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Toxicity and repellent activities of *Thymus pallescens* and *Cymbopogon citratus* essential oils against *Sitophylus granarius*

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Abstract: This study evaluated the toxicity and repellent activities of essential oils (EOs) against *Sitophilus granarius* (Linnaeus, 1758), both *in vitro* and *in vivo* conditions. The EOs obtained from *Thymus pallescens* Noë. (Lamiaceae) and *Cymbopogon citratus* Stapf. (Poaceae) were analyzed by GC-MS, and revealed that carvacrol (56.64%) and geraniol (20.8%) as the respective major components. *T. pallescens* EO was found to be a more effective toxicant, with LC_{50} and LC_{90} values of 9.3 and 34.6 µL/mL, respectively, in the contact test vs 8.2 and 25.3 µL/mL in the fumigation test. *T. pallescens* EO showed also a stronger repellent effect with values ranging from 83.4% to 100%. In the *in vivo* test, the survival probability decreased from 99.9 to 2.48% among insects exposed to *T. pallescens* EO. These data demonstrated that EOs could be considered effective alternatives to chemical insecticides, providing pest control for stored products in an ecologically sustainable manner.

Keywords: contact toxicity; corrected mortality; fumigation toxicity; metabolic profile; survival probability

Cereal grains represent an important resource that provides food for both human and animal consumption (Rosentrater & Evers 2018). Grain production is seasonal, however, grains are consumed continuously (Keskin & Ozkaya 2015; Rosentrater & Evers 2018). Consequently, to assure food security, crops must be stored in warehouses for varying periods of time, ranging from a few days to longer than a year (Proctor 1994). Several biotic and abiotic factors can play decisive roles in grain availability, particularly factors that result in post-production storage losses. Insect pests are considered to be the principal cause of cereal grain losses (Keskin & Ozkaya 2015). During storage, cereal grain losses can reach as high as 50% of the total harvest in some countries (Fornal et al. 2007), and stored product infestations have been associated with 1 663 different insect species (Hagstrum & Subramanyam 2006). Beetles species represent the major group of stored product pests, with approximately 200 identified species that have been associated with food storage problems (Bell 2011). Among beetles, the granary weevil, *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Curculionidae), is one of the most destructive insect pests that affect stored grains worldwide, capable of causing significant economic losses (Keskin & Ozkaya 2015; Plata- Vélez et al. 2017; Rueda et al. 2018). The degree of loss is directly related to the infestation rate, which

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is determined by factors such as the number of eggs laid and the survival and fecundity of the hatched offspring (Nawrot et al. 2010). Pest infestations affect the quantity and quality of cereal grains and can result in the deterioration of seed germination capacity due to the ability of pests to penetrate into the grain mass (Benelli et al. 2012; Plata-Rueda et al. 2018). *S. granarius* infestations can also provide access to secondary species of detritivorous pest, the most frequent of which is the rust-red flour beetle, *Tribolium castaneum* (Herbst, 1797). The infestation of *S. granarius* in stored grain can indirectly infect food with saprophytic and mycotoxigenic microbes, such as *Aspergillus* and *Penicillium* (Magan et al. 2003). The global control of stored-product insects, including *S. granarius*, primarily depends on the application of organophosphorus- and pyrethroid-based insecticides and fumigants, such as phosphine (PH_3) (Zettler & Arthur 2000; Isman 2006; Aulický & Stejskal 2015). Although the use of conventional chemical insecticides is effective, they can cause toxicity to non-target organisms, resulting in residual contamination on the stored products. In addition, these chemical agents present a high risk of toxicity to the farmers and farm workers. Morevover, their intensive and indiscriminate applications have caused the emergence of new pests and resistant populations (Camaroti et al. 2018; Gong and Ren 2020). The increasing resistance of many storage insects, including *S. granarius*, against a variety of insecticides, such as deltamethrin, malathion, tetrachlorvinphos, fenitrothion, and phosphine, has been reported (Tyler & Binns 1982; Kljajic & Ilija 2005; Mutlu et al. 2019). In addition, the resistance of various populations of harmful insects is now the most restricting factor affecting chemical control practices, resulting in diminished efficacy (Denholm & Devine 2013). These undesirable effects have increased the necessity for more selective and biodegradable products, and the use of biopesticides might facilitate the decreased use of insecticides in warehouses (Isman et al. 2011). Recently, the development of biological insecticides has become a sustainable strategy for pest control (Isman et al. 2011; Marsin et al. 2020). Substances derived from natural origins, especially essential oils (EOs), currently represent alternative solutions to protect stored food products from insect infestations. There are growing reports showing that the use of plant extracts as natural insecticides for the control of stored grain insects present several benefits compared with chemical compounds, including increased biodegradability, low toxicity to humans, and maximized insecticidal activity while maintaining environmental conditions inside the storage systems (Lengai et al. 2020). EOs extracted from aromatic plants can represent potential sources of alternative bioactive compounds that can be used to manage stored grain insect pests. Several plant species, belonging to various families, have been reported as being capable of managing insect pest species that affect stored products, particularly *S. granarius* (Campolo et al. 2018; Trivedi et al. 2018; Yang et al. 2020; Guettal et al. 2021). However, few experimental studies have been designed to examine the effects of EOs and their modes of action against *S. granarius*.

Therefore, the aim of this study was to determine the chemical compositions of EOs derived from *Thymus pallescens* Noë and *Cympobgon citratus* (DC. ex Nees) Stapf and to examine their toxicity and repellent activities against *S. granarius*. The second objective of the present study was to evaluate the potential modes of action through which these EOs affect the insect metabolism, especially the effects on the levels of carbohydrates, proteins, and lipids in treated *S. granarius* adults.

MATERIAL AND METHODS

Plant materials. The leaves of the aerial parts of *T. pallescens* and *C. citratus* were collected during the flowering stage from various localities in Mascara (35°22'60" N, 0°9'0" E) and Algiers (36°46'34" N, 3°3'36" E), North Algeria. Plant materials were partially dried for 15 days at room temperature (25 ± 3 °C).

Culturing of insects. *S. granarius* initial colonies were obtained from the wheat storage farm Bordj Bou Arreridj, Algeria, (36°4'0" N, 4°46'0" E) in 2018. The insect pests were reared separately on clean and uninfested wheat and rice grains. Two hundred adult insect pests of each were released into a 1 000 g mixture of wheat, *Triticum aestivum* Linnaeus (Poaceae) and rice *Oryza sativa* Linnaeus (Poaceae) (1 : 1, v/v) grains (Stephensons 1983) in plastic trays (60 × 40 × 12 cm), which were covered with a muslin cloth to allow ventilation. The trays were maintained in incubation chambers at a controlled temperature of 26 ± 3 °C, relative humidity of 70 ± 5%, and 12 : 12 h (light : dark) photoperiod. Adult *S. granarius*, 4–6 days old, were used for each experiment. All experiments were conducted under

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identical environmental conditions that were used to maintain the cultures.

Essential oil extraction and gas chromatography-electron ionisation mass spectrometry (GC-MS) analysis. The EOs were extracted by a hydrodistillation process using a Clevenger-type apparatus (Glassco, India). The extracted oils were dried over anhydrous sodium sulphate, and stored at 4 °C until used for GC-MS analysis and biological activity tests.

GC–MS analyses were performed to determine the compositions of EOs. The GC–MS analysis was performed with an apparatus system, using a VF WAX and HP-5 capillary column (60 m × 0.25 mm × 0.5μm film thickness) Hewlett-Packard computerized MS system comprised of a 6890 gas chromatograph, coupled to a mass spectrometer (5973A) using a non-polar HP5MS column (60 m × 0.25 mm × 0.5 μm film thickness) apparatus. The EOs were diluted in (1/10 hexane, v/v) and 1 μL were injected by splitting, and the split ratio was 1 : 25. The GC conditions were as follows: injector temperature, 250 °C; column temperature, isothermal at 60 °C and held for 6 min, then programmed to 250 °C at 6 °C/min and held at this temperature for 2 min; ion source temperature, 250 °C; and detector temperature, 320 °C. Helium was used as the carrier gas at a rate of 0.5 mL/minute. The mass range varied from m/z 30 to 350 atomic mass units (amu). The EO constituents were identified by comparing their retention indices and mass spectra with those of known samples, comparing their linear retention indices against those for a series of *n*-hydrocarbons, and computer matching against both commercial (NIST 98 and ADAMS) and home-made mass spectra libraries, generated from pure substances, the components of known oils, and MS literature data (Tuberoso et al. 2007).

Toxicity activity. The toxicity of both EOs against *S. granarius* was evaluated by performing both contact and fumigation tests. Contact toxicity assays were performed according to a previously described protocol (Tapondjou et al. 2005). Each EO was diluted with acetone to prepare a serial dilution. The tested concentrations were 10, 20, 30, 40, and 50 μL/mL. For the experimental treatments, twenty *S. granarius* adults were introduced into petri dishes (9 cm diameter) containing filter paper. The EOs were applied to the *S. granarius* adults by spraying the insects evenly using 2 mL for each trial. Control insects were treated with an equal volume of acetone mixed with distilled water. The fumigant toxicity of EOs against *S. granarius* adults was evaluated according to a previously described technique (Abdelgaleil et al. 2018). Glass jars, 1 L in volume, were used as fumigation chambers. The EOs were applied at concentrations of 5, 10, 20, 40, and 50 μL/mL to pieces of Whatman No.1 filter paper, which were strongly attached to the underside of the screw caps of jars. The insides of the jars were brushed with vaseline to prevent the insects from being able to make direct contact with the EO. Each cap, containing the treated filter paper, was tightly screwed onto a jar containing twenty *S. granarius* adults. The cover was well-sealed with parafilm. Control insects were maintained under the same conditions, without the application of EOs. Four replicates were used for each assay, and each experiment was performed twice. The insects were considered dead when no movement was recorded. Mortality was recorded after 72 h treatment and corrected using Abbott's formula (Abbott 1925):

$$MC\,(\%) = \frac{100 \times (M - M_t)}{100 - M_t} \quad (1)$$

where: MC – corrected mortality rate; M – mortality rate in treated series; M_t – mortality rate observed among control series.

Half-maximal lethal concentration (LC_{50}) values were assessed by using probit analysis.

Repellent assay. The repulsive effects of EOs against *S. granarius* adults were assessed using the preferential zone method on filter paper, as previously described (McDonald et al. 1970). Filter paper discs (9 cm diameter) were divided into two equal parts. Five EO concentrations were prepared for this assay by dilution with acetone: 10, 20, 30, 40, and 50 μL/mL. After that, 0.5 mL of each EO concentration was placed on one half of the disc, whereas acetone was applied to the other half. Once the acetone has evaporated, the two halves were reaffixed using tape. The filter paper disc was placed in a 9 cm diameter petri dish. Twenty *S. granarius* adults were placed in the center of the filter paper. After 120 min, the number of insects on the filter paper half treated with EOs (N_t) and the number on the half treated with acetone (N_c) were identified. The percentage of repulsion (PR) was calculated using the following formula:

$$PR\,(\%) = \frac{(N_c - N_t)}{N_c} \times 100\% \quad (2)$$

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The average percentage of EOs repellency was calculated and assigned as ranked by McDonald et al. (1970) by a repulsive different classes varying from 0 to V [Class 0 (*RP* < 0.1%), class I (*RP* = 0.1–20.0%), class II (*RP* = 20.1–40.0%), class III (*RP* = 40.1–60.0%), class IV (*RP* = 60.1–80.0%) and class V (*RP* = 80.1–100.0%)]. Four replicates were used for each assay, and each experiment was performed twice.

***In vivo* bioassays and time–mortality evaluation.** To assess the fumigation toxicity of both EOs *in vivo,* sexed *S. granarius* adults (1–2 weeks old) were used. First, 20 male and 20 female weevils were reared in the plastic trays (60 × 40 × 12 cm) containing a mixture of durum wheat and rice grains, at equal volumes (1 : 1, v/v), which were placed in growth chambers. After 45 days of incubation, the number of insects was estimated, and the number of adults was adjusted between both treatments and controls. 220 weevils (unseparated sex) were disposed in the plastic mouths containing a mixture of durum wheat and rice grains, at equal volumes (1 : 1, v/v), which were replaced in growth chambers. After three days of incubation, the mouths were fitted with glass bottles containing 2 mL of each EO. For the control, the glass bottles were filled with acetone. The bottle caps were pierced with fine pores to allow the constant evaporation of the EOs. The treated and untreated mouths were tightly closed to prevent the evaporation of the EOs, and the containers were replaced in the growth chambers. Three replicates were performed for each treatment. The number of live insects was counted every 48 h for 20 days. Three replicates were used for each assay, and each experiment was performed twice.

Effects of EOs on metabolic profile. To measure proteins, lipid and total carbohydrates contents, insect pests treated with LC_{50} of *T. pallescens* and *C. citratus* EOs were explored. The control used in the three experiments is represented by the insects, which have suffered natural death. Four repetitions were retained for each analysis.

In brief, the quantification of proteins was performed using the Coomassie Brilliant Blue G-250 dye-binding method (Bradford 1976). Coomassie Brilliant Blue G-250 (Thermo Scientific, UK) (100 mg) was dissolved in 50 mL 95% ethanol, and 100 mL 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 L. After crushing the individual insects in 400 µL of the Tris-HCl (20 mM) solution, the samples were incubated at 4 °C for 30 min to allow the proteins time to dissolve. A0.1 mL aliquot was transferred to a 12 × 100 mm test tube, 5 mL of Bradford reactive was added to the test tube, and the contents were mixed by inversion or vortexing. The protein concentration was determined by spectrophotometry at 595 nm. The protein concentrations of each sample were determined against a standard curve constructed using 125, 250, 500, 1 000, and 2 000 µg bovine immunoglobulin G (IgG) dissolved in the same buffer as the samples. Before reading, the plates were gently stirred for 5 s to separate the protein aggregates.

For the extraction and quantification of total carbohydrates, previously described methods were used (Van Handel 1985a; Sönmez & Gülel 2008). Insects were ground in 400 µL of sodium sulfate for 2 min, followed by the addition of 2 mL methanol. The tubes containing the homogenate are then centrifuged at 4 °C for 4 min at 2 000 × g for 2 minutes. A 100 µL aliquot was transferred to a new 12 × 75 mm tube, and 2 mL anthorone reactive was added, followed by incubation in a 95 °C water bath for 17 minutes. The tubes were then placed in an ice bath at 10 min, and the optical density at 625 nm was measured. For carbohydrates, a calibration standard curve was generated using a standard glucose solution (1 g/L). The blank was a 0.5 mg/mL glucose solution (5 mg of glucose in 10 mL of distilled water). A series of dilutions were performed to obtain the following glucose concentrations: 10, 20, 40, 60, 80, 100, and 200 µg/mL.

The lipids contents were determined using methods described by Van Handel (1985b) and Plaistow et al. (2003). The insects were crushed in 400 µL chloroform/methanol (1 : 1, v/v) solution. The supernatant was transferred to a clean tube (16 × 100 mm), which were retained inside a fume cupboard, in a 95 °C water bath to allow the remaining solvent to evaporate. Then, 200 µL concentrated (95%) sulfuric acid was added, and the solvent was allowed to evaporate at 90 °C for approximately 10 minutes. The sample was removed from the heating bath, allowed to cool, and 5 mL vanillin-phosphoric acid reagent (85%) was added. The samples were vortexed and then exposed to open air for 5 min to allow coloration to develop. The optical density of each sample was measured after 25 min at 525 nm. The lipid concentration for each sample was determined against a standard curve constructed using 25, 50, 100, 200, 400, 800, and 1 200 µg of commercial vegetable oil.

Statistical analyses. Generalized linear models (GLMs) were used to analyze the corrected mortality

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[*CM* (%)] and energy biomarker values. The *CM* (%) was subjected to probit analysis to obtain the 50 and 90% lethality (LC_{50} and LC_{90}, respectively) values, which were estimated using a binomial model with a complementary log-log link function. The results are expressed as the mean ± SE, and significant differences between treatments and control conditions were compared using an ANOVA at the 5% probability level, followed by Tukey's post hoc test. The data from the time-mortality (survival) bioassays were subjected to a non-parametric survival analysis using Kaplan-Meier estimators to obtain survival curves and estimates of the median survival time (LT_{50}). All analyses were performed using the statistical software RStudio 1.2.5019-R (version 3.6.1.)

RESULTS

Chemical compositions of essential oils. A total of 36 compounds were identified in the two EOs, which accounted for 96–97% of the total composition. The chemical analysis of the *T. pallescens* EO revealed more than 16 compounds, representing 98.96% of the total oil. The results revealed that phenols were the most important portion of the EO of *T. Pallescens* (Table 1). The major constituents were carvacrol (56.64%) and p-cymene (16.36%), followed by thymol (8.71%), γ-terpinene (5.4%), α-pinene (3.33%), and linalool (3.12%). *C. citratus* EO consisted of 26 compounds with the highest percentage of aliphatic alcohols (Table 2). The chemical profile of *C. citratus* EO revealed the major components geraniol (20.8%), limonene (10.5%), camphene (7.8%), citronellal (4.67%), borneol (4.72%), and geranyl acetate (3.39%).

Table 1. Chemical compositions of the *Thymus pallescens* essential oils as analyzed by gas chromatography-mass spectrometry

Number	Compounds	Ret. time (min)	*T. pallescens* (%)
1	α-thujene	5.68	0.35
2	α-pinene	5.85	3.33
3	camphene	6.2	0.30
4	α-pinene	6.84	0.36
5	α-terpinene	7.79	1.15
6	β-myrcene	7.11	0.65
7	limonene	13.8	0.73
8	1.8-cineole	14.2	0.34
9	γ-terpinene	16.3	5.40
10	p-cymene	17.6	16.36
11	α-terpinolene	18.2	0.19
12	linalol	34.6	3.12
13	borneol	43.8	0.76
14	caryophyllene	59.5	0.57
15	thymol	69.6	8.71
16	carvacrol	71.7	56.64
Total (%)			98.96

Table 2. Chemical compositions of the *Cymbopogon citratus* essential oils as analyzed by by gas chromatography-mass spectrometry

Number	Compounds	Ret. time (min)	*C. citratus* (%)
1	α-pinene	5.85	2.18
2	camphene	6.2	7.80
3	β -myrcene	7.11	0.50
4	limonene	13.8	10.5
5	1.8-cineole	14.2	t
6	γ-terpinene	16.3	t
7	p-cymene	17.6	0.12
8	α -terpinolene	18.2	0.40
9	citronellal	30.3	4.37
10	linalol	34.6	0.70
11	β-elemene	37.2	1.07
12	β-caryophyllene	37.5	1.02
13	citronellyl acetate	41.6	0.89
14	α-terpineol	43.6	2.19
15	borneol	43.8	4.72
16	germacrene D	44.5	0.87
17	geranial	45.6	0.92
18	bicyclogermacrene	45.7	1.60
19	δ-cadinene	47.2	4.18
20	geranyl acetate	47.3	3.39
21	bitronellol	47.7	2.81
22	geraniol	52.4	20.86
23	caryophyllene	59.5	0.32
24	methylisoeugenol	60.9	7.10
25	elemol	64.6	1.37
26	carvacrol	71.7	t
Total (%)			79.18

t – trace; ret – retention

Toxicity activity. The obtained results showed that both EOs exerted very potent toxicity against *S. granarius* populations (Table 3). Behavioral changes were observed among the treated insect pests, as indicated by the aggregation and couplings between males and females. The intensities of the mortality rates varied according to the used toxicity test [F (1, 60) = 35.44; P = 0.000], the tested

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Table 3. Insecticidal activity of *Thymus pallescens* and *Cymbopogon citratus* essential oils against *Sitophilus granarius*

Essential oils	Concentration (µL/mL)	Toxicity test: Corrected mortality (%)	
		contact	fumigation
Cymbopogon citratus	50	86.25 ± 3.75^{abcd}	95.00 ± 2.04^{abc}
	40	78.75 ± 4.27^{bcde}	92.50 ± 3.23^{abc}
	30	70.00 ± 3.54^{defg}	80.00 ± 5.40^{abcde}
	20	41.25 ± 8.50^{ghi}	63.75 ± 5.54^{efg}
	10	32.50 ± 3.23^{i}	57.50 ± 5.95^{fgh}
Thymus pallescens	50	97.50 ± 2.50^{ab}	100.00 ± 0.00^{a}
	40	93.75 ± 4.73^{abc}	97.50 ± 2.50^{ab}
	30	78.75 ± 1.25^{bcde}	96.25 ± 2.39^{ab}
	20	81.25 ± 3.15^{abcde}	75.00 ± 2.04^{cdef}
	10	53.75 ± 4.27^{gh}	63.75 ± 3.75^{efg}
F-value (4, 60) = 2.67; *df* = 4; *P*-value = 0.041*			

*Significant difference at $P = 0.05$; values represent the mean of four replicates ± SE; values followed by a different letters in a column indicate significant difference at $P < 0.05$

EO [F (1, 60) = 60.12; $P = 0.0000$], and the applied concentration [F (4, 60) = 78.77; $P = 0.000$]. Based on the corrected mortality values, *T. pallescens* EO demonstrated a more toxic effect than *C. citratus* EO. The corrected mortality rate increased gradually with increasing EO concentrations. In addition, fumigant test toxicity showed a higher corrected mortality rate than contact test.

During the fumigant toxicity test, *T. pallescens* and *C. citratus* EOs resulted in 53.75–100% and 57.5–95% *CM* (%) rates, respectively, against *S. granarius*. The *CM* (%) rates were obtained, and two different lethal concentration levels were estimated by probit analysis (χ^2; $P < 0.05$). The LC_{50} and LC_{90} values indicated that *T. pallescens* EO was more toxic against *S. granarius*, with LC_{50} = 8.2 and LC_{90} = 25.3 µL/mL (χ^2 = 37, df = 4), followed by *C. citratus* EO, with LC_{50} = 9.71 and LC_{90} = 44.3 µL/mL (χ^2 = 41.6, df = 4) (Table 4).

In the contact toxicity bioassay, *T. pallescens* EO showed strong toxicity against *S. granarius* adults. At the same application concentrations and the same exposure periods, *T. pallescens* and *C. citratus* EOs achieved *CM* (%) rates of 53.75–97.5% and 32.5–86.25%, respectively, against *S. granarius* adults (Table 3). Furthermore, the LC_{50} and LC_{90} values indicated that *T. pallescens* EO was the most toxic against *S. granarius*, at 6.3 and 34.6 µL/mL (χ^2 = 39.6, df = 4), respectively, compared with values of 12.6 and 71.7 µL/mL (χ^2 = 52.5, df = 4) for the

Table 4. Lethal concentrations of *Thymus pallescens* and *Cymbopogon citratus* essential oils against *Sitophilus granarius* after 72 h exposure

Toxicity test	EO	*LC* (µL/mL)	*EV*	*CI* (%)	(χ^2)
Contact	*C. citratus*	LC_{50}	12 61.4	15.80–21.50	52.5
		LC_{90}	71.711	50.20–93.50	
	T. pallescens	LC_{50}	9 31.3	6.90–11.70	39.6
		LC_{90}	34 64.1	26.60–42.70	
Fumigation	*C. citratus*	LC_{50}	9 711.4	6.90–12.40	41.6
		LC_{90}	44 36.4	31.50–57.03	
	T. pallescens	LC_{50}	8 21.0	6.10–10.30	37.0
		LC_{90}	25 32.5	20.30–30.10	

EO – essential oil; *LC* – lethal concentration causing 50 and 90% mortality; *EV* – estimated value; *CI* – confidence interval; χ^2 – chi-squared value for the lethal concentrations and fiducial limits based on a log scale with significance level at $P < 0.05$

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C. citratus EO (Table 4). Mortality was always < 2% in the control insects.

Repellent activity. The average percentages of repellency of the studied EOs are shown in Table 5. EOs from *T. pallescens* and *C. citratus* presented strong repellant activity against *S. granarius*. The EOs showed varying repellent activity, depending on the used EO [F (1, 30) = 132.4; P = 0.00000] and the applied concentration [F (4, 30) = 39.5; P = 0.00000]. In addition, repellency activity increased with increasing concentrations of EOs. *T. pallescens* EO had a higher repellency, with values ranging from 83.4 to 100%, compared with values ranging from 17.4 to 97.3% for *C. citratus* EO.

***In vivo* test and time-mortality relationships.** In the *in vivo* test, the survival rate was determined 20 days after insect exposure to *T. pallescens* and *C. citratus* EOs (Figure 1). The survival rates differed significantly between the two EOs (log-rank test, χ^2 = 162.9, df = 2; P < 0.001). For treatments at the LD_{50} levels, *S. granarius* survival decreased from 99.9 to 2.48% in the presence of *T. pallescens* EO and to 36% in the presence of *C. citratus* EO. In contrast, insects species treated with both EOs died between 2 and 20 days after exposure. The mean survival times (LT_{50}) for *S. granarius* were 11.58 and 15.85 days for insects treated with *T. pallescens* and *C. citratus* EOs, respectively.

Effect of essential oils on metabolic profile. The protein, lipid, and carbohydrate levels were measured in *S. granarius* adults treated with both EOs, and the results are shown in Figure 2. According to the ANOVA results, the protein [F (2, 18) = 5.15; P = 0.032], carbohydrate [F (2, 18) = 18.96; P = 0.01], and lipid contents [F (2, 18) = 5.41; P = 0.01] were significantly affected by EO treatments. The protein levels of *S. granarius* adults treated with *T. pallescens* and *C. citratus* EOs were measured at 15.56 and 18.50 µg/mg, respectively. Significant decreases in protein contents were observed for *S. granarius* adults treated with both EOs, as these values represented 33.07 and 29.9% decreases relative to the value measured in the untreated control. The carbohydrate levels in *S. granarius* treated with *T. pallescens* and *C. citratus* EOs were measured at 0.74 and 1.26 µg/mg, respectively, which were significantly lower than control, representing 48.5 and 13.9% decreases relative

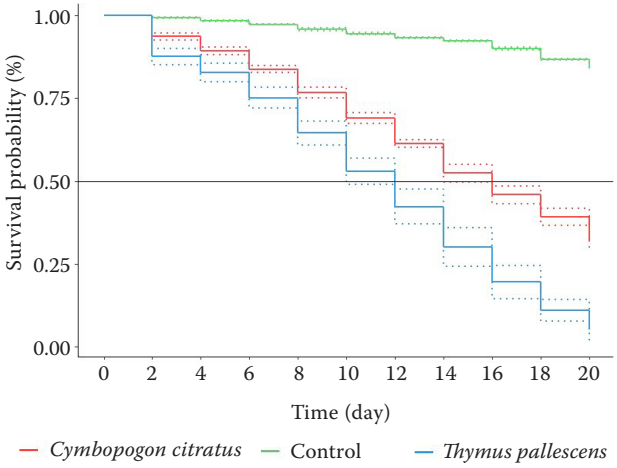

Figure 1. Survival curves of *Sitophilus granarius* adults exposed to *Cymbopogon citratus* and *Thymus pallescens* essential oils, estimated using the Kaplan-Meier log-rank test

Table 5. Repellent effect of the essential oils extracted from *Thymus pallescens* and *Cymbopogon citratus* against *Sitophilus granarius* adult

Essential oils	Concentration (µL)	*RP* (%)	Class
Thymus pallescens	10	83.40 ± 4.9^a	V
	20	91.64 ± 3.0^a	V
	30	95.90 ± 2.6^a	V
	40	100.00 ± 0.0^a	V
	50	100.00 ± 0.0^a	V
Cymbopogon citratus	10	17.42 ± 8.8^d	I
	20	38.70 ± 8.4^c	II
	30	58.81 ± 4.1^b	III
	40	91.49 ± 5.9^a	V
	50	97.30 ± 1.5^a	V
F-value (4, 30) = 17.916; P-value = 0.00000*			

*Significant difference at P = 0.05; values represent the mean of four replicates ± SE; values followed by a different letters in a column indicate significant difference at P < 0.05; *RP* – the percentage of repulsion

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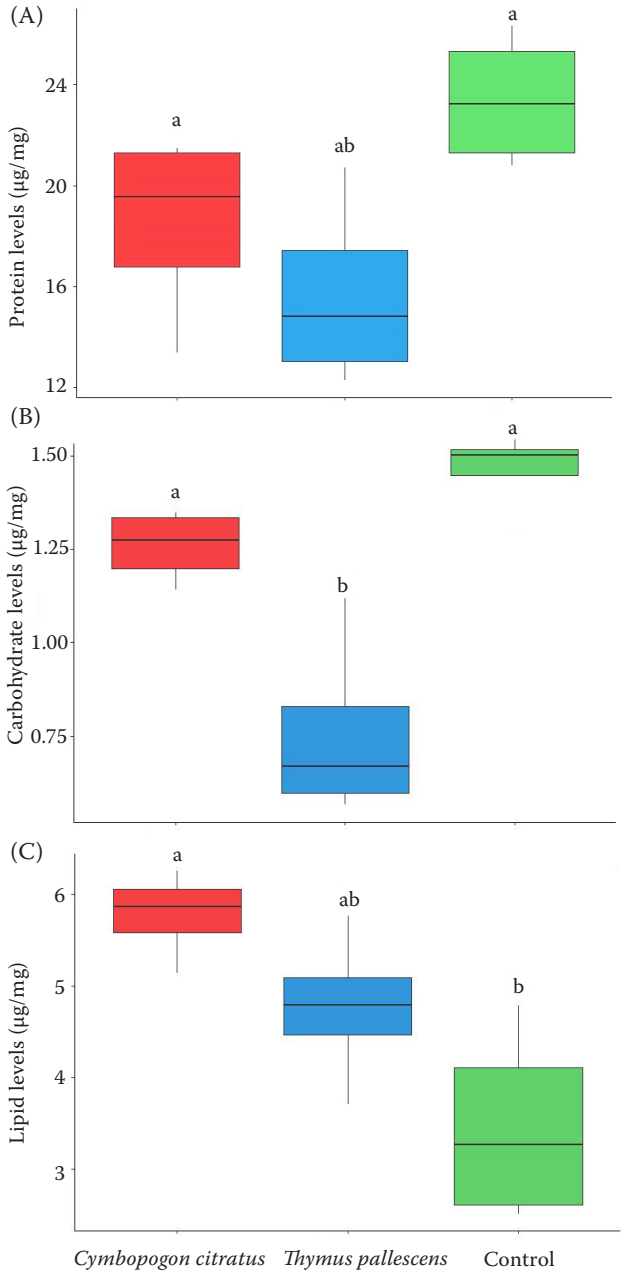

Figure 2. The box plots indicate the quantities of total (A) protein, (B) lipids, and (C) carbohydrates in *Sitophilus granarius* treated with C*ymbopogon citratus* and *Thymus pallescens* essential oils

Values represent the mean of four replicates ± SE; values followed by a different letters in a column indicate significant difference at $P < 0.05$

to the value measured in the control. The obtained results indicated significant increases in the lipid contents of *S. granarius* adults treated with both EOs. Lipid contents increased by 32.55 and 61.88% relative to that of the control, which corresponded to 4.77 and 5.78 µg/mg for treatments with *T. pallescens* and *C. citratus* EOs, respectively.

DISCUSSION

The toxicity, repellency, and physiological effects of *T. pallescens* and *C. citratus* EOs against the granary weevil, *S. granarius,* were determined in this study. The chemical compositions of the EOs were analyzed by GC-MS, which resulted in the identification and quantification of more than 30 compounds. Our analysis showed that carvacrol (67.2%), p-cymene (16.2%), and thymol (4.9%) were the major components of *T. pallescens* EO, which agrees with previous studies (Benchabane et al. 2015; Moutassem et al. 2019). The major volatile constituents of *C. citratus* EO were identified as geraniol (20.8%) and limonene (10.5%), which are similar to those identified in previous studies conducted on the same species, with only slight differences (Bassolé et al. 2011; Moutassem et al. 2019).

Both EOs showed repellent and toxic activities, at varying rates, against the tested stored grain pest. These variations might be explained by differences in the insect pest reactions to the compounds found in the EOs. In addition, both repellent and toxic activities increased with increasing concentrations, and the highest repellant and toxic effects were recorded with *T. pallescens* EO against *S. granarius*. Toxic and repellant activities have previously been described for other EOs derived from various plants against S. *granarius* and other stored insect pests (Kordali et al. 2005; Kim et al. 2010; Kordali et al. 2012; Ziaee et al. 2014; Plata-Rueda et al. 2018**;** Gong & Ren 2020; Plata-Rueda et al. 2020; Teke & Mutlu 2020; Guettal et al. 2021). The toxicity and repellency of both EOs and the differences observed in their efficiencies might be attributed to their different bioactive compounds and their variability, depending on the origin plant species. A literature review revealed several chemical compounds with a wide spectrum of repellency and toxicity effect, including phenols (1.8 cineole and carvacrol), alcohols (α-terpineol, terpinen-4-ol, and linalool), aldehydes, ketones (camphor and citronellal), and monoterpene hydrocarbons (camphene, α-pinene, and p-cimene) (Prates et al. 1998; Kim et al. 2010; Oliveira et al. 2018; Gong & Ren 2020; Plata-Rueda et al. 2020). Based on the GC-MS analyses, carvacrol, thymol, and linalool were the most abundant components of *T.pallescens* EO, and geraniol, citronellol,

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and methylisoeugenol were the most abundant components of *C. citratus* EO. These compounds have previously been reported to exert repellent and toxic activities against stored pests, especially *S. granarius* (Huang et al. 2002; Kim et al. 2010; Benelli et al. 2012; Oliveira et al. 2018; Abouelatta et al. 2020; Plata-Rueda et al. 2020). However, the presence of minor constituents might also play roles in the activities of the EOs (Essoung et al. 2020). Our results corroborate previous findings showing that three *Artemisia* species and their components displayed the potential to serve as control agents against *S. granarius* (Kordali et al. 2006). A more recent study of Plata-Rueda et al. (2020) reported that *C. citratus* EO and its components including citral and geranyl acetate produced high repellent**,** contact, and fumigant toxicity against *Ulomoides dermestoides* (Fairmaire, 1893) (Coleoptera, Tenebrionidae) adults. A similar study reported a repellency of 66.5% and a fumigant toxicity of approximately 92.52% for *Citrus limonum* EO against *S. granarius* (Guettal et al. 2021). Another study of Plata-Rueda et al. (2018) reported that cinnamon and clove EOs possessed significant toxic and repellent activities against *S. granarius*. Morevover, EOs derived from *Rosmarinus officinalis* Linnaeus, *Laurus nobilis* Linnaeus, *Echinacea purpurea, Origanum majorana* Linnaeus, *Ocimum basilicum* Linnaeus, and *Foeniculum vulgare* Mill. exhibited varying degrees of repellent effect against granary weevil (Teke & Mutlu 2020).

In the present study, *T. pallescens* and *C. citratus* EOs induced *S. granarius* mortality *in vivo*, and the LT_{50} were 11.58 and 15.85 days after exposure, respectively. The rapid effects of these EOs against *S. granarius* indicate the potential of *T. pallescens* and *C. citratus* EOs to protect stored products. Both EOs have comparable effects as those reported for chemical insecticides. Previous study showed that *S. granarius* and *S. zeamais* insects treated with spinosad died between 12 and 13 days after exposure (Vélez et al. 2017). As described by other authors, a survival rate as low as 48 h after *U. dermestoides* exposure to *C. ciratus* EO and its components has been reported for some lethal doses (Plata-Rueda et al. 2020). Similar effects were observed when other coleopteran pests of stored grains were exposed to plant terpenoids, such as *Rhyzopertha dominica* (Fabricius, 1792) (Bostrichidae), *S. granarius*, and *Tribolium castaneum (*Herbst 1797) (Tenebrionidae) (Huang et al. 2002; Prates et al. 1998; Plata-Rueda e al. 2018).

One important finding of this study is the particular toxicity of the tested EOs both *in vitro* and *in vivo* against insect pests which may be related to perturbation of various biochemical and physiological mechanisms. Several studies have demonstrated that natural compounds can cause symptoms that indicate neurotoxic activity in insects, such as hyperactivity, seizures, and tremors, followed by paralysis and death (Gaire et al. 2019). The toxicity of both EOs against *S. granarius* may be associated with acetylcholinesterase (AChE) inhibition. In addition, carvacrol has also been shown to inhibit AChE activity or bind to nicotinic acetylcholine receptors (Tong et al. 2013; Lu et al. 2020). The potential for EOs to control insect pests might be due to the synergy between compounds and their abilities to penetrate the insect body or affect the insect respiratory system (Plata Rueda et al. 2020). Therefore, the repellency effects of both tested EOs can be explained by altered spatial perceptions through the olfactory or gustatory systems upon contact (Papachristos & Stamopoulos 2002; Germinara et al. 2015; Martínez et al. 2018), which modulate the functions of olfactory neurons in the sensilla and disrupt chemosensory receptors (Ditzen et al. 2008; Lee et al. 2010), stimulating or reducing the mobility of the insects and affecting walking patterns (Plata-Rueda et al. 2018).

Plant-derived EOs can affect insect metabolism and development through various biochemical and physiological processes (Senthil-Nathan 2013). This study also found that the metabolic rate of *S. granarius* was affected by exposure to *T. pallescens* and *C. citratus* EOs, demonstrating physiological stress, which may explain the toxicity effects of *T. pallescens* and *C. citratus* EOs against *S. granarius*. We observed that treatment with EOs resulted in significant decreases in the levels of proteins and carbohydrates and a significant increase in lipid levels compared with untreated controls. This decrease in protein content could be attributed to one or more factors, such as a decrease in protein synthesis or an increase in protein degradation, and the detoxification of the molecules affected by the bioactive principles present in the EOs (Vijayaraghavan et al. 2010; Ranjini et al. 2016). Similar results were obtained using *C. limonum* EO on the hemolymph protein of *S. granarius* (Guettal et al. 2021). In our study, the carbohydrate contents of the tested pest insect were significantly decreased after EO treatments. Insects typically convert carbohydrates into lipids and glycogens (Sönmez & Gülel 2008), which could explain

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the observed reduction in carbohydrate levels and the increased lipid levels observed in the treated insects. Similar results were obtained in *S. granarius* treated with *C. limonum* EO (Guettal et al. 2021) and in *T. castaneum* and *Callosobruchus maculatus* (Fabricius, 1775) treated with cardamom, cinnamon, and nutmeg EOs (Tarigan & Harahap 2016). The increase in lipid levels can be explained by the establishment of the tegument in insects to prevent the harmful effects of EOs. The external protective layer of insects consists of an impermeable lipid layer, typically composed of alkanes, methyl branched alkanes, and alkenes (Morgan 2004). This lipid layer is important for the prevention of dehydration and to repel rain and in social insects (bees, wasps, and termites). The increase in the lipid levels of insects may therefore reflect the initiation of resistance mechanisms against the stress of EOs. Fatty acids are primary metabolites and also serve as the source of many secondary metabolites, such as phenols and quinones (Morgan 2004). The obtained results are also in accordance with previous observations showing that treatments with plant extracts from *Lantana camara* Linnaeus were able to significantly enhance the lipid contents of the pine processionary moth *Thaumetopea pytiocampa* (Bouzar Essaidi et al. 2014).

CONCLUSION

The EOs of *T. pallescens* and *C. citratus* revealed potent toxicity and repellency activities against *S. granarius* adults, confirming their potential as natural alternative pest control agents with effects comparable to those obtained using chemical insecticides for the control of stored product pests. Our results provide an interesting avenue for the development of bioinsecticides and repellent formulations based on EOs.

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