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Research Article

Bioassays of Fumigant Toxicity and Repulsiveness of *Haplophyllum tuberculatum* Extracts

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Food grains were severely damaged by stored goods insects. The red flour beetle *Tribolium castaneum*, the cigarette beetle *Lasioderma serricorne* and the rice weevil *Sitophilus orysae* are some of the most destructive pests which attack raw cereals throughout the world. In stored grain mill, fumigation with synthetic fumigants was the most economical tool for managing stored pests. Nevertheless, the application of chemical pesticides caused adverse effects on humans and environment. Chloroform, acetone, methanol and water extracts and essential oil of *Haplophyllum tuberculatum* aerial parts were tested for their fumigant and repellent effect against adults of the three stored products pests (*T. castaneum*, *L. serricorne* and *S. oryzae*). GC/MS analysis of the essential oil resulted in determination of 19 compounds. The major constituents were β-phellandrene (16.17%), *trans-p*-menth-2-ene-1-ol, (13.22%) and *cis-p*-menth-2-ene-1-ol (8.29%). Thus, it seemed that the essential oil had the highest fumigant activity against *L. serricorne* after 12 h of exposure compared to the two other insects with a LC_{50} equal to 38.59 $\mu.l^{-1}$ air. The best repellency was observed for the chloroform and methanol extracts against *T. castaneum*. The respective repellent doses RD_{50} values were = 0.03 and 0.04 $mg.ml^{-1}$ after 24 h of exposure. However, after 24 h of exposure acetone extract seems to be more repellent with respective repellent dose values of = 0.14 $mg.ml^{-1}$ (RD_{50}) against *L. serricorne*. *H. tuberculatum* essential oil and extract proved to be effective in control pest infestations in grains and dry food products.

Keywords: *Haplophyllum tuberculatum*, fumigant, repellent, extracts

1 Introduction

Grain farming on a vast scale is necessary to meet the rising global food demand. Sadly, bugs harm roughly one-third of the world's grain reserves (Murugesan et al., 2021). One of the best ways to quickly get rid of insects infesting food storage was by fumigation. It was crucial to develop innovative management methods that do not involve using traditional pesticides and are environmentally friendly. Many insect pests were thought to be manageable with the help of plant essential oils (Ghasemzadeh et al., 2018). Fumigants were frequently used, according to prior experiences, to control pests in stored goods. According to Terziev and Petkova-Geogieva (2019), chemical pesticides resulted in numerous problems pertaining to our health and environment. Plant-derived products have demonstrated to be excellent sources of a variety of volatiles with the potential for development as alternatives to conventional insecticides. Researchers were looking for natural substitutes that pose less of a threat to the environment or people's health as a result of resistance to synthetic pesticides brought on by their overuse (Abdelli et al., 2016). Essential oils (EOs) have gained attention as a pest management alternative recently, and a number of aromatic and medicinal plants have demonstrated outstanding results in the preservation of grain supplies and the control of pests (Oyedeji et al., 2005; De Ribeiro et al., 2020). EOs are organic compounds that are naturally derived from plants and have a variety of biological characteristics, including antibacterial, antioxidant, and insecticidal

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actions. They can be employed as natural alternatives to synthetic chemicals to combat microbial resistance and to control pests (Atares & Chiralt, 2016). Several studies have investigated the effect of EOs on pest stored products. Among these plants there are for example coriander, caraway, *Artemisia*, and pennyroyal (Bachrouch et al., 2023; Sriti et al., 2017a, 2022; Aouini et al., 2023). It is necessary to look for new extracts of plants that are more active. About 70 species of the Rutacea family's *Haplophyllum* genus can be found in the Sudano-Zambezian, Irano-Turanian, Saharo-Arabian, and Mediterranean regions (Soltani & Khosravi, 2005). In traditional medicine, *Haplophyllum* species were typically used as a treatment for headaches, arthritis, skin discolouration, wart removal, and parasite illnesses.

The objective of this study was:

a) to determine the chemical composition of EO extracted from *H. tuberculatum* aerial parts,
b) to assess its insecticidal effect,
c) to evaluate the fumigant and repellent effect of four organic solvent extracts (chloroform, acetone, methanol, and water) against coleopteran insects: *S. oryzae*, *L. serricorne* and *T. castaneum*.

2 Materials and Methods

2.1 Preparation of Extracts

The aerial parts of *H. tuberculatum* were collected during 2022 from Benguerdaine region (southeast of Tunisia, longitude: 33° 07′ 59″ N, latitude: 11° 12′ 58″ E, altitude: 57 m). The lyophilized powder of *H. tuberculatum* aerial part (20 g of dry matter) was successively extracted to Soxhlet apparatus during 3 h using chloroform, acetone, methanol, and water (Mejri et al., 2022). Between each step, the solvent extracts were filtered (Whatman grade 4), concentrated under rotary evaporator at 40 °C and stored at – 20 °C until used for analyses.

2.2 Extraction and analysis of EOs

The aerial part of plant (300 g of dry matter) was hydrodistilled for three hours. The obtained EO was stored in glass vials in darkness at 4 °C until use.

An Agilent 7,890A gas chromatograph was coupled to an Agilent 5,972 C mass spectroscopy detector with electron impact ionization (70 eV). An HP-5 MS capillary column (30 m × 0.25 mm) was used. The column temperature was programmed as follows: from 60 °C to 260 °C with a rate of 5 °C.min^{-1}, from 260 °C to 340 °C with a rate of 40 °C.min^{-1}. The carrier gas was helium N60 with a 0.9 ml.min^{-1} flow rate; split ratio was 100 : 1. Scan time and mass range were 1 s and 50–550 m.z^{-1}, respectively. The identification of compounds was based on mass spectra.

2.3 Insect Rearing

The rice weevil *S. oryzae*, the cigarette beetle *L. serricorne*, and the red flour beetle *T. castaneum* utilized in our investigation they were all from bulk cultures kept in lab settings: a temperature set at 25 ±1 °C and relative humidity (RH) maintained of 65 ±5%. In all trials, the adult insects used were of mixed-sex and 7–14 days old.

2.4 Fumigant Toxicity

Fumigants were considered non-residual treatments in that they do not leave residues to prevent future pest infestations. The fumigant was confined for the planned exposure period to achieve the lethal dosage required to control the target pests, prior to the fumigant being actively or passively aerated. Whatman No. 1 filter paper with a diameter of 0,02 m was impregnated with oil dosages calculated to provide comparable fumigant concentrations ranging from 12.5 to 625 µl.l^{-1} air in order to assess the fumigant toxicity of EOs. Next, a 40 ml Plexiglas bottle's screw closures were used to secure the impregnated filter paper to the bottom. Caps were screwed tightly on the vials containing 10 adults for each species. The mortality of insects was calculated after 1, 6, 12, 18, 24, 30, 36, 40, 48, 64 and 72 h of exposure. When no leg or antennal movements were observed, insects were considered dead. The mortality was calculated using the Abbott correction formula (Abbott, 1925).

2.5 Bioassay for Repellent Activity

This assay was used to study the repulsive effect of the extract tested against the three beetles by determining the percentage of repulsion of each extract as well as the median dose of repulsion. The repellent effect of extracts on coleopteran was evaluated using the preferential filter paper method described by Jilani and Saxena (1990). Thus, the 0.08 m diameter Whatman filter paper discs were used. For this purpose, they were cut into two equal parts. Four concentrations of extracts were used for the three insects: 1 mg.ml^{-1}, 0.5 mg.ml^{-1}, 0.25 mg.ml^{-1} and 0.125 mg.ml^{-1} by dilution in acetone. Then 0.150 ml of each dilution thus prepared was spread uniformly over one half of the disc while the other half received only 0.150 ml of acetone. Five repetitions were performed for each concentration. After 15 min which was time required for complete evaporation of solvent, the two halves of the disks were attached using adhesive tape. The reconstituted filter paper disc was placed in a Petri dish and 20 insects of mixed sex (ratio 1 : 1) were placed in the center of each disc. The mortality monitoring of the insects on the Petri dish was carried out according to kinetics of 1 h up to 24 h for each tested concentration. Number of insects (*T. castaneum*, *S. oryzae* and *L. serricorne*) presented on the part of filter paper treated with *Haplophyllum* extract (Nt) and the number of those presented on the part treated only with acetone (Nc) were recorded.

2.6 Resistant Dose Median and Percentage

After varying exposure times, the counts of the three insects on the treated and untreated sections of the experimental paper halves were noted. Nerio et al. (2009) determined percentage repellency (PR) in the following way (1):

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100 \qquad (1)$$

where: *Nt* – represents the number of insects on the treated region following the exposure period; *Nc* – represents the number of insects on the untreated area

2.7 Statistical Analysis

Every extraction and calculation were carried out three times. The mean and standard deviation (SD) of the data were calculated. Using SPSS 23.0 (SPSS IBM2017), an analysis of variance (ANOVA) was conducted. The Duncan test was applied to the means to detect significant differences of repellency among concentration sand oils at the 0.05% level. Data were presented in tables as means with standard errors.

3 Results and Discussion

3.1 EO Composition

The average yield obtained from EOs extracted from studied *Haplophyllum* aerial parts was 0.21% (Table1). Our findings concurred with those of Sriti et al. (2017b), who found that the aerial component of *Haplophyllum* had an EO output of 0.24%. The EO yield of *Haplophyllum* from Oman was 0.21% (Al-Burtamani et al., 2005), who also observed that our results were consistent with their findings. However, El-Naggar et al. (2014) investigated the aerial parts essential oil of several Egypt based growing regions and discovered that the production ranged from 0.31% to 0.65%. The primary consequences of geographical and ecological changes among habitats can be used to explain this variation in essential oil output. Additionally, genetics, maturity stage, environmental, ontogenetic, and analytical methodologies can all be blamed for variances in oil output.

Chemical composition identified 19 compounds which β-phellandrene (16.17%) and *trans-p*-menth-2-ene-1-ol were the major compounds (13.22%) (Table 1). Similar results were obtained by Sriti et al.(2017b) who found out that *trans-p*-menth-2-ene-1-ol (18.23%) was the major constitute in EOs from *Tunisia*. *Haplophyllum* EO can be considered an important source of *trans-p*-menth-2-ene-1-ol, which was known for its insecticidal activities and for its effectiveness against numerous Anopheles gambiae spp of mosquitoes where inducing 100% of mortality to both sensitive and resistant *A. gambiae* (De Sousa et al., 2011). EO was characterized by a predominance of monoterpene alcohols (44.84%) (Table 1). This class is followed by monoterpene hydrocarbons (44.07%).

Table 1 Chemical composition of the essential oil of *Haplophyllum tuberculatum* aerial parts

Compound*	IR	(%)
Monoterpene hydrocarbons (%)	44.07 ±0.89	
α-pinene	939	6.35 ±0.34
Camphene	954	2.56 ±0.02
β-pinene	980	3.27 ±0.03
β-myrcene	991	2.21 ±0.04
α-phellandrene	999	8.26 ±0.51
δ-³-carene	1,011	1.79 ±0.03
α-terpinene	1,018	2.46 ±0.00
***p*-cymene**	1,026	1.00 ±0.00
β-phellandrene	1,048	16.17 ±1.02
Oxygenated monoterpenes (%)	44.84 ±1.14	
***cis-p*-menth-2-ene-1-ol**	1,108	8.29 ±0.76
***trans-p*-menth-2-ene-1-ol**	1,123	13.22 ±1.12
***cis*-piperitol**	1,180	3.08 ±0.03
***trans*-piperitol**	1,191	4.59 ±0.04
Piperitone	1,216	4.40 ±0.02
Octyl acetate	1,149	4.16 ±0.01
Bornyl acetate	1,270	4.93 ±0.11
n-octyl 2-methyl butyrate	–	1.11 ±0.00
Butanoic acid 3-octyl ester	–	1.06 ±0.00
Sesquiterpene hydrocarbons (%)	0.07 ±0.00	
Valencene	1,490	0.07 ±0.00
Yield (%)	0.21 ±0.03	

* order of elution in HP-5 MS column

3.2 *Fumigant*

The adults of *T. castaneum*, *S. oryzae*, and *L. serricorne* were fumigated with EO of *H. tuberculatum*, and the results demonstrated that the insecticidal effect depends on dose and the length of exposure (Fig. 1). Thus, the EO of *Haplophyllum* was more toxic for *L. serricorne*, causing a 100% of mortality recorded with the dose 625 $\mu l.l^{-1}$ air already after 12 h of exposure. Furthermore, after 72 h of exposure, *L. serricorne* insects had a 23% mortality rate at the lowest dose (12.5 $\mu l.l^{-1}$ air). Because the main constituents of *H. tuberculatum*'s essential oil were known to be harmful to a variety of mosquitoes, the oil's high toxicity was necessary (De Sousa et al., 2011). The highest concentration (625 $\mu l.l^{-1}$ air) caused 96% mortality after only 72 h of exposure (Fig. 1). Moreover, the lowest concentration (12.5 $\mu l.l^{-1}$ air) caused 6% mortality of *T. castaneum* insects after 72 h of exposure. Indeed, fumigant impact varied according to essential oil concentration and duration exposure. The same pattern was observed when *S. oryzae* was involved, as shown in Fig. 1. Actually, the risk of death rose with increasing dose. On the other hand, it found that the greatest concentration of *H. tuberculatum* caused 90% death on *S. oryzae* after 72 h of exposure, in contrast to the other coleopteran pest (*T. castaneum*). There were no previous citations concerning the insecticidal effects of *H. tuberculatum* aerial part EO from *Tunisia* on adults of *T. castaneum*, *S. oryzae*, and *L. serricorne*. In this context, the insecticidal activity of the aerial parts of *H. tuberculatum* against *Culex quinquefasciatus* has been established by Ghasemzadeh et al. (2018) and these authors showed that the ethanol extract possesses good insecticidal activity. Probit analysis after 24 h for the *Haplophyllum* EO tested was shown in Table 2. *L. serricorne* was more susceptible than other species. Probit analysis showed that *H. tuberculatum* (LC_{50} = 38.59 $\mu l.l^{-1}$ air) was more toxic to *L. serricorne* than *T. castaneum* (LC_{50} = 601.20 $\mu l.l^{-1}$ air) and *S. oryzae* (LC_{50} = 601.20 $\mu l.l^{-1}$ air). According to Aouini et al. (2023), *C. sativum*, *C. carvi* and *A. herba-alba* possess insecticidal effects on *T. castaneum*, *S. oryzae* and *L. serricorne*. These authors reported that *A. herba-alba* EO showed good insecticidal activity with 24-h against

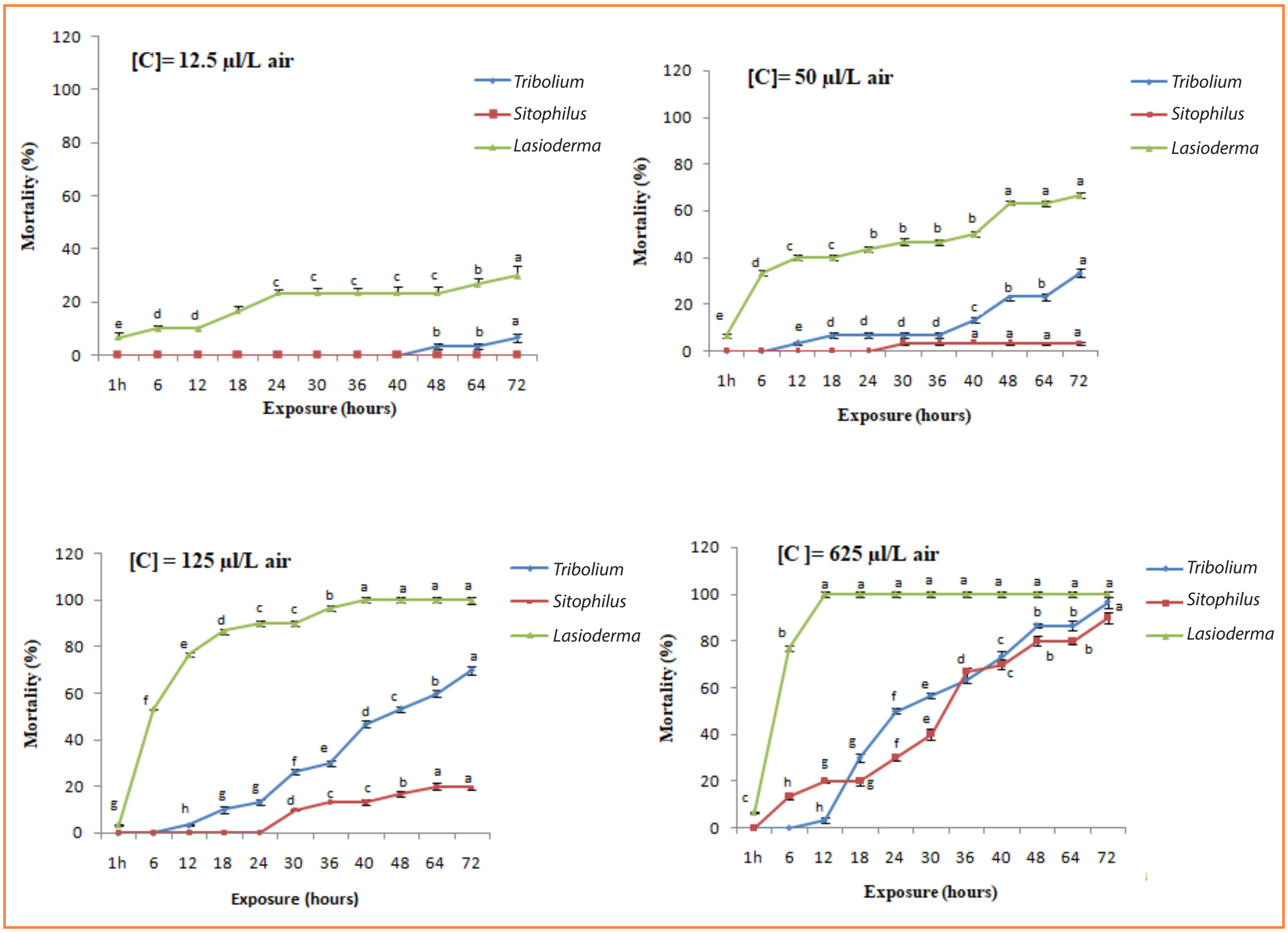

Figure 1 Percentage of mortality of *Tribolium castaneum*, *Lasioderma serricorne*, and *Sitophilus oryzae* exposed for various periods of time to essential oil of *Haplophyllum tuberculatum*
All measurements were done in triplicate (*n* = 3); values followed different letters (a-h) in the same column indicated significant differences by the Duncan test different at $P < 0.05$

Table 2 LC_{50} and LC_{95} (µl.l^{-1} air) values of *Haplophyllum tuberculatum* essential oil against *Tribolium castaneum*, *Sitophilus oryzae* and *Lasioderma serricorne*

Insects	LC_{50} (µl.l^{-1} air)	LC_{95} (µl.l^{-1}) air	Khi-deux χ^2
Tribolium castaneum	601.20^{b}	7981.84^{a}	0.287
Sitophilus oryzae	816.74^{a}	5395.71^{b}	0.39
Lasioderma serricorne	38.59^{c}	267.53^{c}	4.86

Units LC_{50} and LC_{95} µl.l^{-1} air, applied for 24 h at 25°C

L. serricorne (LC_{50} = 29.7 µl.l^{-1} air) and against *T. castaneum* (66.1 µl.l^{-1} air). Ghasemzadeh et al. (2018) proved that the EO of *H. tuberculatum* were strongly toxic against *P. interpunctella* where the Mortality rate reached of 86.7 exposed to different concentrations. Numerous researches (Bachrouchet al., 2015; Sriti et al., 2017a; Aouini et al., 2023) have shown that the insecticidal efficiency of EO varies according on the type of experiment, duration of exposure, source, concentration, stage of the insect, and species.

3.3 Repellent Activity

Based on the RD_{50} values (Table 3), the chloroform and acetone extracts were strongly toxic against *T. castaneum* with 0.03 mg.ml^{-1}. The RD_{50} values for methanol and water fractions at 24 h after treatment were 0.04 and 0.07 mg.ml^{-1}, respectively. The obtained results showed that a polar solvent (chloroform and acetone) at 24 h after treatment was the most noxious against *T. castaneum* and the polar solvent presented lowest repellent activity. However, it appeared that the chloroform extract (0.23 mg.ml^{-1}) of *Haplophyllum* had more repellent activity against *S. oryzae*. The methanol and water extract also maintained an important toxicity, when RD_{50} had 0.40 and 0.56 mg.ml^{-1}, respectively. Acetone was used to elicit a complete adulticide action in *L. serricorne* at a repulsive dose of 0.14 mg.ml^{-1}. The substantial repellent effect was likewise demonstrated by the methanol and chloroform extracts, with RD_{50} values of 0.23 and 0.28 mg.ml^{-1}, respectively. Therefore, all of these extracts showed an intriguing ability to resist *L. serricorne*, *T. castaneum*, and *S. oryzae* beetles. It is obvious that haplophyllum extract had the ability to develop a novel and secure control product against these three insects.

All *Haplophyllum* extracts performed exceptionally well against *T. castaneum* in the repellency test (Table 4). After 24 h of contact, the water extract killed 30% and 90% of the insects for doses of 0.125 mg.ml^{-1} and 1 mg.ml^{-1}, respectively. Activity gradually increased over dosages. This activity did, in fact, grow as a result of exposure concentration. The same insect was impacted by the highest concentration of chloroform extract (1 mg.ml^{-1}) in a lower and more intense way, resulting in 86% fatality. At a dosage of 1 mg.ml^{-1}, the methanol and acetone extracts did indeed exhibit the highest repellency. The proportion of *Haplophyllum* that was tested on *T. castaneum* showed that water, chloroform, acetone, and methanol were the most

Table 3 Repellency doses RD_{50} and RD_{95} values (mg.ml^{-1}) of *Haplophyllum* extract against adults of *T. castenum S. oryzae* and *L. serricorne*

Insect	Repellent	Chloroform	Acetone	Methanol	Water
T. castaneum	RD_{50}	0.03 (0.00–0.08)^{Aa}	0.03 (0.00–0.085)^{Aa}	0.04 (0.00–0.09)^{Aa}	0.07 (0.01–0.12)^{Ba}
	RD_{95}	1.24 (0.65–10.27)	3.02 (1.10–382.85)	2.63 (1.08–71.90)	2.01 (1.0–13.29)
	slope ±SEM	1.06 ±0.31	0.82 ±0.28	0.92 ±0.28	1.13 ±0.28
	χ^2	0.615	1.13	0.252	3.99
S. oryzae	RD_{50}	0.23 (0.12–0.35)^{AB}	1.47 (0.69–234.74)^{Cc}	0.40 (0.22–1.13)^{Bc}	0.56 (0.33–1.94)^{Bc}
	RD_{95}	13.35 (3.66–735.24)	181.81 (11.65–93)	258.92 (19.59–3835.92)	40.084 (5.82–6,032.31)
	slope ±SEM	0.93 ±0.24	0.78 ±0.31	0.58 ±0.17	0.88 ±0.30
	χ^2	0.074	0.519	4.71	0.28
L. serricorne	RD_{50}	0.28 (0.03–0.65)^{BB}	0.14 (0.00–0.27)^{AB}	0.23 (0.001–0.52)^{BB}	0.47 (0.24–2.60)^{CB}
	RD_{95}	12.829 (2.27–1,660)	22.36 (3.29–34)	15.18 (2.27–6,723.13)	101.26 (8.80–5136.65)
	slope ±SEM	0.99 ±0.43	0.74 ±0.30	0.90 ±0.43	0.70 ±0.22
	χ^2	0.054	0.089	0.125	2.24

Applied for 24 h at 25°C. 95% lower and upper confidence limits were shown in parenthesis; values followed by the same letter are not significantly different according to LSD test at *P* ≤0.05

Table 4 Percentage repellency (%) of *Haplophyllum tuberculatum* extracts against *T. castaneum*, *S. oryzae* and *L. serricorne* adults after 24 h of exposure

Extracts	Percentage repellency (%)				Response class			
	0.125 ($mg.ml^{-1}$)	0.25 ($mg.ml^{-1}$)	0.5 ($mg.ml^{-1}$)	1 ($mg.ml^{-1}$)				
Tribolium castaneum								
Chloroform	40 ±2.33^{Ad}	70 ±3.23^{Ac}	76.67 ±2.58^{Ab}	86.67 ±2.33^{Ba}	II	IV	IV	V
Acetone	40 ±2.46^{Ad}	56.67 ±2.17^{Bc}	60 ±1.12^{Cb}	83.33 ±3.14^{Ba}	II	IV	IV	V
Methanol	30 ±1.33^{Bd}	53.33 ±2.06^{Bc}	70 ±1.70^{Bb}	76.67 ±2.78^{Ca}	II	IC	IV	IV
Water	30 ±1.05^{Bd}	40 ±2.15^{Cc}	53.33 ±^{Db}	90 ±2.70^{Aa}	II	II	IV	V
Sitophilus oryzae								
Chloroform	-20 ±1.41^{Bd}	0 ±0.00^{Ac}	26.67 ±3.21^{Ab}	43.33 ±2.44^{Aa}	O	O	II	III
Acetone	-60 ±2.11^{Dd}	-50 ±2.18^{Cc}	-20 ±1.43^{Db}	-15 ±1.11^{Da}	O	O	O	O
Methanol	-6.67 ±0.41^{Ad}	0 ±0.00^{Ac}	3.33 ±0.03^{Bb}	10 ±1.55^{Ca}	O	O	I	I
Water	-45 ±1.62^{Cd}	-20 ±1.57^{Bc}	-10 ±0.77^{Cb}	20 ±1.01^{Ba}	O	O	O	II
Lasioderma serricorne								
Chloroform	-30 ±0.56^{Cd}	0 ±0.00^{Bc}	20 ±1.53^{Cb}	40 ±1.04^{Ba}	O	O	I	II
Acetone	15 ±0.89^{Ad}	35 ±1.72^{Ac}	40 ±2.34^{Ab}	45 ±2.11^{Aa}	I	II	II	III
Methanol	-20 ±1.34^{Bd}	0 ±0.00^{Bc}	30 ±1.76^{Bb}	40 ±1.12^{Ba}	O	O	II	II
Water	-60 ±1.81^{Dd}	-20 ±1.23^{Cc}	-5 ±0.03^{Db}	10 ±1.01^{Ca}	O	O	O	I

Comparison made between mean values from the same column (by letters in uppercase) revealed exposure time impact on insect repellency. Comparison made between mean values from the same row (by letters in lowercase) indicated oil concentration impact on insect repellency. Values followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$
Repellency classes: O = <0.1; class I = 0.1–20; class II = 20.1–40; class III = 40.1–60; class IV = 60.1–80; class V = 80.1–100, based on Juliana and Su (1983)

powerful repellents. Except for methanol extract, which showed class IV repellency status after 24 h of exposure, water, chloroform, and acetone all demonstrated class V repellency status at greater concentrations (1 $mg.ml^{-1}$) against *T. castaneum*. Extracts of *H. tuberculatum* were the most effective, potentially controlling *T. castaneum*. Even at the highest doses (1 $mg.ml^{-1}$), a significant repellent efficacy (43%) against *S. oryzae* was seen after 24 h of contact (Table 4). The water extract did definitely display classes II repellency status for the maximum doses and for 24 h of contact. At a dose of 1 $mg.ml^{-1}$ against *S. oryzae*, the acetone and methanol fractions showed the lowest toxicity (Table 4). Chloroform came out on top over water, acetone, and methanol as the fractions that repelled *S. oryzae* the most. After 24 h of contact, the chloroform, methanol, and acetone extract had a moderate (40%) level of toxicity for *L. serricorne*, but the water (10%) fraction had the lowest level of toxicity at this concentration (Table 4).

4 Conclusions

The current study's results showed that *H. tuberculatum* essential oil possesses insecticidal properties against adults of *T. castaneum*, *S. oryzae*, and *L. serricorne*. These properties can be mostly attributed to monoterpenoids, which are substances with insecticidal action against a variety of insect species. However, the intricate interactions between the essential oil's many components which may even have additive or synergistic effects for those present at low concentrations are what give rise to the inhibitory activity of the oil. The findings of our investigation indicated that the various extracts and essential oils from *H. tuberculatum* oil have strong repellent and fumigant actions against pests in stored products. This research should evaluate the oil residues in food, flavour quality of food, and persistence experiments. However, more research is needed to determine whether *H. tuberculatum* is capable of reducing stored-products insect populations in IPM programs.

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