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Chemical constituents and biological activities of two Citrus species essential oils and aqueous extracts compared to basalt "Farina di Basalto"

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Keywords: Essential oils, extracts, antioxidant, insecticidal, repellent, phytotoxic, basalt

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Chemical constituents and biological activities of two *Citrus* species essential oils and aqueous extracts compared to basalt “Farina di Basalto®”

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ABSTRACT

This work aimed to evaluate the phytochemical composition of *C. aurantium* and *C.sinensis* essential oils and aqueous extracts and their antioxidant, insecticidal, repellent and phytotoxic potential. The chemical composition of essential oils, isolated by hydrodistillation, was analyzed by gas chromatography–mass spectrometry (GC-MS). High percentages of monoterpenes hydrocarbons was revealed (93.53 and 98%). Limonene was identified as main compound (88.77 and 95.12% for *C. sinensis* and *C. aurantium*, respectively) in the two analysed species. Nevertheless, *C. sinensis* essential oil was distinguished by the presence of Linalool (5.63%). Aqueous extracts showed considerable contents of total phenols (27.54 and 46.89 mgEGA/mg DW), total flavonoids (7.47-10.18 mg EQ/gDW), and condensed tannins (3.7 and 4.46 mg EC/gDW). The antioxidant capacity determined by 1,1-diphenyl-1-picrylhydrazyl (DPPH) revealed that aqueous extracts exhibited the best activity (IC_{50}= 33.8 µg/mL and 57.41 µg/mL) which was correlated to their phenolic contents. The insecticidal and repellent activities were also evaluated against insect pest of stored wheat, *Tribolium castaneum* (Coleoptera, Tenebrionidae). The Basalt, “Farina di Basalto® ”, which is a volcanic rock and a natural fertilizer rich in nutriments was also used for this test. Significantly higher insecticidal activity of *C.aurantium* essential oil (LC_{90}= 38.173 mg/ml) and basalt (LC_{90}=56.703 mg/ml) were recorded when compared to aqueous extracts. The best repellent capacity was revealed for *C. aurantium* essential oil. The present findings revealed also that basalt powder showed an important germination rate, and stimulated radicle and hypocotyl length.

Keywords: Essential oils, extracts, antioxidant, insecticidal, repellent, phytotoxic, basalt.

INTRODUCTION

Pest control sector has been largely dominated by synthetic pesticide products, which pose potential risks to public health and the environment. Their prolonged use has disrupted natural biological systems and frequently led to the development of resistance (El-Akhal et al., 2015). *Tribolium* *castaneum* Herbst (Coleoptera: Tenebrionidae) is the most damaging beetle species destroying stored products. Their management are difficult because they are developing resistance to insecticide (Bachrouch et al., 2023). In view of this situation, there is a critical need to explore alternative methods for managing agricultural pests, such as botanical extracts, known for their generally more environmentally friendly characteristics. Also, a number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively used in food industry, although their use has begun to be questioned because of their toxicity. Therefore, the development and use of more effective insecticidal and antioxidants obtained from botanical sources especially, are desired (Scalbert et al., 2005).

Recently, with increasing concern to eco-friendly product, plant essential oils and extracts are getting renewed interest. These natural alternatives are not only effective but also considered safer and more environmentally friendly compared to synthetic counter parts. Several essential oils, including those derived from Citrus species, have been designated as "GRAS" (Generally Recognized as Safe) by the US Food and Drug Administration, underscoring their favorable safety profiles. Consequently, studies on the biological activities of *Citrus* essential oils are increasing (Manzur et al., 2005; Khanikor et al., 2021).

The genus *Citrus* (*Rutaceae*) is one of the ancient, most traded, and most popular crops. It is widely grown in the tropical and subtropical areas of the world. On the other hand the genus *Citrus*, which includes several important fruits such as oranges, mandarins, limes, and lemons, is the largest fruit crop in the world (Velázquez-Nuñez et al., 2013). There are 33 recorded species of *Citrus* worldwide with many recorded and unexplored varieties present in different parts of the world (Khanikor et al., 2021). *Citrus* essential oils and extracts are rich sources of useful phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds (Zhao et al., 2012). Linalool, linalyl acetate, terpineol, and limonene are major active components of *Citrus* essential oils and hydrosol which have different bioactive properties (Değirmenci et al., 2020). Among the main phenolic compounds found in orange juice is hesperidin and narirutin, followed by naringin and didymin (Roussos et al., 2016). The *Citrus* genus marks its presence in daily life in the fields of traditional medicine, cosmetics, food and perfumery industry.

Citrus aurantium L. (bitter orange or sour orange) is an ever green tree of 2–2.5 m high, having white perfumed flowers and orange fruits, cultivated in tropical and subtropical zones. Many products are made from bitter orange flower: the essential oil, the absolute, and the hydrosol. Composition of essential oils from bitter orange is not the same and it depends, to a large extent, on the geographic origin and parts of the plant (zest, fruit, and flowers) (Değirmenci and Erkurt., 2020). It is known for its antimicrobial, antioxidant, cytotoxic, anxiolytic, antidiabetic, anti-obesity, gastroprotective and ulcer healing actions, potent fumigant, anti-cholinesterase and anti-inflammatory activities (Badalamenti et al., 2022).

Citrus sinensis (L.) Osbeck (sweet orange) is an evergreen tree or shrub (3–15m). The tree has a rounded, spherical, compact canopy with regular branches, and angled twigs when young. Thorns are generally present, especially when the tree is in its juvenile phase (Swingle eand Reece., 1967). Sweet orange essential oil was reported to inhibit the growth of several bacteria and fungal species, larvicidal activity against the malaria vector, effective anthelmintic agent against gastrointestinal nematodes, potent fumigant against house flies, and mosquitoes (Kammoun et al., 2021).

Thus, this work aims to determine and to compare essential oils compositions and phenolic contents of *C.aurantium* and *C. sinensis* peels cultivated in Tunisia. Moreover, we attempt to determine their antioxidant, insecticidal, repellent and phytotoxic capacities.

2 MATERIAL AND METHODS

2.1 Plant material

C. aurantium and *C. sinensis* were collected from Mograne region (Altitude: 158m-262m, Latitude : 36°23-36°25’, Longitude : 10°7-10°10’) in Tunisia. Peels were than air dried at room temperature for two weeks and then finely powdered.

2.2 Essential oil extraction and GC-MS analysis

The essential oil extraction was obtained from 100 g of powdered air dried peels by steam distillation during 3h using a Clevenger type apparatus. The essential oil was collected and dried over a small amount of anhydrous sodium sulphate. The yields were calculated as the quantity of the essential oil compared to the air-dried material (% w/w).

GC-MS analyses were conducted using an Agilent 7890A gas chromatograph equipped with a HP-5MS capillary column (30 m length, 0.25 mm diameter, 0.25 µm film thickness) coupled to an Agilent 5975C mass selective detector (MSD). Helium was employed as the carrier gas at a flow rate of 0.8 ml/min. The oven temperature was ramped from 60°C to 240°C at a rate of 4°C/min. The injector temperature was maintained at 250°C. The quadrupole and ion source temperatures were set to 150°C and 230°C, respectively. Mass spectra were scanned from 50 to 550 m/z at 70 electron volts (eV). Identification of components in the essential oils was achieved by comparing their retention times with those of authentic standards analyzed under identical chromatographic conditions, by comparing their retention indices with literature values, and by co-injecting the essential oils with available authentic standards. Furthermore, identification was confirmed by comparing the mass spectra of terpenic compounds with those stored in the W8N08 and NIST08 libraries.

2.3 Preparation of plant extracts

1g of dried peels were extracted with 10 mL of water at ambient temperature for 24 h. The samples were filtered and stored at +4 °C for general analysis.

2.4 Total phenolic, flavonoid and condensed tannins content

The total phenolic content was determined using the Folin–Ciocalteu colorimetric assay as described by Chetoui et al. (2013). A volume of 0.5 mL of diluted sample was mixed with 2 mL of Folin-Ciocalteu reagent, followed by the addition of 2.5 mL of sodium carbonate solution (7.5%). After incubating for 90 minutes, in the dark, the absorbance was measured at 760 nm. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried plant weight (mg GAE/g DW).

Estimation of total flavonoid content values was revealed with the aluminum chloride (AlCl3) colorimetric method as described before by Chetoui et al. (2013). 1mL of each diluted sample was mixed with 1 mL of AlCl3 solution (2%), followed by the reading of absorbance at 430 nm after 15 min of incubation. The total flavonoid content was expressed as mg quercetin equivalents per g of plant dried weight (mg QE/gDW).

Condensed tannins estimation, as catechin equivalent, was evaluated using the vanillin-HCl method (Sun et al., 1998) with slight modifications. 500 mL of extract was added to 1.5 mL of 4 % methanol vanillin solution and 1.5 mL of concentrated HCl. The absorbance was measured at 500 nm, after 15min of incubation in dark at room temperature. Total condensed tannins was expressed as mg catechin equivalent per g of dried weight (mg CE/gDW). The analyses of total phenolic, flavonoid, and condensed tannins content were performed in triplicate.

2.5 Antioxidant activity

The antioxidant capacity was determined using the free radical scavenging method (DPPH; 1,1-diphenyl-2-picryl-hydrazil radical (DPPH•), as described by Jaouadi et al. (2023). Briefly, DPPH radical scavenging was determined from 1 mL of essential oil/ extract (at different concentrations) mixed with 3ml of a freshly prepared methanol solution of DPPH (4.10-2 mM). The mixture was allowed to stand in dark at room temperature for 30 min. Then the absorbance values were measured at 517 nm. Results were expressed as IC_{50} (concentration required to inhibit 50% of DPPH•).

2.6 Insecticidal activity against *Tribolium castaneum*

2.6.1 Insects rearing

The adults *Tribolium castaneum* were collected from the infested wheat kept at the Laboratory of Entomology at the High School of Agriculture of Mograne.

2.6.2 Contact bioassay

Filter paper disc (9 cm) was placed in a Petri dish and soaked with different concentrations of essential oils diluted in acetone (1, 5, 10 and 25mg/mL) and aqueous extracts (25, 50 and 100mg/mL). A group of 10 adults of *T. castaneum* were placed in each Petri dish. After 24 hours, the number of dead insects was recorded. Acetone and water were used as a negative control. All Petri dishes were stored in a climate room at 25±1°C, 60- 70% Relative Humidity, and a photoperiod of 16:8 (L:D) h. Mortality rates of different treatments were estimated and corrected using the Abbott's formula (Abott., 1925). Three doses (1.5, 3 and 5%) were also applied with Basalt, "Farina di Basalto®" (micronized by Basalti Orvieto srl). Basalt is an effusive volcanic rock family characterized by a predominantly mafic chemical composition.

There are various types of basalt, each with unique characteristics related to their composition, structure and origin. Based on its chemical composition, mineralogy and physical characteristics, this basalt used for the production of Farina di Basalto® (FdB) can be classified as phonolitic tephritic basalt and has unique characteristics. The Basalt used for the production of Farina di Basalto® is particularly prized for its unique composition with micro and lean elements that are useful for plants. Indeed, it contains natural mineral elements, such as Silicon oxide (SiO2) 47%, Aluminum oxide (Al2O3) 21%, Potassium oxide (K2O) 9%, Iron oxide (Fe2O3) 6.85%, Calcium oxide (CaO) 8%, Magnesium oxide (MgO) 2.25%, Sodium oxide (Na2O) 3.55%, Phosphorus pentoxide (P2O5) 0.65%, Titanium dioxide (TiO2) 0.6%, Manganese (Mn) 636 mg/Kg, Sulfur (S) 536 mg/Kg, Boron (B) 81 mg/Kg, Copper (Cu) 51 mg/Kg and Zinc (Zn) 68mg/Kg (Elimem et al., 2021).

2.6.3 Repellent activity

Essential oils and aqueous extracts were evaluated for their repellent capacity using the area preference method (Zhang et al., 2022). Filter paper (6cm in diameter) was cut in half and 500µl of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 µl of acetone or water. The numbers of insects present on different sides of the paper were recorded after 1,3, 5 and 24h.

2.7 Phytotoxic activity

The phytotoxic activity was evaluated on germination, radical and hypocotyl elongation of Triticum aestivum L. Seeds were surface sterilized with diluted sodium hypochlorite for 15 min, then rinsed four times with deionized water. 2 mL of different doses of essential oils (0.1, 0.3, 0.6 and 1 mg/mL), aqueous extracts (2.5,5 ,10 , 20 mg/mL) and Farina di Basalto® type XF (1.5, 3, 5 and 10% of the weight of the sampled seeds) were dispersed in sterile Petri dishes (9 cm diameter) lined with double-sterile filter paper. 20 seeds from each target species were arranged in each Petri dish and stored in the dark at 25 ◦C for 7 days. Distilled water was used as a control. All phytotoxic assays were conducted in triplicate. After 7 days, the germination percentage was determined.

At the end of the experiment, final germination rate (GR) was calculated according to the formula cited by El Rasafi et al. (2016). GR= Germinated seeds x 100/ Total seeds

Hypocotyl and radicle length were measured after 7 days, using a digital calliper.

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4.7 Statistical analysis

The analysis of variance (ANOVA procedure) followed by Duncan's multiple range tests (SPSS software version 26.0 for Windows) wad used to determine the variation in essential oil compositions and phenol contents and their biological activities. Correlations among data obtained were calculated using Spearman coefficient (r-Spearman) (at $p < 0.001$). For the insecticidal activity, bioassay data were obtained using the Probit analysis to find out LT_{90} values.

3 RESULTS

3.1 Yields and chemical composition of the essential oils

C. aurantium peels revealed the highest essential oil yield (3.77%). Selected species were found to be rich in monoterpenes hydrocarbons (93.53 and 98%). Sesquiterpenes hydrocarbons (0.34 and 0.44%) were observed in lower levels (Table 1, Figure 1). *C. sinensis* was distinguished by the presence of oxygenated monoterpenes (5.99%).

Table 1. Mean percentage of *C. aurantium* and *C. sinensis* essential oils compounds.

Compounds	**RIa**	**KI**	***C.aurantium***	***C.sinensis***
Yield (%)			3.77	2.69
α-pinene	933	939	0.85	1.63
Sabinene	972	975	0.11	1.30
β-pinene	976	979	0.07	-
β-myrcene	989	990	1.85	1.83
Limonene	1041	1039	95.12	88.77
Linalool	1101	1095	-	5.63
β-fenchol	1112	1115	-	0.36
β-caryophyllene	1411	1419	0.25	-
Germacrene-D	1474	1480	0.09	-
Valencene	1486	1496	-	0.44
Total identified (%)			98.34	99.96
Monoterpenes hydrocarbons (%)			98.00	93.53
Oxygenated monoterpenes (%)			-	5.99
Sesquiterpenes hydrocarbons (%)			0.34	0.44

Means in each column followed by different letters are significantly different ($p < 0.05$).
RI: Retention indices relative to n-alkans on HP-5MS column.

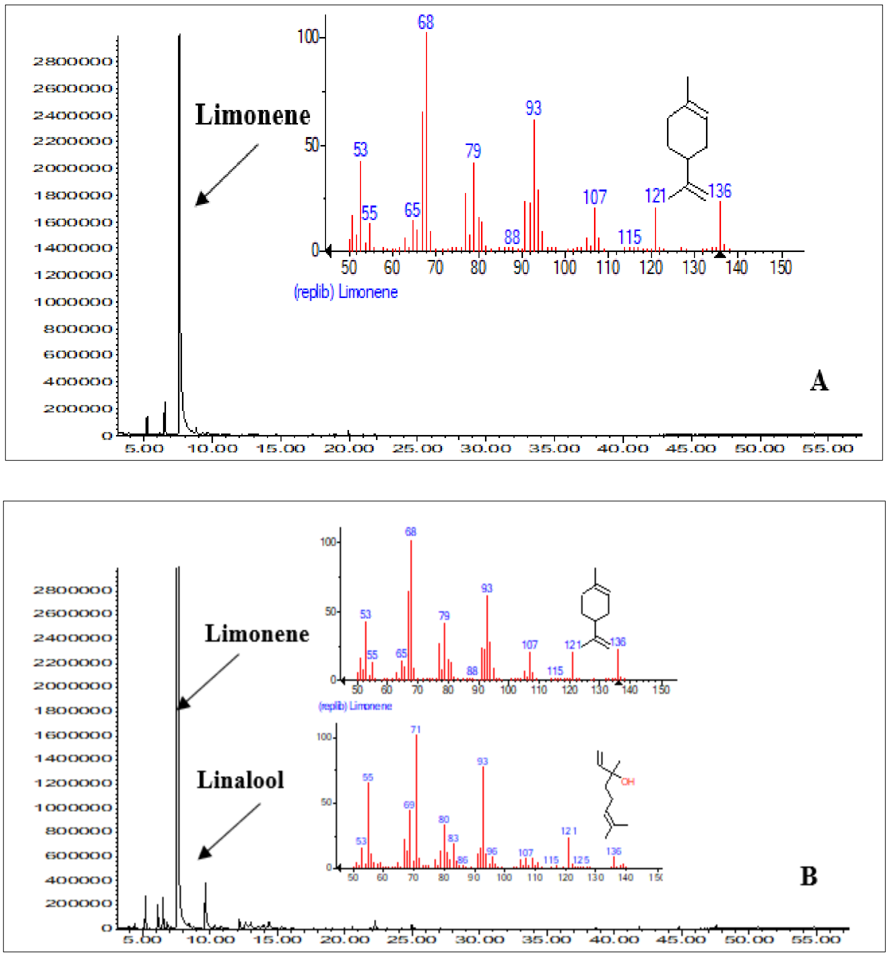

Figure 1. Chromatograms of *C. aurantium* (A) and *C. sinensis* (B) peels essential oils.

Chemical composition analysis of *C.aurantium* essential oil showed the existence of seven different compounds representing 98.34% of the total oil. The main monoterpene was limonene (95.12 %), followed by β-myrcene (1.85%), α-pinene (0.85%), Sabinene (0.11) and β-pinene (0.07) (Table 1, Figure 2). Eight compounds were identified accounting for 99.96 % of the total oil composition of *C.sinensis*. Limonene (88.7%) was also the major component. On the other hand, this essential oil was distinguished by the presence of Linalool (5.63%) and Valencene (0.64%).

3.2 Phenolic contents and antioxidant activity of *C. aurantium* and *C. sinensis* aqueous extracts

The total phenolic, flavonoid, and tannin contents varied significantly among tested aqueous extracts (Table 2). The highest amount of total phenolic content was revealed for *C.aurantium* extracts (46.89 mgEGA/mg DW). The highest flavonoid content was noted for *C.aurantium* sample (10.18 mgEQ/gDW). Regarding condensed tannins, the highest value was observed for *C.sinensis* extracts (4.46 mg EC/gDW).

Table 2. Total phenolics, flavonoids and tannins content and antioxidant activity of *C. aurantium* and *C. sinensis* aqueous extracts and essential oils (EO).

	Sample	**Total polyphenols (mgEGA/mg DW)**	**Flavonoids (mg EQ/gDW)**	**Condensed tanins (mg EC/gDW)**	**DPPH IC50 (µg/ml)**
C.aurantium	EO	-	-	-	580.4± 3,2a
	AE	46.89 ± 1.6^a	10.18 ± 0.21^a	3.7 ± 0.19^a	33.8± 1,4^d
C.sinensis	EO	-	-	-	600.3± 3,2^b
	AE	27.54 ± 1.22^b	7.47 ± 0.01^b	4.46 ± 0.09^b	57.41± 3,2^c
Standard	TROLOX	-	-	-	23.6± 0,8^e

Means in each column followed by different letters are significantly different ($p < 0.05$).

The antioxidant properties of tested essential oils and aqueous extracts was assessed by measuring the free radical scavenging activity (DPPH) (Table 2). This activity is expressed by the antioxidant concentration required for a 50 % DPPH· reduction (IC_{50}). Values differed significantly among samples. Tested essential oils and extracts were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H. The highest activity was revealed for *C. aurantium* and *C.sinensis* aqueous extracts (IC_{50}= 33.8 µg/mL and 57.41 µg/mL, respectively). The lowest effective activity was noted for C. aurantium followed by *C. sinensis* aqueous extracts (IC_{50}= 580.4 and 600.3 µg/mL, respectively).

3.3 Insecticidal activity

3.3.1 Contact toxicity

Tested essential oils and aqueous extracts were evaluated for their insecticidal effect against the red flour beetle, *T. castanaeum*. The degree of activity of tested samples varied according to the dose used and the duration of the treatment (Figures 2, 3). In all cases, significant differences were observed in mortality of *T.castaneum* exposed to essential oils, aqueous extracts and Farina di Basalto® (FdB). The mortality increased with increasing concentrations and exposure time. Results revealed that percent of mortality in control petri dishes were very low during the six first days of observation and they ranged between 0 and 10 %. Significantly higher insecticidal activity of *C.aurantium*, *C. sinensis* essential oils and “Farina di Basalto® ” (LC_{90}= 38.173, 49.369 and 56.703mg/ml, respectively) was recorded when compared to aqueous extracts (Table 3).

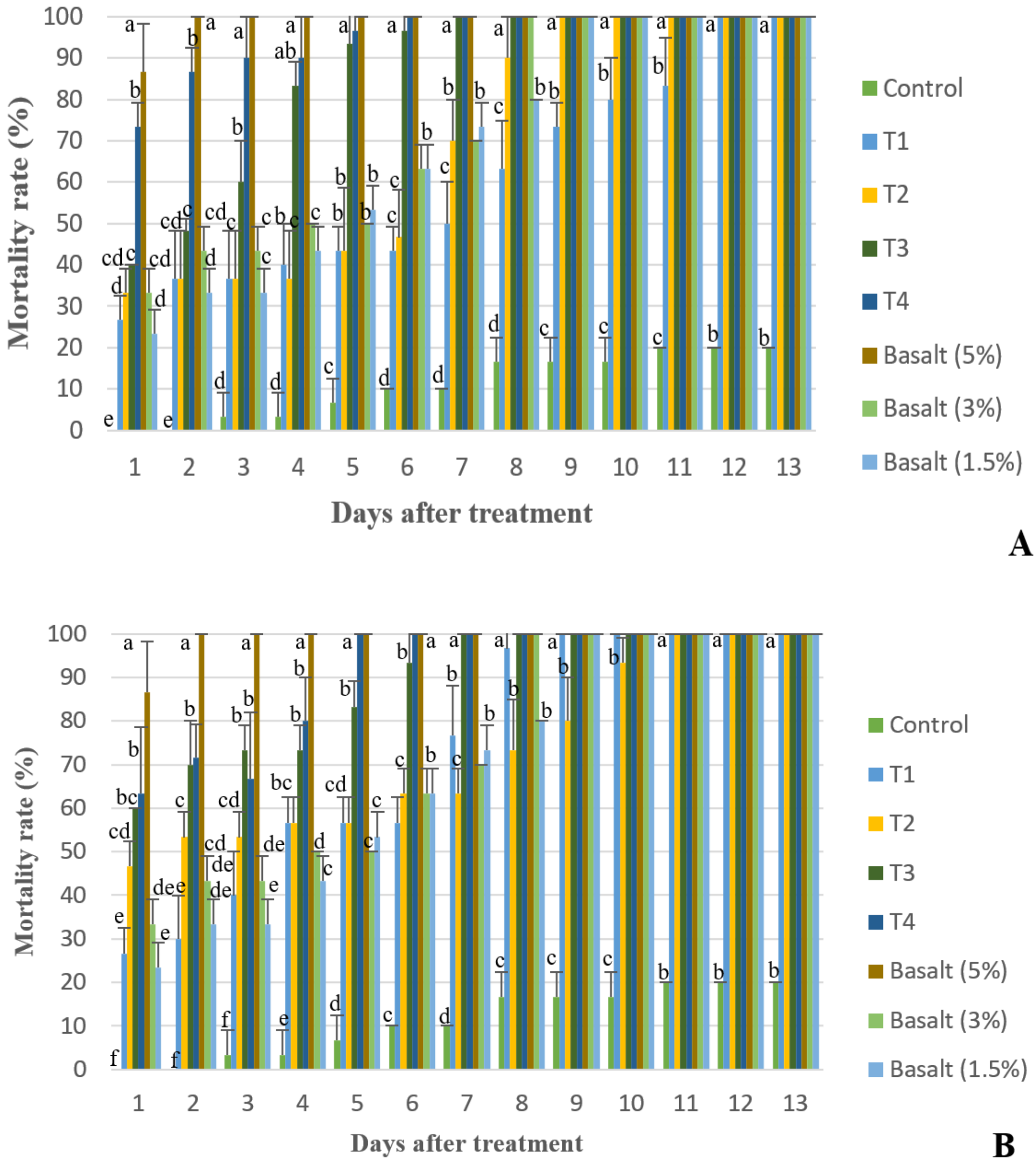

Figure 2. Effect of *C. aurantium* (A), *C. sinensis* (B) essential oils and Farina di Basalto® (FdB) on *T. castaneum.* (T1 : 1 mg/mL, T2 : 5 mg/mL, T3 : 10 mg/mL, T4 : 25 mg/mL).

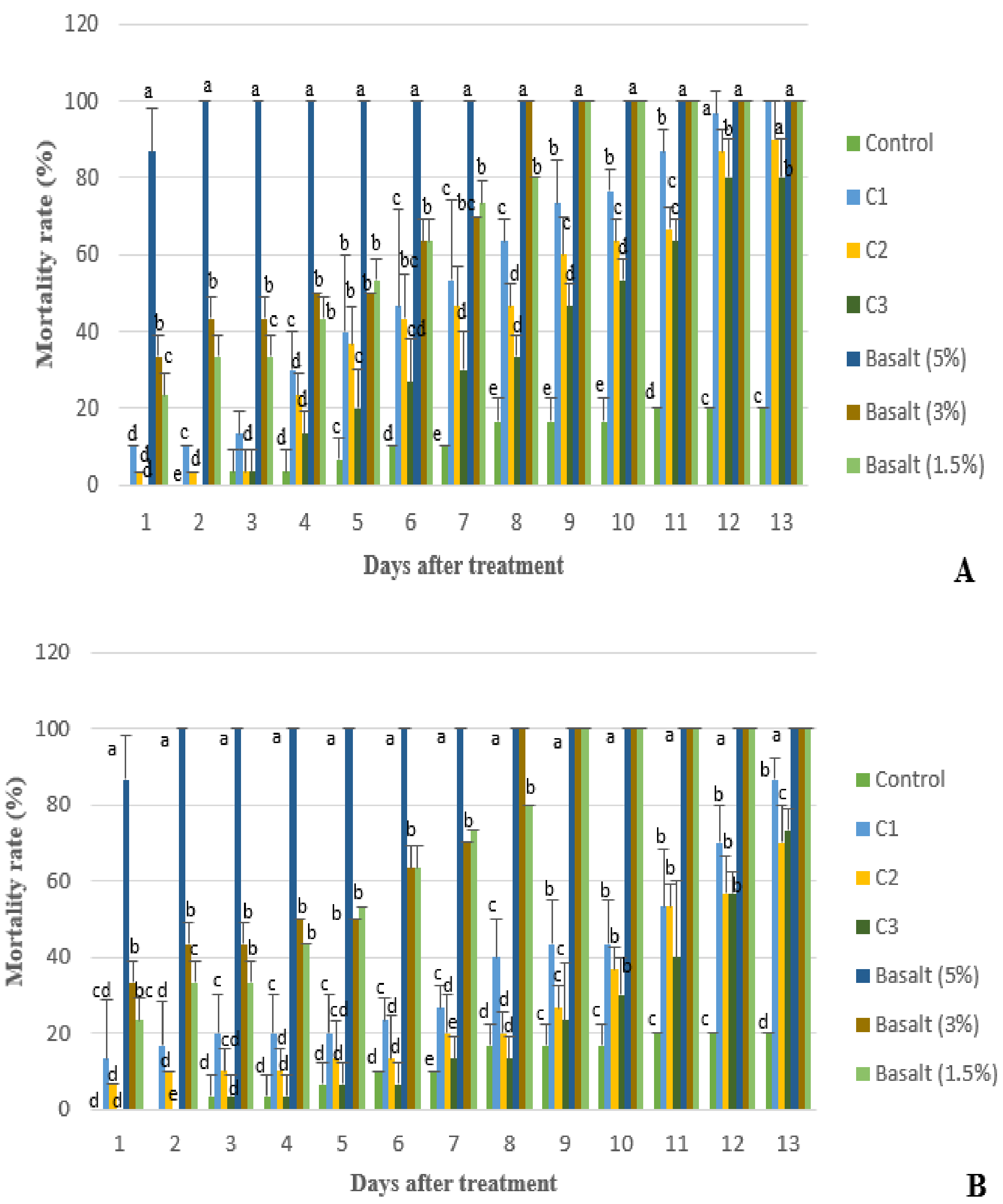

Figure 3. Effects of *C. aurantium* (A), *C. sinensis* (B) extracts and Farina di Basalto® (FdB) on *T. castaneum*. (C1 : 100 mg/mL, C2 : 50 mg/mL, C3 : 25 mg/mL).

Table 3. LC_{90} (mg/mL) values of contact toxicity of tested essential oils and Farina di Basalto® (FdB).

	Essential oils		**Aqueous extracts**		**Farina di Basalto® (FdB)**
	C.aurantium	***C. sinensis***	***C. aurantium***	***C. sinensis***	
LC_{90}	38.173	49.369	260.023	264.368	56.703

3.3.2 Repellency bioassay

The repellent activity was also revealed against *T.castaneum*. Highest activity was observed at higher concentration of the all the plant extract. Essential oils revealed a greater degree of repellence in comparison to aqueous extracts (Table 4).

Table 4. Repellent effects of the essential oils and aqueous extracts from *C.aurantium* and *C.sinensis*.

	Period of exposure (h)				**Response class**			
Concentrations (mg/mL)	1	3	5	24				
Essential oils								
C. aurantium								
1	33.33	26.67	13.33	6.67	II	II	I	I
5	60.00	40.00	20.00	13.33	IV	III	II	I
10	100.00	73.33	33.33	26.67	V	IV	II	II
25	100.00	86.67	46.67	33.33	V	V	III	II
C. sinensis								
1	26.67	20.00	13.33	6.67	II	II	I	I
5	46.67	33.33	26.67	20.00	III	II	II	II
10	86.67	46.67	33.33	26.67	V	III	II	II
25	100.00	80.00	60.00	46.67	V	V	IV	III
Aqueous extracts								
C. aurantium								
25	6.67	33.33	13.33	40.00	I	II	I	II
50	13.33	20.00	33.33	26.67	I	II	II	II
100	13.33	20.00	40.00	53.33	I	II	II	III
C. sinensis								
25	6.67	13.33	13.33	38.33	I	I	I	II
50	11.67	33.33	40.00	46.67	I	II	III	III
100	11.67	26.67	33.33	60.00	I	II	II	IV
Farina di Basalto® (FdB) (%)								
1.5	26.67	13.33	13.33	6.67	II	I	I	II
3	33.33	33.33	26.67	13.33	II	II	II	I
5	86.67	46.67	33.33	26.67	V	III	II	II

Repellency classes: O = < 0.1: is not repulsive; class I = 0.1–20: Very weak repulsive; class II = 20.1–40: weakly repulsive; class III = 40.1–60: moderately repulsive; class IV = 60.1–80: repulsive; class V = 80.1–100: very repulsive.

Results revealed that tested essential oils showed the best repellent effect to *T. castaneum* using percentage of repellency. The highest repellency potential (100 %) was detected at dose of 10 mg/mL after 1 h of exposure, for *C. auarantium* essential oil. However, after 24 h of exposure time to the all concentrations, mean percentage repellency values were ranged between 6.67 to 33.33% (classes II repellency status).

3.4 Phytotoxic activity

Phytotoxic potential of *C. aurantium* and *C. sinensis* essential oils, aqueous extracts and Farina di Basalto® (FdB) basalt were tested in terms of germination rate and inhibition in seedling growth of *Triticum aestivum* L. (Table 5). Generally, they exhibited germination and seedling growth effect that was concentration-dependant. The highest germination rate (100%) was detected in presence of FdB basalt treatment. However, the lowest germination rate (5%) was revealed in presence of C. sinensis essential oil. Essential oils seems also to decrease hypocotyl and radical elongation. Nevertheless, a stimulation of hypocotyl and radicle lengths was observed in presence of Farina di Basalto® (FdB) basalt powder.

Table 5. Phytotoxic activity of the essential oils, aqueous extracts and Farina di Basalto® (FdB) on *Triticum aestivum* L. seedlings.

		Germination rate	**Hypocotyl length (cm)**	**Radicle length (cm)**
Essential oils (mg/mL)				
C. aurantium	0	100±1	5.05±0.74	8.94±0.49
	0.1	56.67±7.64	4.95±0.23	5.92±0.34
	0.3	46.67±10.41	4.84±0.97	4.98±0.43
	0.6	31.67±10.41	4.52±1.01	4.67±0.96
	1	16.67±2.89	3.01±0.35	4.40±0.20
C. sinensis	0	100±1	5.05±0.74	8.94±0.49
	0.1	48.33±2.89	3.45±0.04	4.42±0.46
	0.3	33.33±2.70	3.34±0.03	3.48±0.41
	0.6	28.33±5.77	3.02±0.25	3.17±0.26
	1	5±1.00	1.51±0.11	2.90±0.19
Aqueous extracts (mg/mL)				
C. aurantium	0	100±2.00	3.84±0.21	6.44±0.35
	2.5	90±5.00	3.96±0.23	5.93±2.84
	5	73.33±7.64	4.52±0.47	9.99±0.93
	10	51.67±2.87	4.2±0.25	7.65±0.26
	20	25.00±5.00	2.02±0.35	4.22±1.05

C. sinensis	0	100±2.00	3.84±0.21	6.44±0.35
	2.5	60.00±13.23	5.77±1.00	6.45±1.08
	5	55.00±5	4.35±0.55	5.08±0.55
	10	33.33±2.87	2.84±0.59	3.72±0.51
	20	21.67±5.77	1.83±0.28	1.33±0.27
“Farina di Basalto® type XF’’				
Farina di Basalto® (FdB)	0	100±1	2.67±0.43	6.1±1.73
	1.5%	90±1	3.48±1.44	8.71±2.37
	3%	90±2	3.76±0.31	9.12±0.53
	5%	100±0	4.56±0.20	10.02±0.80
	10%	100±0	3.47±0.80	8.67±1.21

4 DISCUSSION

Limonene was the dominant compound revealed in analysed essential oils. Our results were similar to those reported by Dosoky and Setzer (1966), Sarrou et al. (2013) and Trabelsi et al. (2014), who reported that *C.aurantium* essential oils consist mainly of Limonene. Nevertheless, these findings are not in agreement with those performed by Abderrazek et al. (2014) on Algerian *C. aurantium* essential oil characterized by Linalool, trans-Carveol, and cis-Linalool Oxide richness. For *C. sinensis* essential oil the same finding was reported by Oyedji et al. (2020) and Manzour et al. (2005), revealing that Limonene was the major compound. However, a varied amount of essential oils constituents in *C. sinensis* have been previously reported (González-Mas et al., 2019). Qualitative and quantitative differences variations compared to this study may be due to various factors such as genotype, season, maturational stage, and pedoclimatic factors that occur in the location of the plant’s origins Dosoky and Setzer (1966).

C. aurantium extract revealed the best total phenol and flavonoid contents. In line with that, our previous work on *C. aurantium*, revealed the identification of seven flavonoid derivatives, including five flavanones (eriodictyol, hesperidin, naringenin-rutinoside, eriocitrin, brutieridin), one flavonol (quercetin), and one limonoid (limonin), where hesperidin was the major one (Elimem et al., 2023). However, our results, were lower than that reported for other *Citrus* species such as *C. reticulata*, *C. Unshiu* (Ghasemi et al., 2009) and *C. aurantifolia* (Loizzo et al., 2012). Also, our results were lower than that revealed for methanol extracts from Turkey (Değirmenci and Erkurt., 2020). This variation may be due to solvent used or the extraction process.

Essential oils and aqueous extracts were also screened for their antioxidant activity. Several studies on the chemical composition and bioactivity of different *Citrus* oils reported their strong radical scavenging activity (El-Akhal et al., 2015). Total antioxidant capacity of plant extracts/essential oils is influenced by their chemical composition and content of antioxidants. According to our results, all the investigated aqueous extracts showed stronger antioxidant capacity than essential oils. A direct correlation, between DPPH activity, total polyphenols, flavonoids and condensed tannins (r=-0.952, r=-0.979, r=-0.961, p<0.01) was observed. In fact, phenols are very significant plant compounds and have prominent antioxidant activity owing to their hydroxyl groups. Several studies reported that phenolic content are rich in *Citrus* extracts and have different levels of free radical scavenging. In line with that, Zou et al. (2016) reported that a high correlation was found between the scavenging activity and the flavonoid content in *Citrus* branches. Among the *Citrus* flavonoids, the antioxidant effect of naringin, hesperidin and naringenin are commonly studied. Also, different limonoids have variable antioxidant capacities and some are even better than vitamin C. However, the capacity of phenolic compounds to reduce DPPH radical varied from a compound to another and there is a synergy between them and/or other present constituents in the extracts (Lagha-Benamrouchea and Madani., 2013). Analysed essential oils revealed lower activity. Indeed, Essential oil was usually characterised by its complex composition, and it is difficult to attribute the antioxidant effect of a total essential oil to one or few active compounds. Both minor and major compounds should make a significant contribution to the oil’s activity (Jaouadi et al., 2023). It is suggested that, even at low concentrations, authentic flavor components such as γ-terpinene, terpinolene, geraniol, β-pinene and myrcene have high antioxidant activities (Sarrou et al., 2013).

Insecticidal activity against *Tribolium castaneum* indicated that essential oils and “Farina di Basalto® ” were significantly more effective than aqueous extracts. Our findings highlight recent works that revealed Farina di Basalto® (FdB)’s basalt powder's role in enhancing crops' tolerance to pests (Elimem et al., 2021; Elimem et al., 2023). Concerning essential oils, good efficacy in terms of toxicity is generally known. Essential oils are part of natural plant defense system and many of them are proved effective and some are exploited for integrated management practices of pest and pathogens. It is already established that *Citrus* essential oils of different *Citrus* species are effective against wide range of pest and pathogens (Khanikor et al., 2021). In line with that, a good number of studies reported insecticidal potential of *Citrus* essential oils extracted from different *Citrus* sp. and their constituents at different times, a few of which are commercialized to be used by the consumers against insect pests. The seed essential oils of *Citrus reticulate* var. kinnow, *Citrus reticulate* var. freutrall, *Citrus sinensis* and *Citrus jambhiri* were tested against *Tribolium castaneum* with promising efficacy in terms of LC_{50} for *Citrus jambhiri* followed by *Citrus reticulate* and *Citrus sinensis* (Bilal et al., 2015). Similarly Oboh et al. (2017) recorded insecticidal efficacy of *C. sinensis* peel essential oil against *Callosobruchus mamulatus, Tribolium confusum, Sitophilus oryzae* (Khanikor et al., 2020) [5]. In this line, Oyedeji et al. (2020) revealed also that the peel contains essential oils that exhibit insecticidal activities against different insects such as houseflies, mosquitoes, rice weevils and red flour beetles. Indeed, insecticidal capacity is justified by the mechanism of action of their individual major compounds (Benelli et al., 2019). In our case, Limonene may be the principal components exerting good insecticidal efficacy. Individual assessment for insecticidal property of this common constituent compound have been performed by different researchers and some of them were found active against insect pest. Indeed, Limonene and other *Citrus* limonoids are reported as insect repellents, feeding deterrents, growth disruptors, and reproduction inhibitors against a wide range of pest complexes Khanikor et al., (2020).

Furthermore, essential oil compound such as linalool, α-pinene, β-mycrene, have been reported in literature to have contact toxicity against *S. zeamais* and *T. castaneum* (Kim and Lee., 2014). In fact, Monoterpenes have been well documented to be active as fumigants, repellents or insecticides toward stored products insects. However, the potential of essential oil compounds could lead to the development of synergists, used in combinations to increase the lethality and effectiveness against insect pests (Isman., 2006; Ismail., 2021).

The overall repellent efficacy of tested essential oils was greater than that of aqueous extracts. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. In line with that, a previous study conducted by Deletre et al. (2016) confirmed that essential oils could exhibit repellent efficacy even without any direct contact with the insects. Such a repellent action could be achieved by the penetration of the volatile constituents of essential oils into the respiratory system of the target insect. In fact, some monoterpenes such as α-pinene, Limonene present in our tested essential oils are common constituents of a number of essential oils described in the literature, as presenting mosquito repellent activity (2010). Khanikor et al. (2020) revealed that Limonene and *Citrus* limonoids are reported as insect repellents, feeding deterrents, growth disruptors, and reproduction inhibitors against a wide range of pest complexes. Also, insecticidal activity of this compound was reported effective against *Tuta asoluta* (Lepidoptera: Gelechiidae). Also, Da-Camara et al. (2015) reported that Limonene had a strong repellent activity against *Tribolium confusum* and TconOR93 gene was determined to be a major effector in perception of this compound. Nevertheless, the higher insecticidal or repellent potencies of the tested oils may refer to synergistic, antagonistic or additive effects between limonene and the other minor constituents in those oils.

The present study revealed also that increasing the concentration of tested essential oils, delayed seed germination and reduced the radicle growth and the hypocotyl length of *Triticum aestivum* L. In our work, Limonene was the main constituent of studied essential oils. In the literature, several studies have reported about possible phytotoxicity of Limonene the main constituent of studied essential oils. Indeed, Caputo et al. (2020) revealed that this component was able to inhibit, in a significant way, the germination of *Raphanus sativus* L. and radical elongation of *L. sativum*. However, this compound showed no significant effects against *Avena fatua*, *Echinochloa crus-galli*, *Phalaris minor* and *Zea mays* seeds. However, Limonene was weakly phytotoxic against *Amaranthus retroflexus* L., *Centaurea salsotitialis* L., *Raphanus raphanistrum* L., *Rumex nepalensis* Spreng., *Sinapis arvensis* L. and *Sonchus oleraceus* L.. In light of this, it is possible to postulate that the phytotoxic activity observed for *Citrus* essential oil could be related to its main constituent, although a synergistic activity with other minor compounds cannot be excluded.

5 CONCLUSIONS

Comparative analysis of chemical composition of Tunisian *C. aurantium* and *C. sinensis* essential oils revealed their Limonene richness. Aqueous extracts showed the best radical scavenging capacity, correlated to their phenolic contents, which suggests that they should be considered as a natural source for beneficial and healthy food. On the other hand, tested essential oils and Farina di Basalto® (FdB) revealed an important insecticidal activity against *T. castaneum*. Therefore, they could represent natural and safe alternatives for pest control. Our results revealed also that Farina di Basalto® (FdB) have a positive impact on growth parameter of *Triticum aestivum* L. These findings emphasize the importance of the phytochemical composition of *Citrus* species and Farina di Basalto® (FdB) and could support their utilization in a large field of application.

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