. For the control, ten adult insects were placed into empty spittoons in the same conditions as the treated one and didn't receive any treatment. Each treatment was replicated five times. Insect mortality was recorded every 3, 6, 24, 48, 72, 96 and 120 hours. Insects were considered dead when it is completely motionless with no movement in the legs and antennae.

The tests are conducted to determine median lethal concentrations LC_{50} and median lethal time LT_{50}.

The values of LC_{50} and LT_{50} are determined using Probit analysis (Finney 1971).

2.6 Antifeedant activities on wheat treated with essential oils

Batches of 20g of uninfested wheat were weighed and placed in vials of 250 ml. Two EOs doses were added 8 and 10 µl corresponding to 160 and 200 µl/l air, respectively. EOs doses were dissolved in 1ml of acetone. Wheat grains were treated with the different doses. The vials were sealed, well shaken for 5 minutes to obtain a homogeneous mixture. Then, grains were air-dried for 20 minutes. The Whole were transferred into a 50 ml glass vials to which were added 20 adult insects. The control was treated only with acetone. The glass vials were sealed and kept in the dark at 29 ° C and 65% RH. Each treatment was replicated five times and insect mortality was recorded every 24h until 120h.

2.7 Data Analysis

Mortality rates were corrected using Abbott's formula (Abbott 1925). (M_C) designate the corrected insect mortality, (M_0) is the insect mortality in the treated population insects and (M_t) is the insect mortality in controls: $M_C = (M_0-M_t / 100-M_t) * 100$

All data were subjected to the analysis of variance and means were processed using the Statistical Analysis System (SAS, 2007) and the PDMix procedure to detect the difference between insects, essential oils, concentrations and time at the 5% probability level. Probit analysis (Finney 1971) is

used to estimate the concentrations that kill 50% of the insects population (LC_{50}) and the time that kills 50% of the population (LT_{50}).

3. Results and discussion

3.1 Essential oils extraction yields

Essential oil yields were presented in (Table 1).*M. pulegium* presented the most important essential oil yield (0.685%) followed by *L. angustifolia*. Both plant species belong to Lamiaceae family. The distillation of the leafy branches and berries of *J. communis* yielded 0.22 %. 0.03% was the essential oil yield of *S. officinalis* and it was the lowest in comparison with other plant species distilled in this study.

3.2 Repellent effect of essential oils

3.2.1 *Rhyzopertha dominica*

The Chi-2 test (chi-square) shows that the fourteen EO have significant repellent activity against adults *R. dominica* (Table 2).Some essential oils are repulsive at the lowest concentration (0.038µl / cm²) and the shortest exposure time (3h and 6h). Indeed EO of *L. angustifolia*, *A. arborescens* L. and *R. officinalis* have shown an effective repellent activity against *R. domoinica*.

M. pulegium EO showed a significant repellency at the low-dose and after a short time of exposure of 3 to 6 hours. The doses 0.076 and 0.31µl / cm² were highly repulsive after 6 hours of exposure (Table 2).

T. capitata EO recorded a slight repellent activity during the first hours of exposure to 0.038 and 0.076µl / cm². This repellency turned into attractiveness with the higher doses. Indeed, (-44%), (-24%) and (-16%) repellency percentage were obtained after 24 hours of exposure to 0.15 µl / cm², 0.31 µl/cm² and 0.38 µl/cm², respectively. *T. capitata* EO has an attractive activity on *R. dominica* adults that can be interesting for the oral toxicity tests (Table 2).

The EO extracted from *R. chalepensis* showed no repellent activity against *R. dominica* at the concentrations 0.038 and 0.076µl/cm². This repellency was manifested at the dose 0.15µl /cm² with 80% recorded after 24 hours of exposure (Table 2).

EOs from *L. nobilis*, *P. lentiscus*, *J. communis*, *P. graveolens*, *C. sempervirens*, *A. herba-alba*, *M. communis* and *C. aurantium* showed very repellent activities at different doses tested and after different exposure periods against *R. dominica*.

3.1.2 *Tribolium castaneum*

The Chi-2 test (chi-square) (χ^2) revealed that the fourteen essential oils have a significant repellent effect on *T. castaneum*. Repellent activity of EO was manifested by their migration into the control part of the filter paper disc.

Indeed, EOs from *C. aurantium*, *L. nobilis*, *A. herba-alba*, *A. arborescens*, *P. lentiscus*, *C. sempervirens*, *R. officinalis*, *P. graveolens* exhibited highly significant repellency against *T. castaneum* for the various tested doses (0.038; 0.076; 0.15; 0.31 and 0.38 µl/cm²) and different exposure times (3, 6, 24 hours) (Table 2). *R. chalepensis* EO leaded a very important repellency except at the dose 0.076 µl/cm² where the repellent activity was not significant after 24 hours of exposure to treatment. Moreover, EO of *L. angustifolia* was very repellent after 3 hours of exposure starting from the dose 0.15µl/cm². Besides, after 3 hours of exposure to different concentrations, *M. pulegium* EO showed a significant repellent activity against *T. castaneum* with the highest percentage at the high dose 0.38µl/cm².

EO of *T. capitata*, *M. communis* and *J. communis* leaded a highly significant repellency after various periods of exposure towards *T. castaneum* at the different doses.

3.2 Fumigant toxicity test

3.2.1 *Rhyzopertha dominican*

The screening of essential oils and their fumigant effect on *R. donimica* had identified EOs showing an important insecticidal effect at low-dose and a short exposure time. LC_{50} and TL_{50}values are reported in Table 3.

Generally the mortality rate of *R. dominica* increases with the dose applied for the fourteen essential oils tested (*P. lentiscus*, *A. arborescens*, *A. herba-alba*, *J. communis*, *P. graveolens*, *L. angustifolia*, *M.*

pulegium, *R. officinalis*, *S. officinalis*, *T. capitata*, *L. nobilis*, *M. communis*, *C. aurantium*, *R. chalepensis*) except the essential oil of *C. sempervirens*, which remains a constant mortality (Table 3). *J. communis* and *C. sempervirens* belonging the Cupressaceae family showed the least effective effect with percentage mortality not exceeding 50%. These EO didn't have an insecticidal effect against *R. dominica* even at high doses and extended of exposure period.

Table 2: Effect repellent essential oils on adults of *Tribolium castaneum* (Tc) and *Rhyzopertha dominica* (Rd) depending on the dose and exposure time.

Oil	Dose (µl / cm²)	3h				6h				24			
		Tc		Rd		Tc		Rd		Tc		Rd	
		χ^2r	χ^2s	χ^2r	χ^2s	χ^2r	χ^2s	χ^2r	χ^2s	χ^2r	χ^2s	χ^2r	χ^2s
C.aurantium	0.038	38.74	39.7 **	23.14	27.3 **	23.14	24.9 **	8.02	10.9 *	11.54	42.1 **	3.94	5.7ns
	0.076	46.10	46.9 **	18.02	22.5 **	35.30	36.1 **	23.90	27.3 **	38.74	39.7 **	25.94	29.3 **
	0.15	25.94	28.5 **	28.90	31.3 **	28.90	30.5 **	28.90	33.7 **	42.34	43.3 **	38.74	40.5 **
	0.31	35.30	36.9 **	6.50	9.7 *	38.74	39.7 **	2.90	5.2ns	35.30	37.7 **	2.02	3.2ns
	0.38	35.30	36.9 **	5.14	17.2 **	32.02	34.9 **	0.00	10.8 *	28.90	30.5 **	0.34	7.6ns
R.chalepensis	0.038	2.02	16.0 **	6.50	8.1ns	13.54	19.3 **	6.50	12.9 *	9.70	13.6 **	13.54	15.3 **
	0.076	38.74	39.7 **	3.94	9.6 *	23.14	24.1 **	2.02	12.9 *	0.34	7.6ns	9.70	12.1 *
	0.15	38.74	40.5 **	15.70	18.1 **	23.14	26.5 **	11.54	15.6 **	20.50	27.7 **	32.02	34.1 **
	0.31	28.90	30.5 **	11.54	15.6 **	13.54	20.9 **	11.54	21.3 **	11.54	17.2 **	6.50	8.1ns
	0.38	6.50	14.5 **	11.54	14.1 **	18.02	21.7 **	3.94	5.6ns	3.94	14.5 **	13.54	15.3 **
L.nobilis	0.038	42.34	44.1 **	9.70	12.1 *	38.74	40.5 **	23.14	25.7 **	25.94	30.1 **	20.50	23.7 **
	0.076	38.74	40 .5 **	5.14	12.5 *	28.90	32.1 **	11.54	23.7 **	32.02	33.3 **	15.70	23.7 **
	0.15	42.34	44.1 **	5.14	10.9 *	23.14	24.1 **	2.02	12.1 *	50.02	50.5 **	2.02	19.3 **
	0.31	25.94	28.5 **	11.54	14.9 **	18.02	27.3 **	18.02	19.3 **	25.94	27.7 **	11 .54	14.8 **
	0.38	42.34	44.1 **	18.02	20.1 **	25.94	27.7 **	0.74	9.7 *	23.14	24.9 **	0.10	11.3 *
A.herba-alba	0.038	42.34	43.3 **	9.70	16.0 **	38.74	40.5 **	8.02	17.1 **	28.90	30.5 **	23.14	24.1 **
	0.076	32.02	32.5 **	2.02	4.4ns	32.02	33.3 **	1.30	4.2ns	23.14	24.1 **	15.7	18.1 **
	0.15	46.10	46.9 **	0.00	9.3ns	35.30	36.9 **	0.34	2ns	32.02	33.3 **	5.14	9.1ns
	0.31	18.02	19.3 **	2.90	9.2ns	25.94	27.7 **	0.10	11.3 *	13.54	18.4 **	0.74	11.1 *
	0.38	20.50	26.0 **	2.90	9.2ns	13.54	17.6 **	2.90	11.6 *	18.02	22.5 **	0.00	1.0ns
T.capitata	0.038	6.50	8.8ns	2.02	16.0 **	11.54	20.5 **	9.70	14.4 **	9.70	12.9 *	5.14	12.5 *
	0.076	13.54	18.4 **	0.74	7.1ns	2.02	8.9ns	20.50	23.7 **	3.94	4.9ns	0.74	8.7ns
	0.15	25.94	28.5 **	3.94	13.6 **	2.90	10.9 *	18.02	19.3 **	9.70	16.9 **	9.70	17.7 **
	0.31	3.94	12.8 *	0.10	3.2ns	2.90	4.4ns	3.94	17.6 **	6.50	14.5 **	2.90	4.4ns
	0.38	9.70	19.3 **	1.30	3.4ns	0.74	7.1ns	23.14	27.3 **	11.54	14.1 **	1.30	4.4ns
L.angustifolia	0.038	0.10	10.2 *	5.14	9.2ns	15.70	18.1 **	5.14	6.9ns	6.50	20.0 **	0.34	4.2ns
	0.076	0.74	18.5 **	0.74	25.7 **	9.70	12.8 *	2.90	22.1 **	8.02	10.1 *	1.30	7.6ns
	0.15	20.50	22.1 **	0.10	12.1 *	0.10	4.9ns	3.94	11.2ns	0.10	3.3ns	1.30	4.4ns
	0.31	18.02	23.2 **	0.34	10.1 *	2.90	9.3ns	0.10	8.9ns	6.50	10.4 *	0.34	4.3ns
	0.38	25.94	26.9 **	0.74	8.1ns	11.54	13.3 **	2.90	10.9 *	15.70	22.0 **	0.34	11.6 *
A.arborescens	0.038	25.94	32.5 **	8.02	11.6 *	42.34	44.1 **	13.54	16.9 **	35.30	36.9 **	13.54	14.5 **
	0.076	32.02	34.9 **	5.14	10.9 *	32.02	34.9 **	8.02	13.3 **	35.30	36.9 **	8.02	10.1 *
	0.15	35.30	36.9 **	1.30	7.6ns	35.30	36.9 **	0.34	7.7ns	28.90	31.3 **	0.10	12.9 *
	0.31	13.54	16.9 **	2.90	6.0ns	13.54	16.9 **	0.10	6.4ns	32.02	33.3 **	0.10	6.4ns
	0.38	32.02	34.1 **	1.30	10.8 *	32.02	34.1 **	0.34	19.7 **	28.90	31.3 **	0.74	8.9ns

P.lentiscus	0.038	9.70	17.7 **	2.90	16.5 **	0.74	17.7 **	1.30	5.2ns	6.50	29.6 **	8.02	11.6 *
	0.076	42.34	43.3 **	25.94	29.3 **	25.94	27.7 **	28.90	32.1 **	0.74	20.8 **	25.94	30.9 **
	0.15	32.02	33.3 **	20.50	22.1 **	32.02	37.3 **	5.14	9.9 *	23.14	26.5 **	6.50	7.3ns
	0.31	38.74	39.7 **	15.70	18.1 **	46.10	46.9 **	28.90	32.1 **	25.94	27.7 **	20.50	23.7 **
	0.38	46.10	46.9 **	35.30	36.9 **	32.02	33.3 **	25.94	29.3 **	20.50	25.3 **	20.50	26.9 **
J. communis	0.038	2.02	12.9 *	9.70	18.3 **	0.74	11.3 *	11.54	18.1 **	23.14	34.5 **	0.74	3.2ns
	0.076	32.02	33.3 **	9.70	12.9 *	0.74	11.3 *	18.02	19.3 **	11.54	23.7 **	6.50	9.6 *
	0.15	38.74	40.5 **	9.70	10.5 *	42.34	43.3 **	9.70	14.4 **	18.02	22.5 **	2.90	10.9 *
	0.31	38.74	39.7 **	23.14	25.7 **	35.30	36.1 **	28.90	30.5 **	20.50	22.1 **	23.14	24.9 **
	0.38	46.10	46.9 **	6.50	12.1 *	35.30	37.7 **	9.70	13.6 **	15.70	24.4 **	0.74	12.9 *
M.pulegium	0.038	35.30	38.5 **	8.02	15.7 **	32.02	33.3 **	9.70	16 **	8.02	15.7 **	2.90	6.0ns
	0.076	13.54	16.1 **	2.90	9.3ns	6.50	12.1 *	0.34	14.1 **	5.14	10.1 *	0.00	10.9 *
	0.15	2.90	5.1ns	1.30	7.6ns	0.34	5.3ns	0.10	6.4ns	0.10	11.3 *	0.10	6.4ns
	0.31	15.70	19.7 **	1.30	7.6ns	15.70	18.1 **	5.14	20.5 **	8.02	10.8 *	3.94	21.7 **
	0.38	23.14	27.3 **	0.74	4.9ns	11.54	19.7 **	0.34	10.9 *	2.90	13.3 **	0.74	12.1 *
M.communis	0.038	13.54	15.3 **	20.50	40.5 **	25.94	28.5 **	13.54	18.5 **	28.90	32.1 **	32.02	33.3 **
	0.076	38.74	39.7 **	28.90	32.1 **	23.14	25.7 **	35.30	40.9 **	18.02	20.9 **	42.34	43.3 **
	0.15	28.9	33.7 **	28.90	31.3 **	23.14	25.7 **	38.74	40.5 **	9.70	12.1 *	32.03	34.1 **
	0.31	38.74	40.5 **	42.34	43.3 **	25.94	28.5 **	38.74	40.5 **	18.02	20.1 **	23.14	30.4 **
	0.38	38.74	39.7 **	38.74	40.5 **	42.34	44.1 **	35.30	36.1 **	42.34	43.3 **	38.74	39.7 **
C.sempervirens	0.038	42.34	43.3 **	6.50	11.2 *	42.34	43.3 **	18.02	19.3 **	32.02	37.3 **	0.74	7.1ns
	0.076	38.74	40.5 **	13.54	15.3 **	42.34	43.3 **	3.94	18.5 **	32.02	34.1 **	5.14	11.6 *
	0.15	35.30	38.5 **	8.02	12.4 *	50.02	50.5 **	6.50	8.9ns	28.90	30.5 **	8.02	10.8 *
	0.31	42.34	43.3 **	8.02	16.5 **	46.10	46.5 **	13.54	17.7 **	35.30	38.5 **	6.50	14.5 **
	0.38	50.02	50.5 **	32.02	34.9 **	42.34	43.3 **	23.14	29.6 **	42.34	43.3 **	20.50	22.1 **
R.officinalis	0.038	42.34	43.3 **	15.70	27.7 **	28.90	31.3 **	23.14	33.7 **	38.74	39.7 **	0.10	12.9 *
	0.076	50.02	50.5 **	8.02	23.7 **	38.74	40.5 **	6.50	22.5 **	46.10	46.9 **	3.94	16.0 **
	0.15	38.74	40.5 **	2.02	5.4ns	46.10	46.9 **	2.90	9.2ns	42.34	43.3 **	0.10	16.9 **
	0.31	23.14	28.9 **	6.50	12.9 *	35.30	36.9 **	8.02	16.5 **	32.02	34.1 **	2.90	18.1 **
	0.38	25.94	26.9 **	0.10	1.5ns	32.02	34.1 **	0.10	1.5ns	20.50	22.9 **	0.74	1.5ns
P.graveolens	0.038	35.30	36.9 **	8.02	11.7 *	32.02	33.3 **	2.90	13.3 **	25.94	26.9 **	8.02	13.9 **
	0.076	50.02	50.5 **	1.30	5.9ns	50.02	50.5 **	2.02	12.1 *	50.02	50.5 **	2.02	8.0ns
	0.15	50.02	50.5 **	3.93	11.8 *	35.30	36.9 **	2.90	11.6 *	42.34	43.3 **	2.90	16.5 **
	0.31	38.74	39.7 **	0.00	22.0 **	35.30	37.7 **	2.90	12.4 *	38.74	40.5 **	13.54	16.1 **
	0.38	35.30	36.9 **	0.74	17.7 **	23.14	24.1 **	0.10	17.6 **	28.90	30.5 **	8.02	14.0 **

**, significant differences at $p < 0.05$ and $p < 0.01$

Data are tested by applying the Chi-2 test (chi-square test);

The total number of insects for each concentration is 50 individuals.

Table 3: LC_{50} and LT_{50} values of essential oils from Tunisia plant species against adults of *R. dominica* and *T. castaneum*

	R. dominica		*T. castaneum*	
	LC_{50}(µl/lair)	**LT_{50} (h)**	**LC_{50}(µl/lair)**	**LT_{50} (h)**
L. angustifolia	11,14	3,624	>150	>150
R. chalepensis	14,82	3,595	21.033	12,324
M.pulegium	16,6	7,011	49.844	7,519
R. officinalis	16,66	26,779	>150	>150
T. capitata	35,41	37,471	>150	>150
M.communis	46,35	172,792	>150	>150
S.officinalis	49,4	141,026	>150	>150
L.nobilis	60,12	429,737	>150	>150
A. herba-alba	62,95	14,492	>150	>150
A. arborescens	105,86	84,776	>150	>150
P.lentiscus	120,69	>150	>150	>150
P. graveolens	137,81	>150	>150	>150
C. aurantium	>150	>150	>150	>150

*TL_{50} presented in the table were calculated at the concentration 23,58(µl/l)

Results indicated that EOs extracted from *L. angustifolia*, *M. pulegium*, *R. officinalis*, *R. chalepensis* appear to be the most effective against *R. dominica*. Fifty percentage of insects mortalities were reached at lower concentrations 11.14 µl/l air, 14.82 µl/l air of *L. angustifolia* and *R. chalepensis*, respectively. EOs of *C. aurantium*, *P. graveolens*, *A. arborescens* and *P. lentiscus* presented the highest LC_{50} values and they were the less effective against *R. dominica*.

3.2.2 *Tribolium castaneum*

In most cases, *T. castaneum* mortality percentages increased with the concentration except the essential oils of *T. capitata* and *C. aurantium* which there was no mortality recorded even at the highest dose after 24 hours of exposure. Under the same conditions, essential oils of *J. communis* and *P. graveolens* didn't exceeded 5% of mortality (Table3).

Essential oils that showed over 50% of mortality after 24 hours (*R. chalepensis*, *M. pulegium*, *A. herba-alba*, *R. officinalis* and *M. communis*) seemed to be interesting to be used as an alternative to synthetic insecticides. The rest of EO requires higher concentrations to cause the mortality of the insect and did not present an economically profitable insecticidal interest. At the lowest concentration (23.58µl /l air) *M. pulegium* was more effective than *R. chalepensis*, causing 50% of mortality after about 8h and 12h , respectively (Table3).

Except *M. pulegium* and *R. chalepensis* essential oils, the rest of EOs recorded TL_{50} higher than120h. At 235,85(µl/l) *A. herba-alba* , *M. communis, R. officinalis* and reached TL_{50} equal to11,879; 19,595 and 29,929 h , respectively.

3.3 Antifeedant activities on wheat treated with essential oils

Based on the results of fumigant toxicity bioassays, essential oils extracted from *A. arborescens* , *M. pulegium* and *R. chalepensis* were chosen following their effectiveness on both insects *R. dominica* and *T. castaneum*, to be tested for their antifeedant activities. Mortality rates reached almost 100% for the three EOs tested after 120 hours of exposure at the dose 235.85µl / l air.

R. chalepensis essential oil was very effective against *R. dominica*. It caused 76% and 94% of mortality after 24 hours and 48h and reached 100% mortality after 72 hours at 160µl/l air.

M. pulegium EO caused 95% of mortality after 24 hours at the dose of 160 µl /l air. 48h later, the mortality reached 98%. However, the EO from *A. arborescens* caused a mortality rate of 76% after 24 hours of treatment at the dose 160 µl/l air (Table 4).

Results indicated that EO from *R. chalepensis* was more toxic than the EO from *M. pulegium* against *T. castaneum*. Indeed, after 24 hours of exposure at 160µl/l air, *R. chalepensis* caused 80% of mortality. Whearas *M. pulegium* achieved only 37%. Thus, the EO from *R. chalepensis* caused the largest antifeedant activity in comparison with those from *A. arborescens* and *M. pulegium* (Table 4).

Table 4: Percentage of mortality of *R. dominica* (R.d) and *T. castaneum* (T.c) in wheat grain treated with essential oils

		24		48h		72h		96h		120h	
EOs	**Doses (µl /l)**	***R.d***	***T.c***	***R.d***	***T.c***	***R.d***	***T.c***	***R.d***	***T.c***	***R.d***	***T.c***
R. chalepensis	160	76	80	94	90	100	100	100	100	100	100
	200	59	88	90	100	93	100	95	100	97	100
M. pulegium	160	95	37	95	71	98	86	98	91	98	94
	200	91	62	98	90	98	98	98	100	98	100
A. arborescens	160	76	2	28	5	33	7	39	7	49	12
	200	43	5	65	8	67	11	67	14	72	19

The EO which had the most important toxic activity required minimal time to kill half of the tested population. The lethal time 50% of the population depended upon the concentration. It is inversely proportional with the latter (Table 5). *M. pulegium* had an immediate effect on *R. dominica* at 200µl/l. CL_{50} = 55,49 µl/l air was the lowest and it represents the TL_{50}=0,069h. The LC_{50} and LT_{50} of EO of *A. arborescens* were very high, it exceeded 150 hours for the two insects therefore it does not show any interest antifeedant activity (Table 5).

Table 5: LC50 and LT50 essential oils applied to wheat grain against *R. donimica* (Rc) and *T. castaneum* (Tc)

	LC50 (µl / l air)		**LT50 (h)**			
			160µl / l air		**200 µl / l air**	
	R. d	***T. c***	***R. d***	***T. c***	***R. d***	***T. c***
A. arborescens	296.039	477.08	153.096	> 150	33.165	> 150
M. pulegium	55.49	178.46	1.613	31.186	0.069	19.995
R. chalepensis	131.859	123.818	16.522	20.83	14.367	8.304

Discussion

Several scientific researchers were investigated to study essential oils yields and activities against many arthropods (Abderrahim et al. 2019;Ait-Ouazzou et al. 2012; Attia et al. 2012; Blažekovic et al. 2018; Cardia et al. 2018; Lakehal et al. 2016). Studies reported that variations in EOs yields considerably depend on plant species, geographic location, the method or extraction time, the plant parts used the collecting period, etc (Mejri et al. 2010; Teles et al. 2013).

In this study plant species with the most important essential oil yields were *L. angustifolia* (0.67%), *T. capitata* (0.35%) and *J. communis* (0.23%).

L. angustifolia essential oil yield (0.67%) was higher compared to a study carried out by Cardia et al. (2018) which was (0.14%). While Blažekovic et al.(2018) showed a higher essential oil yield (0.9%).

In the present study the yield of *T. capitata* EO was 0.35% , whereas, Aazza et al. (2016) presented that its EO yield was 1.3%. Moreover, Abderrahim et al. (2019) showed differences in essential oil yields from *A. arborescens* growing in three areas in Bejaia and in comparison with EO yield in this study.

Many essential oils from plant species were investigated for their insecticidal activities to control insect pests of stored grain (Ben Chaaban et al. 2019; Campolo et al. 2018;Chiluwal et al. 2017). They are tested for their repellent (Bougherra et al. 2015, Taban et al. 2017), fumigant (Bachrouch et al. 2010) and antifeedant activities (Lee et al. 2004, Upadhyay et al. 2018).

In the current study *R. dominica* seems more tolerant to the repellent effect of EOs than *T. castaneum* which showed greater sensitivity. *Pistacia lentiscus* esential oil showed repellent activity against *R. dominica and T. castaneum*. Our results are in accordance with a study investigated by Bougherra et al. (2015) showing that *P. lentiscus* exerted repellent activities on *R. dominica, Sitophilus zeamais, Tribolium confusum* with a superior resistance of *R. dominica*. Similarly, Bachrouch et al. (2010), recorded the insecticidal activity of *P. lentiscus* on the third instar larvae and the adult of *T. castaneum* with LC_{50} equal to 112.12 and 28.03 µl / l air, respectively. However, in this study we noted a very lower efficiency against *T. castaneum*. This difference in efficiency may be explained by the geographic origin of plants and therefore the essential oil composition.

Furthermore, in 2012, Mediouni-Ben Jemâa et al. (2012) recorded significant variation in repellent and fumigant activities of three *L. nobilis* essential oils from Morocco, Algeria and Tunisia against *R.*

dominica and *T. castaneum* with a higher repellency against the latter. The insecticidal effects of EOs could be attributed to the geographic origin of plant and the tolerance of insect species to EOs (Teles et al. 2013; Tunç et al. 2000).

In the same context, Bett et al. (2016) showed the insecticidal and repellent of two essential oils extracted from the leaves of *Cupressus lusitanica* Miller and *Eucalyptus saligna* Smith against adult *Tribolium castaneum*, *Acanthoscelides obtectus*, *Sitotroga cerealella* and *Sitophilus zeamais* with highest repellency of the four EOs against *T. casaneum* (65–92.5%).

A study investigated by Cosimi et al. (2009) showed that 24h after treatment *Citrus bergamia* EO(or *Citrus aurantium*) carried the highest repulsion on maize weevil and *Cryptolestes ferrgineus*.

R. dominica adults (CL_{50}=11,14 µl/l) were significantly more susceptible than *T. castaneum* (CL_{50}>150 µl//l) to the fumigant effect of essential oils from *L. angustifolia*. This susceptibility was confirmed by Ebadollahi et al. (2010) with LC_{50} = 5.66 µl/l and 39.685 µl/l 24 h after treatment against *R. dominica* and *T. castaneum*, respectively.

M. communis investigated in this study seems less effective against *R. dominica* and *T. castaneum*. According to Ayvaz et al. (2010), *M. communis* essential oil showed an insecticidal effect against three different stored product insects *Ephestia kuehniella*, *Plodia interpunctella* and *Acanthoscelides obtectus* with LC_{50} values of 12.74; 22.61 and 49.58µl / l air 24h after treatment , respectively.

Several scientific researchers were investigated to show the insecticidal effects of essential oils such *P. graveolens* (Kabera et al. 2011), *R. officinalis* (Ben Slimane et al. 2015, Lee et al. 2002) , *R. chalepensis* (Majdoub et al.2014) and *M. pulegium*(Aziz and Abbass 2010; Ben Chaaban et al. 2019), against pest insects of stored grains (Upadhyay et al. 2018).

Another study investigated by Taban et al. (2017) showed the insecticidal and repellent activities of essential oils on *T. castaneum*. In fact, EOs from of three species of *Satureja* spp. (*S. Khuzestanica*, *S. rechingeri* and *S. bachtiarica*) were strongly repellent against *T. castaneum* adults at the concentration tested (1% v / v) with a highest repellency of *S. khuzestanica* (98% to 100%) after 4 hours of exposure and fumigant toxicity at the lowest dose with 2.51 mg /L air.

In contrary to our results, Lee et al. (2002) showed that *R. officinalis* was potentially toxic to *T. castaneum* with LC_{50}=7.8µl/l air whereas in the present study LC_{50} is highly superior(199,6 µl/l air). On the other hand, efficiency of both *Thymus vulgaris* were important with LC_{50}>100 µl/lair.

T. castaneum seems to be more resistant to the fumigant activity than *R. dominica*. In this regards, Shaaya et al. (1997) showed that a large number of EOs were assessed against four major stored-product insects *S. oryzae*, *R. dominica*, *Oryzaephilus surinamensis* and *T. castaneum*. The latter was found to be the most resistant to the fumigant activity of EOs(Nenaah 2011). Our findings were confirmed with a study carried out by Rozman et al. (2007) and showed that *T. castaneum* is very tolerant in comparison to *R. dominica* and *S. oryzae* exposed to EOs extracted from *L. angustifolia*, *R. officinalis*, *T. vulgaris* and *L. nobilis*. Another study investigated by Lee et al. (2004) recorded that *S. oryzae* was more tolerant than *T. castaneum* and *R. dominica* to essential oils from Myrtaceae for their fumigant activities with and without wheat.

Previous studies showed that the geographical origin and climate factors, the seasonal and genetic variation and stage of development can influence the chemical composition of the essential oils (Anwar et al. 2009; Milios et al. 2001; Shahat et al. 2011; Teles et al. 2013) and therefore their biological activities. In 2010, Mejri et al. demonstrated that the chemical composition of the essential oil could be influenced by the method of distillation, the distilled part of the plant also its state(fresh or dried). These could explain the differences recorded in their biological effects between scientific research.

To summarize, the biological activities of essential oils considerably depended upon their phytochemical profile and the insect species, concentrations and time of exposure to the treatment.

In this study, several essential oils were tested for their insecticidal and repellent activities against two major insect pest of stored grain. Essential oils from *M. pulegium*, *R.chalepensis* were the most effective against both insects Future research efforts should be directed towards the method of application of essential oils since they are volatile, looking for other plant extracts more effective preserving human and environmental health.

4. Conclusion

This study was carried out to determine the insecticidal effects of fifteen essential oils from Tunisia throughout three bioassays: Repellent, fumigant and antifeedant activities against two pest major of stored-grains *R.dominica* and *T. castaneum*. Most essential oils showed significant insecticidal activities against both insects depending upon plant species, insect tolerance, concentrations and exposure time.

R. chalepensis and *M. pulegium* were the most effective essential oils towards both insects. Future research efforts should be focused on investigate chemical compounds of essential oils, toxicity of major compounds on human, mammal and non-target organisms.

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