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Research Article

Toxicity, repellency and chemical composition of essential oils from aerial parts of *Pistacia lentiscus* (L) against *Tribolium casatneum* (Coleoptera: Tenebrionidae).

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Abstract

Tribolium castaneum (*Tc*) is one of the principal pests affecting cereals, they provoke considerable quantitative and qualitative losses of grains. The purpose of this investigation was to compare for the first time, the antioxidant and insecticidal activities and chemical composition of aerial parts of *Pistacia lentiscus* essential oils (PLEOs). The major components are α-myrcene, limonene and α-pinene, for leaves, stems and fruits respectively. Concerning antioxidant activity, the results indicated a high activity of PLEOs with IC_{50} of 5, 46±0, 12 mg/ml, 4, 67±0,18 mg/ml and 2, 75±0,12 mg/ml of DPPH assay compared to the control groups for leaves, stems and fruits respectively. Also, complementary assays: FRAP and ABTS revealed an important antioxidant capacity confirming those funded by the DPPH assay. Contact toxicity demonstrated that PLEOs possess strong insecticidal efficiency against reed flour beetle with LD_{50} values 0, 77 µL/cm², 0, 53 µL/cm² and 0, 49 µL/cm² for leaves, stems and fruits respectively. The LT_{50} values ranged 42, 80 to 76 hours for leaves, 5, 5 to 60, 23 hours for stems and 6 to 51 hours for fruits. About locomotor activity, insects tend to spend more time in the untreated half arena. However, their instantaneous growth rate was significantly reduced which is caused by the presence of the essential oil in the arena.

1. Introduction

The world population is growing it will be more than 8,5 billion in 2025 [1], according to economists this is an alarming situation in the face of the demand that will be increasing for food products. However, stored foodstuffs are often attacked and deteriorated by insect pests during the storage period. *Tc* (Coleoptera, Tenebrionidae) is one of the species worldwide that causes significant economic losses for stored products such as cereals [2]. Today, several synthetic insecticides are ineffective against a wide range of insect pests.

Previous studies have shown that these synthetic products cause problems in human health and environmental pollution [3]. To this end, the management of this pest is focused on using eco-friendly products such as essential oils due to their biomolecules as well as biological activities as antioxidant, antimicrobial, antifungal and insecticidal activities [4]. Essential oils have been proposed as alternative products to control stored pests because of their mechanisms of action, they act on GABAergic receptors [5], octopamine system [6], inhibition of

acetyl cholinesterase [7] and destruction of neurophysiologic functions in the insect nervous system.

Pistacia lentiscus (*Pl*) is a plant belonging to the Anacardiaceae family that grows on all types of soil and is widely distributed in the Mediterranean circuit. *Pl* is currently considered an interesting plant for its biological effects, which justifies its extensive use in traditional pharmacopoeia for the treatment of several diseases in Morocco including digestive [8], cardiovascular and diabetes illnesses [9]. One of the most remarkable biological effects of *Pl* is anticancer effect due to the resin [10-14].

The insecticidal activity of PLEOs against *Tc* was mentioned in previous studies [15-16] which is based solely on the fumigant toxicity. However, the potential effect involved on locomotor strategies used by these pests to mitigate the effects of these oils. Locomotor responses and instantaneous development rate were completely neglected. The objective of our investigation was. Firstly, comparison of the chemical composition of PLEOs from aerial parts (stems, fruits and leaves) and antioxidant activity. Secondary, evaluation of insecticidal activity (contact and fumigant toxicity), locomotor responses and instantaneous rate of development.

2. Materials and methods

2.1. Plant material

Aerial parts (fruits, leaves and stems) of *Pistacia lentiscus,* were collected in the region of Ourika (31°23' latitude N/7° 42 ' longitude W; 35 KM from Marrakech, Morocco). They were harvested in March 2018. The samples were identified by Professor CHAIT Abderrahman and stored as a voucher specimen (P 18) in the plant herbarium of laboratory, department of biology, faculty of sciences Semlalia, Marrakech, Morocco. The plant was dried in the open air at room temperature until the weight stabilization for extraction of essential oils.

2.2 Extraction of PLEOs

The essential oils were obtained from dry material by hydro-distillation, Clevenger type apparatus. Also, 500 g, 300 g and 500 g of ground plant materials (leaves, fruits and stems respectively) were added to distilled water. Then, essential oils were collected manually, using sodium sulfate to remove water. Finally, the PLEO samples were conserved in a refrigerator at 4°C.

2.3 GC-MS analysis

The analysis protocol of PLEOs was mentioned in our previous study (Terrafe et al., 2022). Identification of chemical compounds was based on the Adam's library.

2.4 Antioxidant activity

2.4.1 DPPH activity

The free radical activity of PLEOs was determined by the stable radical (DPPH), according to similar method described by Sahin [17]. Briefly, 1,0-10 µL/ml of PLEOs was added to 2 ml of 60 µM methanol solution of DPPH and incubated at ambient temperature in obscurity. After thirty minutes, the absorbance was recorded against methanol as a blank at 517 nm. Quercetin and BHT (butylated hydroxyanisole) were used as positive control. The concentration of the PLEOs that neutralizes 50% of DPPH (IC_{50}) was estimated using the following formula:

$$I\ (\%) = [(A-B)/A] \times 100$$

Where, A is the absorption of the control at thirty minutes. B is the absorption of the PLEOs after thirty minutes.

2.4.2 FRAP assay

The free radical activity of PLEOs was determined by inhibition of the formation of the Fe (II) -Ferrozine complex after incubation of the samples with the divalent iron according to the method described by [18]. The method was based on the chemical conversion reaction of Fe^{3+} to Fe^{2+}. Briefly, the sample and control substance were mixed with phosphate buffer (2,5 ml, 0,2M, ph= 6,6) and potassium ferricyanide [K3Fe (CN) 6] (2.5 ml, 1 %). Thirty minutes later, trichloroacetic acid was added (2,5 ml of 10 % (w/v)). The obtained mixture was centrifuged 650 ^{x} g for 10 min. Finally, the upper layer was mixed with 2,5 ml distilled water and 0,5 ml of FeCl3 (ferric chloride 1%). The absorbance was measured at 700 nm after 15 min time of reaction. Quercitine and BHT were used as positive control. Three replications were performed to calculate the mean value of the IC_{50}.

2.4.3 ABTS assay

The ABTS test was used to assess the free radical scavenging activity of the essential oil samples. Briefly, 100 µL of PLEOs or water (control) were mixed with

1 mL of diluted ABTS+ solution, and the absorbance was measured at 734 nm six minutes later [19].

2.5 Insecticidal activity

2.5.1 Insect cultures

Tc colonies were housed in the laboratory without any exposure to insecticides. Sixty insects of both genders are reared on a mixture of wheat flour, wheat germ and yeast extract (13:6:1 w/w/w) in borosilicate glass jars (16cm [diameter] × 22 cm [height]). These jars are covered with mosquito netting to allow insects to breathe. The cultures were kept in a growth room at 26 ± 1°C, a relative humidity and a photoperiod of 16:8 hours (light: darkness). Only adults are used for biological tests by contact and fumigation toxicity bioassays. All tests were carried out under conditions identical to those of the breeding.

2.5.2 Contact toxicity assay

The insecticidal activity of PLEOs against *Tc* adults was measured by contact toxicity assay. Four doses were prepared for each aerial part (leaves, stems and fruits) by diluting each time in 1 ml of acetone the respective volumes of 40, 50, 60 and 70 µL of essential oil. These volumes correspond to doses of 0,62, 0,78, 0,94 and 1,10 µL/cm² for leaves. Then, four doses 30, 35, 40 and 45 µL of essential oil. These volumes correspond to doses of 0,47, 0,55, 0,62 and 0,71µL/cm² for stems. Other four doses were prepared 10, 15, 25 and 25 µL of PLEOs. These doses correspond to 0,15, 0,23, 0,31 and 0,39 for fruits. Each of solutions prepared was spread uniformly over a 9 cm diameter (i.e. 63,62 cm ² surface area) filter paper washer (Whatman No. 1) placed in a glass Petri dish of the same diameter. After fifteen minutes, (solvent was evaporated) ten unsexed adults freshly collected from their breeding environment and 7 to 14 days old was added to each Petri dish and these boxes were immediately closed. Three replicates were performed for each dose. Mortality was recorded after 2, 4, 6, 8, 24, 48 and 72 hours. The signs of mortality are: absence of antennal movements and leg. Bio-tests were designed to determine the lethal dose LD_{50}, LD_{90} values doses and the lethal time LT_{50}, LT_{90} values of exposed insects [19].

2.5.3 Fumigant toxicity assay

Fumigant toxicity was evaluated using filter paper (Whatman No.1) that was impregnated with different PLEOs doses. The filter paper was attached to the inside of the small bottles of a volume of 60 ml, each of them containing 10 individual insects aged between 1 to 7 days. Repetitions of 3 times were carried out for each dose. The mortality was noted after 2 h, 4 h, 6 h, 8 h, 12 h, 24 h,48 h and 72 h of exposure, we consider a dead individual when no movement of the antennal and leg was observed [19].

2.5.4 Population growth bioassay

The PLEOs were used to evaluate their effect on the biological development rate of the insect population. The experiment consisted of exposing the insects to the different doses of the essential oils (60, 120, 250 and 500 µL/ kg) in glass jars (0,8 L) each containing 250 g of the barley grains. Twenty insects were released in each jar to colonize the grain mass for sixty days. Control was treated only by acetone under standard conditions mentioned previously. After this period, the number of living insects and mass of grains were measured. Four replications were performed for each dose. The instantaneous growth rate has been calculated according to the following formula: $ri = [\ln (Nf / Ni)] / \Delta T$, where Nf and Ni are the final and initial numbers of living (adult) insects, respectively, and ΔT is the duration of experience in days [20].

2.5.5 Behavioral and locomotor responses

The behavioral bioassays of PLEOs against *Tc* in circular arenas that were either half or fully treated were evaluated by similar method described by Braga, Haddi, Correa, Pereira, Guedes [21-25]. Briefly, the filter was impregnated with 3 mL of PLEOs (1,05 µL of PLEOs/cm²) at the concentration corresponding to the estimated LC_{90}. The control was treated with acetone only. The filter papers were placed in Petri dishes (135x20 mm), after drying for 20 min. The Teflon® was used to coat the inner walls of each Petri dish to prevent movement of each insect from escaping. Two parameters were recorded for the fully treated arenas for 10 min such as, number of stops and walked distance (cm) in the arena. In order to calculate the proportion of time spent in each compartment of the arena (completely or half-Treated), bioassays were carried out with individual insects adults, twenty insects were used and for each replicate, the filter paper was replaced and the side which the insects were released was randomly selected.

2.6 Statistical analysis

The PROC PROBIT procedure was used to estimate

dose-mortality curves in probit analysis. The finding of the survival bioassays was subjected to a survival analysis with Sigma Plot software, which used the Kaplan-Meier estimators (Log-rank method). A paired student's t-test ($p<0.05$) was used to compare pairwise differences in walking behavior in the half treated arenas.

3. Results and discussion

3.1. Chemical composition

The clear and yellowish essential oils obtained by hydrodistillation of aerial parts of PL yielded 0, 61 %, 0, 45 % and 0, 78 % respectively for leaves, stems and fruits. PLEOs Analysis by GC-MS reveal 22 compounds for all parts representing a total 98,41 %, 98,35 % and 98,05 % for leaves, fruits and stems respectively. The results are shown in Table 1. Regarding, yield is significantly lower than those obtained by Congiu [28] in Italy and Zrira et al [29] in Morocco. The yield of essential oil seems to depend on the nature of the parts of plants used, method of extraction, period of harvest, altitude and climatic conditions.

Our results are consistent with previous studies in terms of quality but not quantitatively. Concerning fruits oil, the main components are α-myrcene (13,89 %), α-pinene (24,12 %), 4-terpineol (4,37 %), verbenol (6,25 %), o cymene (2,28%). On the other hand, the major components present in the stems oil are: limonene (31,54 %), α-myrcene (12,07 %). Our results differ slightly with those reported by Amhamdi et al [26] on the same species from the east of Morocco. However, obtained results are in agreement with those reported by Zrira et al [29] from three regions of Morocco, in this study the major oil components are terpinene-4-ol (43,80%), α-pinene (38,50%), β-myrcene (11.50%), and limonene (9,8%). Nevertheless, they are in agreement with those reported by Bouyahya et *al* [30] because the study was conducted on the same aerial parts. These findings are in accordance with those reported by Amhamdi et *al*., Castola et *al* [26-27]. The comparison of essential oil composition from leaves, stems, fruits of Pl was not previously reported and therefore our study can be considered as the first report on the PLEOs.

3.2. Antioxidant activity

To evaluate the antioxidant properties of PLEOs, three assays were used: FRAP, ABTS and DPPH assays. As listed in Table 2, in comparison to stems and then leaves. In fact, PLFEO IC_{50} values, recorded by DPPH, FRAP and ABTS tests were 2,75, 6,75 and 5,75 mg/mL respectively. These results suggest a high antioxidant activity of the PLEOs since the IC_{50} values obtained are comparable to those of the standard antioxidants quercitine and BHT. Meanwhile, PLSEO presented IC_{50} equivalent to 4,67, 6,24 and 9,16 mg/mL by DPPH, FRAP and ABTS assays. Whereas, PLLEO exhibited a significant antioxidant activity with IC_{50} equal to 5,46, 7,59 and 8,16 mg/mL by DPPH, FRAP and ABTS respectively.

Recently, the investigation of a novel antioxidant biomolecules is a crucial research topic due to their implication in treatment of several diseases. Few studies were conducted on the antioxidant property of *Pistacia* lentiscus essential oils. Our outcomes are in accordance with those described by many studies [30-32]. Another study [33] reported a low antioxidant activity of *Pl* essential oil of leaves, also [34] mentioned that essential oil of *Pl* var Chia did not possess antiradical activity evaluated by the DPPH test. On the other hand, *Pistacia atlantica* essential oil possesses a low capacity of neutralizing free radicals and interesting ferric reducing power [35]. The essential oils contain several classes of terpenes such as; oxygenated monoterpenes, diterpenes, triterpenes and sesquiterpenes. Monoterpenes provide redox properties to essential oils and consequently antioxidant capacities. This activity could be due according to Aissi et *al* [31] to the presence of several bioactive compounds as p cymene and muurolene for DPPH assay, muurolol and cadinol for FRAP assay

3.3 Insecticidal activity

3.3.1 Fumigant and contact toxicity bioassays

The insecticidal effect of PLEOs was evaluated by the fumigant and contact toxicity assays against adults of *Triobolium castaneum*. As mentioned in Tables 3 and 4, the LD_{50} values were 0,77 µL/cm², 0,53 µL/cm² and 0,49 µL/cm² respectively for leaves, stems and fruits. The highest insecticidal activity against this stored pest was recorded by PLFEO, PLSEO and PLLEO. Concerning the fumigant toxicity, LD_{50} values were 176 µL/L air, 107,83 µL/L and 89,16 µL/L respectively for leaves, stems and fruits. On the other hand, LT_{50} reduced as oil concentrations increased which is reported in Table 4 and 5. The lowest LT_{50} 39,20 h, 42,80 h perfectly correspond to the highest

Table 1. Chemical composition of PLEOs from aerial parts

Peak n°	RT (min)	Compounds	PLSEO (%)	PLLEO (%)	PLFEO (%)
1	1,86	Tricyclene	1,45	1,14	1,12
2	2,32	α-thujene	3,11	2,06	2,42
3	2,43	α-pinene	5,16	20,75	24,12
4	2,56	Camphene	3,57	2,54	5,31
5	2,78	α-phellandrene	4,21	3,23	0,66
6	4,17	α-myrcene	10,26	36,18	13,89
7	6,83	o cymene	2,73	2,46	4,89
8	8,12	Terpinene	5,13	3,45	0,28
9	8,92	Limonene	31,54	3,15	0,96
10	9,34	Verbenol	6,23	3,07	6,25
11	10,84	Borneol	0,45	2,15	2,92
12	11,15	Terpinen-4-ol	1,19	4,11	2,14
13	13,3	α-terpineol	1,63	0,78	3,41
14	14,46	Terpineol	5,36	6,49	16,94
15	16,52	Bornyl acetate	4,65	1,87	1,54
16	17,44	Caryophyllene	3,28	0,17	1,64
17	18,85	ç muurolene	0,65	1,23	4,11
18	19,05	Germacrene D	2,81	0,45	0,15
19	21,68	α-muurolene	0,89	0,34	1,36
20	22,84	α-cadinol	1,56	0,47	0,24
21	24,37	p-camphrene	0,14	1,57	1,19
22	26,69	Cadinene	2,35	0,75	2,51
	Total (%)		98,35	98,41	98,05
	Oil yeld (%, w/w)		0,62	0,45	0,78
	Grouped compounds (%)				
	Monoterpene hydrocarbons		68,25	74,96	53,65
	Oxygenated monoterpenes		19,16	19,98	36,14
	Sesquiterpene hydrocarbos		9,38	2,94	8,02
	Oxygenated sesquiterpenes		1,56	0,53	0,24

50 m × 0.25 mm × 1.0 mm; Nd= non determined (p<0.05%). LEO= leaves essential oil, EOF= fruits essential oil, EOS= stems essential oil.

Table 2. The antioxidant capacity of PLEOs expressed in value of IC_{50} (mg/mL).

	Essential oils IC_{50} (mg/mL)			Standard antioxydants IC_{50} (µg/mL)	
	Leaves	Stems	Fruits	Quercetine	BHT
DPPH	5,46±0,12	4,67±0,18*	2,75±0,12	2,10±0,06	4,25±0,09
FRAP	7,59±0,07	6,24±0.05	6,75±0,12	3,65±0,01	3,65±0,01
ABTS	8.16±1.13	9,16±0,03	5,75±0,12	2.57±0.25	3.34±0.05

*Values represent means standard deviations for three replicates

Table 3. LD50 and LD90 values for contact toxicity and fumigant toxicity of PLEOs against adults of *Tc*

Essential oils		LD_{50} (95%CI)	LD_{90} (95%CI)	Slope±S.E	X^2	Df
Contact	Leaves	0,77(0,66-0,87)	1,05(0,91-1,58)	2,43±0,21	1,88	2
Toxicity	Stems	0,53(0,45-0,58)	0,67(0,61-0,85)	3,26±0,01	1,32	2
µL/cm2	Fruits	0,49(0,18-0,61)	0,84(0,69-1,37)	1,25±0,10	0,77	2
Fumigant	Leaves	176(160,34-191,03)	466,24(346,19-588,2)	3,14±0,35	2,59	2
Toxicity	Stems	107,83(74,5-136,66)	179,5(147,66-267,5)	2,16±0,27	1,1	2
µL/cm2	Fruits	89,16(48,83-118,16)	167,16(133,33-284,33)	2.23±0,10	0.10	2

X^2: Chi square test; CI: Confidence intervals at 95%; Df: Degree of freedom

Table 4. LT_{50} and LT_{90} values of PLEOs against *Tc* in contact toxicity.

	Oil concentrations	LT_{50} (CI 95%)	LT_{90} (CI 95%)	Slope±S.E	X^2	Df
	Contact toxicity µL/cm2					
Leaves	0,62	76.00 (37,58-114,41)	136,80 (114,41-160,45)	3,12±0,28	4,26	3
	0,72	69,20 (32,37-106,02)	124,50 (106,02- 141,52)	2,34±1,32	2,8	3
	0,94	48,08 (18,34-77,81)	86,54 (77,81-96,35)	2,57±1,40	3,29	3
	1,11	42,80 (37,58-72,54)	77,04 (72,54- 85,34)	1,89±1,78	2,75	3
Stems	0,47	60,23(36,48-83,52)	108,00 (42,5-132)	3,12±0,28	3,15	3
	0,55	18,15(13,76-22,23)	32,40 (12,43-39,15)	2,34±1,32	2,16	3
	0,62	13,42(11,21-15,64)	24,15(8,11-32,11)	2,57±1,40	4,13	3
	0,71	5,50(1,10-3,32)	9,90 (3,4-12,9)	1,89±1,78	3,65	3
Fruits	0,15	51(24,97-77,02)	91,80 (73.5-110)	3,12±0,28	1,26	3
	0,23	16,66 (12,33-21,23)	29,88(22.43-37,35)	2,34±1,32	3,29	3
	0,31	13,42(11,21-15,64)	24,15(12,43-39,15)	2,57±1,40	4,26	3
	0,39	6,00 (3,67-8,23)	10,81(4,423-13,25)	1,89±1,78	2,63	3

X^2: Chi square test; CI: Confidence intervals at 95%; Df: Degree of freedom. Time mortality values were obtained using Kaplan-Meier estimators (Log-rank method).

Table 5. LT_{50} and LT_{90} values of PLEOs against *Tc* in fumigant toxicity

Oil concentrations µL/L air		LT_{50} (CI 95%)	LT_{90} (CI 95%)	SLOPE	X^2	Df
Fumigant toxicity µL/L air						
Leaves	83,33	96,60(67,58-125)	173,8(125-221,4)	3,43±2,1	4,26	3
	166,66	85,20(52,37-118,12)	153,3(118,1-189,2)	2,24±0,5	2,83	3
	250	53,60(19,34-87,81)	96,4(87,81-106,12)	2,50±1,3	3,29	3
	333,33	46,59(15,52-77,27)	83,8(77,27-143,02)	2,47±0,4	6,75	3
Stems	50	61(56,13-65,86)	109,8(43,4-132,80)	5,53±1,8	5,62	3
	75	51,88(46,31-57,46)	93,33(75,32-111.23)	3,36±0,3	3,45	3
	100	37,28(30,49-44,08)	67,14(29,65-75.12)	2,35±0,0	2,11	3
	125	13,65(9,34-17,95)	24,57(9,21-31,12)	4,49±1,3	4,32	3
Fruits	25	73(71,04-74,96)	131,4(106,12-157,1)	3,86±1,2	3,84	3
	50	54,57(51,06-58,07)	98,22(69,63-126.83)	4,30±2,6	5,69	3
	75	39,74(35,89-43,60)	71,53(69,21-73,72)	2,44±0,4	1,79	3
	100	14,66(11,36-17,96)	29,38(21,19-37.50)	4,35±1,7	4,58	3

X^2: Chi square test; Df: Degree of freedom; CI: Confidence intervals at 95%, Time mortality values were obtained using Kaplan-Meier estimators (Log-rank method).

concentrations 1.10 µL/ cm^2 and 333.33 µL/ L in contact and fumigant toxicity tests respectively.

The obtained outcomes demonstrate that PLEO exerts a significant insecticidal effect. These findings are in accordance with previous study [15]. Few studies [2-15-16], demonstrated the insecticidal effect of *Pl* against *Tc*. However, in other studies [36-37] *Tc* is a victim model for a large spectrum of several essential oils such as *Artemisia vulgaris* and *Artemisia tridentate* [36]. In addition, Ebadollahi et *al* [37] reported an important insecticidal effect of *Lavandula stoechas* essential oil and suggested that it may be used as bio-insecticide against red flour beetle. Furthermore, PLEO possesses an extensive insecticidal effect when combined to some essential oils especially, *Mentha microphylla* and *Myrtus communis* against *Culex pipiens*. The fumigant and contact toxicity could be explained according to Regnault et *al* [38] to oxygenated monoterpenes (e.g. terpineol, linalool) which induce mortality by inhibition of acetylcholine-esterase.

3.3.2. Behavioral locomotor responses and population growth bioassay

The locomotor behavior of insects on the arenas half treated or untreated by essential oils is shown in Fig. 1. The results indicate that insects spending more time in the untreated half of arena. Statically, there is a significant difference between the untreated and treated arena in stems LD_{50} (F=1,25; p<0,01; t-test), as well as between untreated and treated halves of arena

Table 6. Regression analysis of the curves presented in Figure 2

Variable	Oil	Model	Estimated parametrs±S.E		F	p	R^2
			a	b			
	Leaves	y = a·exp (−bx)	0,04±0,001	0,006±0,005	44,26	<0,001	0,98
ri	Stems	y = a·exp (−bx)	0,02±0,008	0,009±0,001	20,15	<0,001	0,97
	Fruits	y = a·exp (−bx)	0,05±0,003	0,004±0,006	32,15	<0,001	0,99

for LD_{50} of fruits (F=2, 65; p<0,001; t-test). However, no significant difference was observed between the LD_{50} of essential oil from leaves.

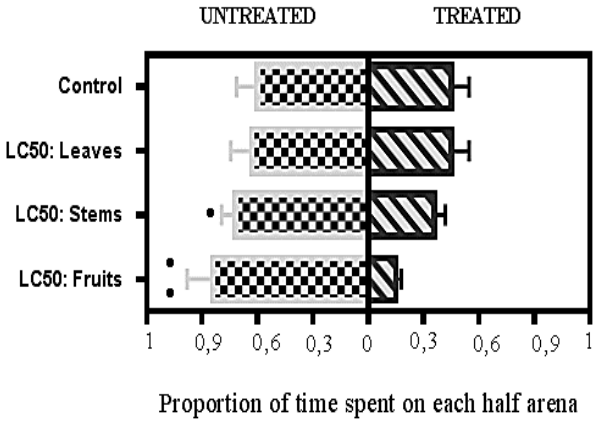

Figure 1. Proportion of time spent by *Tc* in the untreated and treated half arena with PLEOs. The mean of 20 repetitions is shown by each histogram bar. The points show significant differences between the arena halves that was treated with PLEOs and those untreated (paired t-test p<0, 05).

In this investigation, we have clearly demonstrated that the essential oils have a neurotoxic effect immediately after their application, which is manifested by the appearance of certain symptoms as paralysis, hyperactivity and trembling. These outcomes are in agreement with those reported by Zhu et *al* [39]. The physiological effect of PLEOs, could be related to the presence of monoterpenoids (terpineol, myrcene, and D-limonene) acting by disrupting the aminergic [40], and GABAergic [41] transmission as well as their inhibition on acetylcholine esterase in the insect nervous system is one of their possible mechanisms as demonstrated in previous studies [40-41]. These results suggest that PLEOs have effective insecticide potential against *Tc*.

The second figure represents the effect of PLEOs on the instantaneous rate of development. The significant differences were noted between the oil doses (F=98,21; p<0,001) and oil types (F=104,51; p<0,001), the instantaneous rate of development declines by increasing concentration. However, the insects treated by essential oil of leaves were less affected, while the other essential oils (stems and fruits) affects the insects considerably. All of the adjustment parameters for the curves presented in Fig. 2 are illustrated in Table 6.

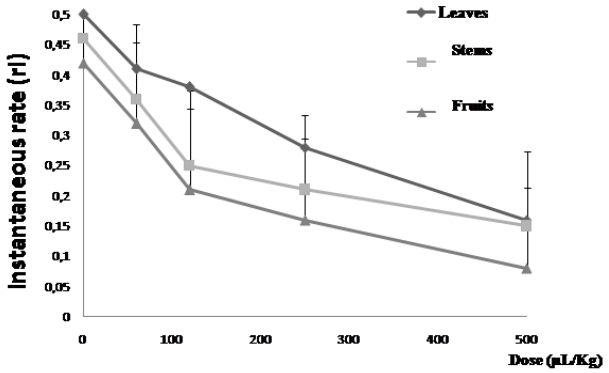

Figure 2. Instantaneous rate of development of *Tc* exposed to different essential oils doses of *Pl*.

4. Conclusions

The present study demonstrates that PLEOs have quantitative differences in chemical compounds between leaves, fruits and stems. The outcomes of this investigation revealed several volatile compounds in PLEO that were very effective against *Tc*. Therefore, the fruits exhibited a higher insecticidal activity followed by stems then leaves. Regarding the antioxidant potential, the PLEOs possess an interesting antioxidant capacity. This was validated by DPPH, FRAP and ABTS assays. As a conclusion, PLEOs could be used as a powerful antioxidant and eco-friendly solution to control *Tc* in stored grains.

Abbreviations

PLEOs: *Pistacia lentiscus* essential oils
DPPH: Diphenyl-1-picrylhydrazyl
FRAP: ferric reducing ability power
LD_{50}: Lethal dose that kills 50%
LD_{90}: Lethal dose that kills 90%
LT_{50}: Time required killing 50
LT_{90}: Time required killing 90
PLFEO: *Pistacia lentiscus* fruits essential oil

PLSEO: *Pistacia lentiscus* stems essential oil
PLLEO: *Pistacia lentiscus* leaves essential oil
Pl: Pistacia lentiscus
Tc: Tribolium castaneum

Authors' contributions

Methodology, writing original draft preparation, C.T.; Conceptualization writing-review, M.B.; Essential oils analysis and compounds identification, S.M.; Data analysis and acquisition, M.A.; Validation, writing review & editing, R.A.; Methodology, Validation, Writing original draft preparation & editing, A.C.

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Conflicts of interest

There are no conflicts of interest declared by the authors.

References

1. Babu, R.M.; Sajeena, A.; Seetharaman, K.; Reddy, M.S. Advances in genetically engineered (transgenic) plants in pest management—an over view. J. Crop Prot. 2003, 22, 1071–1086.
2. Upadhyay, N.; Dwivedy, A K.; Kumar, M.; Prakash, B.; Dubey, N.K. Essential oils as eco-friendly alternatives to synthetic pesticides for the control of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Essent. Oil-Bear. Plants. 2018, 21, 282–297.
3. Kumar, P.; Mishra, S.; Malik, A.; Satya, S. Repellent, larvicidal and pupicidal properties of essential oils and their formulations against the housefly, Musca domestica. Med. Vet. Entomol. 2011, 25, 302–310.
4. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils–a review. Food Chem. Toxicol. 2008, 46, 446–475.
5. Priestley, C.M.; Williamson, E.M.; Wafford, K.A.; Sattelle, D.B. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABAA receptors and a homo-oligomeric GABA receptor from Drosophila melanogaster. Br. J. Pharmacol. 2003, 140, 1363–1372.
6. Enan, E.E. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Arch. Insect Biochem. 2005, 59, 161–171.
7. López, M.D. ; Pascual-Villalobos, M.J. Mode of inhibition of acetylcholine-esterase by monoterpenoids and implications for pest control. Ind. Crops Prod. 2010, 31, 284–288.
8. Bouyahya, A.; Abrini, J.; Et-Touys, A.; Bakri, Y.; Dakka, N. Indigenous knowledge of the use of medicinal plants in the North-West of Morocco and their biological activities. Eur. J. Integr. Med. 2017, 13, 9–25.
9. Jamila, F.; Mostafa, E. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. J. Ethnopharmacol. 2014, 154, 76–87.
10. Bozorgi, M.; Memariani, Z.; Mobli, M.; Salehi Surmaghi, M.H.; Shams-Ardekani, M.R.; Rahimi, R. Five Pistacia species (*P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci. World J. 2013.
11. Maxia, A.; Sanna, C.; Frau, M.A.; Piras, A.; Karchuli, M.S.; Kasture, V. Anti-inflammatory activity of *Pistacia lentiscus* essential oil: involvement of IL-6 and TNF-α. Nat. Prod. Commun. 2011, 6(10), 1934578X1100601033.
12. Giaginis, C.; Theocharis, S. Current evidence on the anticancer potential of Chios mastic gum. Nutr Cancer. 2011, 63, 1174–1184.
13. Mharti, F.Z.; Lyoussi, B.; Abdellaoui, A. Antibacterial activity of the essential oils of *Pistacia lentiscus* used in Moroccan folkloric medicine. Nat. Prod. Commun. 2011, 6, 1934578X1100601024.
14. Catalani, S.; Palma, F.; Battistelli, S.; Benedetti, S. Oxidative stress and apoptosis induction in human thyroid carcinoma cells exposed to the essential oil from *Pistacia lentiscus* aerial parts. PloS one, 2017, 12, e0172138.
15. Bachrouch, O.; Jemâa, J.M.B.; Chaieb, I.; Talou, T.; Marzouk, B.; Abderraba, M. Insecticidal activity of Pistacia lentiscus essential oil on Tribolium castaneum as alternative to chemical control in storage. Tunis. J. Plant Prot. 2010, 5, 63–70.
16. Bougherra, H.H.; Bedini, S.; Flamini, G.; Cosci, F.; Belhamel, K.; Conti, B. Pistacia lentiscus essential oil has repellent effect against three major insect pests of pasta. Ind Crops Prod. 2015, 63, 249-255.
17. Şahin, F.; Güllüce, M.; Daferera, D.; Sökmen, A.; Sökmen, M.; Polissiou, M.; Özer, H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food control. 2004, 15(7), 549-557.
18. Oyaizu, M. Studies on products of browning reaction anti-oxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet. 1986, 44, 307–315.
19. Kasrati, A.; Alaoui Jamali, C.; Bekkouche, K.; Wohlmuth, H.; Leach, D.; Abbad, A. Comparative evaluation of antioxidant and insecticidal properties of

essential oils from five Moroccan aromatic herbs. J. Food Sci. Technol. 2015, 52, 2312-2319.
20. Walthall, W.K.; Stark, J.D. Comparison of two population-level ecotoxicological endpoints: The intrinsic (rm) and instantaneous (ri) rates of increase. Environ. Toxicol. Chem. 1997, 16, 1068–1073.
21. Braga, L.S.; Correa, A.S.; Pereira, E.J.G.; Guedes, R.N.C. Face or flee? Fenitrothion resistance and behavioral response in populations of the maize weevil, Sitophilus zeamais. J. Stored Prod. Res. 2011, 47, 161–167.
22. Haddi, K.; Mendonça, L.P.; dos Santos, M.F.; Guedes, R.N.C.; Oliveira, E.E. Metabolic and behavioral mechanisms of indoxacarb resistance in *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 2015, 108, 362–369.
23. Corrêa, A.S.; Pereira, E.J.G.; Cordeiro, E.M.G.; Braga, L.S.; Guedes, R.N.C. Insecticide resistance, mixture potentiation and fitness in populations of the maize weevil (*Sitophilus zeamais*). J. Crop Prot. 2011, 30, 1655–1666.
24. Pereira, C.J.; Pereira, E.J.G.; Cordeiro, E.M.G.; Della Lucia, T.M.C.; Tótola, M.R.; Guedes, R.N.C. Organophosphate resistance in the maize weevil *Sitophilus zeamais*: magnitude and behavior. J. Crop Prot. 2009, 28(2), 168-173.
25. Guedes, N.M.P.; Guedes, R.N.C.; Ferreira, G.H.; Silva, L.B. Flight take-off and walking behavior of insecticide-susceptible and–resistant strains oil of *Sitophilus zeamais* exposed to deltamethrin. Bull. Entomol. Res. 2009, 99, 393–400.
26. Amhamdi, H.; Aouinti, F.; Wathelet, J.P.; Elbachiri, A. Chemical composition of the essential of *Pistacia lentiscus* L. from Eastern Morocco. Rec. Nat. Prod. 2009, 3(2).
27. Castola, V.; Bighelli, A.; Casanova, J. Intraspecific chemical variability of the essential oil of *Pistacia lentiscus* L. from Corsica. Biochem. Syst. Ecol. 2000, 28, 79–88.
28. Congiu, R.; Falconieri, D.; Marongiu, B.; Piras, A.; Porcedda, S. Extraction and isolation of *Pistacia lentiscus* L. essential oil by supercritical CO2. Flavour Fragr. J. 2002, 17, 239–244.
29. Zrira, S.; Elamrani, A.; Benjilali, B. Chemical composition of the essential oil of *Pistacia lentiscus* L. from Morocco a seasonal variation. Flavour Fragr. J. 2003, 18, 475–480.
30. Bouyahya, A.; Assemian, I.C.C.; Mouzount, H.; Bourais, I.; Et-Touys, A.; Fellah, H.; Bakri, Y. Could volatile compounds from leaves and fruits of *Pistacia lentiscus* constitute a novel source of anticancer, antioxidant, antiparasitic and antibacterial drugs? Ind Crops. Prod. 2019, 128, 62-69.
31. Aissi, O.; Boussaid, M.; Messaoud, C. Essential oil composition in natural populations of *Pistacia lentiscus* L. From Tunisia: Effect of ecological factors and incidence on antioxidant and anti-acetylcholine-esterase activities. Ind. Crops Prod. 2016, 91, 56–65.
32. Yosr, Z.; Imen, B.H.Y.; Rym, J.; Chokri, M.; Mohamed, B. Sex-related differences in essential oil composition, phenol contents and antioxidant activity of aerial parts in *Pistacia lentiscus* L. during seasons. Ind. Crops Prod. 2018, 121, 151–159.
33. Barra, A.; Coroneo, V.; Dessi, S.; Cabras, P.; Angioni, A. Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus* L. from different origins and its antifungal and antioxidant activity. J. Agric. Food Chem. 2007, 55, 7093–7098.
34. Bampouli, A.; Kyriakopoulou, K.; Papaefstathiou, G.; Louli, V.; Krokida, M.; Magoulas, K. Comparison of different extraction methods of Pistacia lentiscus var. chia leaves: Yield, antioxidant activity and essential oil chemical composition. J. Appl. Res. Med. Arom. Plants. 2014, 1(3), 81-91.
35. Gourine, N.; Yousfi, M.; Bombarda, I.; Nadjemi, B.; Stocker, P.; Gaydou, E. M. Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria. Ind. Crops Prod. 2010, 31(2), 203-208.
36. Wang, D.; Collins, P. J.; Gao, X. Optimising indoor phosphine fumigation of paddy rice bag-stacks under sheeting for control of resistant insects. J. Stored Prod. Res. 2006, 42, 207–217.
37. Ebadollahi, A.; Safaralizadeh, M.; Pourmirza, A. Fumigant toxicity of *Lavandula stoechas* L. oil against three insect pests attacking stored products. J. Plant Prot. Res. 2010, 50(1).
38. Regnault-Roger, C.; Hamraoui, A. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res. 1995, 31, 291–299.
39. Zhu, B.C.R.; Henderson, G.; Yu, Y.; Laine, R.A. Toxicity and repellency of patchouli oil and patchouli alcohol against Formosan subterranean termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J. Agric. Food Chem. 2003, 51, 4585–4588.
40. Kostyukovsky, M.; Rafaeli, A.; Gileadi, C.; Demchenko, N.; Shaaya, E. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest Manag. Sci. 2002, 58, 1101–1106.
41. Tong, F.; Coats, J.R. Quantitative structure–activity relationships of monoterpenoid binding activities to the housefly GABA receptor. Pest Manag. Sci. 2012, 68, 1122–1129.