

Insecticidal efficacy and chemical composition of *Balanites aegyptiaca* (L.) Delile seed oils against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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ABSTRACT

Botanical pesticides are a new trend for pest management because they are an environmentally safe alternative for synthetic chemicals. The aim of this study was to investigate the insecticidal activity of desert date (*Balanites aegyptiaca* [L.] Delile) seed oils against the red flour beetle (*Tribolium castaneum* Herbst) and determine the oil chemical compounds by gas chromatography-mass spectrometry. Oils were extracted by chloroform, hexane, and ethanol, and they were tested on the pest by the film residue method at doses of 1.131, 0.566, 0.283, and 0.142 mg cm⁻² after 12, 24, 36, and 48 h. Results showed that chloroform achieved a 100% mortality rate at 1.131 and 0.566 mg cm⁻² after 12 and 36 h exposure, respectively. Meanwhile, the hexane extract caused a similar effect after 24 and 48 h at the same doses, respectively. The chloroform extract scored the lowest median lethal dose (LD₅₀, 0.134 mg cm⁻²) against the pest after 48 h of exposure. This finding indicated that chloroform extract was the most toxic for *T. castaneum* compared with the other extracts. The results of the oil analysis revealed that (9Z,12Z)-octadeca-9,12-dienoic acid, hexadecanoic acid, (Z)-octadec-9-enoic acid, and (E)-octadec-6-enoic acid were the main components, but the concentration differed from one extract to another. These results suggest that chloroform and hexane extracts have potent insecticidal activity and could be used in grain storage to control pests.

Key words: Chloroform extract, contact toxicity, desert date, LD₅₀, *Tribolium castaneum*.

INTRODUCTION

Stored product pests can cause damage and economic loss for grains and commercial products. Insects damage the stored products by direct feeding or contaminating commodities with body parts and setae, and can render them unpalatable or unmarketable. Moreover, insect pest infestation reduces the rate of seed germination and viability and promotes infections involving bacterial and fungal diseases (Lazzari and Lazzari, 2012; Johnson, 2013; Yaseen et al., 2019). Therefore, many pesticides, particularly fumigation pesticides, have been used to control stored grain pests (Wijayarathne et al., 2018; Agrafioti et al., 2019). This approach has created many problems, the most important being toxicity to humans and animals, the increasing cost of pesticides, and emergence of insect strains resistant to most pesticides (Maksymiv, 2015; Damalas and Koutroubas, 2016).

The previously mentioned problems and many other known drawbacks of synthetic pesticides have created the urgent need to develop new alternatives of chemical control, provided that new measures are economically viable, environmentally safe, and ecologically compatible. Therefore, various alternative measures were studied to resolve pesticide problems, such as the evaluation of botanical extracts (Satti and Elamin, 2012; Dhaniya and Dayanandan, 2016; Chiffelle et al., 2019; Mendoza-García et al., 2019; Rahim and Iqbal, 2019). This method has several advantages;

for example, easily applied, safe for humans and the environment, easily biodegradable plant extracts, acceptable price affordable by traditional farmers, selectivity, and high efficacy (Rozman, 2015; Trivedi et al., 2018). Several plants, such as the desert date, contain bioactive compounds that can be used to control pests.

The desert date (*Balanites aegyptiaca* [L.] Delile; Zygophyllaceae) is found in most arid to sub-humid areas of Africa and South Asia (Chothani and Vaghasiya, 2011). The tree is widely distributed in the drylands of Africa from Mauritania to Nigeria and eastward to Ethiopia, Somalia, and East Africa (Manji et al., 2013). It contains many secondary metabolites such as alkaloids, saponins, steroids, flavonoids, tannins, and phenolic compounds (Al-Thobaiti and Abu Zeid, 2018; Satpute and Vanmare, 2018; Hu et al., 2019). These multiple chemicals possess different biological activity such as mosquitocidal, larvicidal, and insecticidal properties (Djeghader et al., 2018; Jatau et al., 2018). Therefore, the aim of the study was to evaluate the insecticidal activity of *B. aegyptiaca* seed oils against *Tribolium castaneum* Herbst and determine the oil chemical compounds by gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant materials and insects

Balanites aegyptiaca (L). Delile fruits were collected in September 2018 from the ground under naturally grown trees in the Alsondodab zone (15°22'21" N, 32°43'22" E; 397 m a.s.l.), Khartoum state, Sudan. The collected fruits were identified by Professor Abdalla Abdelrahim Satti, Department of Alternatives to Pesticides and Biocontrol, Environment and Natural Resources Research Institute, National Center for Research, Khartoum, Sudan. Powders were prepared from seed kernels by crushing fruits with a mortar and pistil (locally made in Khartoum, Sudan) and pulverized in an electric blender (Panasonic, MX-J220P, Shanghai, China). These powders were kept in dark bottles to protect them from light degradation, and they were taken to the laboratory at the Institute of Resources and Environmental Engineering, Shanxi University, Shanxi, China. Meanwhile, temperature and relative humidity (RH) at the laboratory ranged from 20 to 25 °C and 25% to 35%, respectively.

Tribolium castaneum Herbst, already identified by the College of Life Sciences at the university, was obtained from a crop and kept at the laboratory of the Institute of Resources and Environmental Engineering, Shanxi University. The insects were reared in glass jars (1200 mL) inside an incubator (Lichen, HSP-70BE, Shanghai, China) at 30 ± 2 °C and 65 ± 8% RH and fed wheat flour (Mishra et al., 2016). For the experiment, 1-7 d old adult insects of mixed sex were selected.

Oil extraction and analysis

The maceration method was used to extract the oils of *B. aegyptiaca* with the three extraction solvents hexane, chloroform, and ethanol. Seed powders (50 g) were placed in conical flasks (500 mL) and 200 mL of each solvent was added separately. The conical flasks were stirred with a magnetic mixer for 30 min and allowed to soak at room temperature for 7 d (Gupta et al., 2016). The filtrate was obtained with a vacuum filter pump with Whitman filter paper. The solvent was evaporated under reduced pressure at 15 to 35 °C with a rotary evaporator (Yar, RE-5299, Shanghai, China) to obtain the extract. The resulting oils were kept in vials (50 mL) and placed in a refrigerator until further use.

The extracted oils were analyzed by gas chromatography-mass spectrometry (GC-MS) equipment (7890B/5977A, Agilent, Santa Clara, California, USA). A capillary column (HP-5MS, 30 m, 0.25 mm ID, df ¼ 0.25 mm) was used for the separation system (Ren et al., 2019). Oven temperature was set at 40 °C (3 min) and reached 250 °C (5 °C min⁻¹), and the carrier gas was highly pure helium (99.999%) with a 1 mL min⁻¹ flow rate. The GC-MS analysis was performed with the MS system (5977A MSD, Agilent). The ion source temperature was 270 °C and the transmission line temperature was 300 °C. The ionization energy was 70 eV and a mass range from 50 to 550 amu. Compounds were identified by comparing their mass spectrum with the mass spectrum of known components from the National Institute of Standards and Technology (NIST) library (NIST, Gaithersburg, Maryland, USA).

Treatment preparations

Treatment preparations started as a pre-step for biological assays. The first step aimed to prepare the concentrations, and the oils were filtered with a syringe filter (13 mm diameter 0.45 µ filter) to improve quality. Afterward, 500 mg of each type of oil was diluted with 10 mL acetone to obtain the highest concentration (50 mg mL⁻¹) for the present study. Other

concentrations (25.0, 12.5, and 6.25 mg mL⁻¹) were prepared by serial dilutions. To calculate the dose per area (mg cm⁻²), the concentration (mg mL⁻¹) in 1 mL (applied on a Petri dish) was divided with the area of the Petri dish (44.20 cm²). Accordingly, the calculated doses were 1.131, 0.566, 0.283, and 0.142 mg cm⁻².

Toxicity assay

The residual film method was selected to determine the contact toxicity of *B. aegyptiaca* oils against *T. castaneum* (Sabiha et al., 2017). Accordingly, 1 mL of each concentration was applied in Petri dishes (7.5 cm) and the solvent (acetone) was left to evaporate for 30 min, 10 *T. castaneum* adults were released in each Petri dish, dishes were placed in the incubator, and each treatment was replicated four times, including the control with only acetone (Paramasivam and Selvi, 2017). Insect mortality was recorded after 12, 24, 36, and 48 h. The experiment used a completely randomized design. Data were analyzed by one-way ANOVA and the level of significant differences was $P < 0.05$. Duncan's multiple range test was used. Probit analysis (Finney, 1971) was used to determine median lethal dose (LD₅₀) values with SPSS version 16.0 (IBM, Armonk, New York, USA).

RESULTS AND DISCUSSION

Toxicity assay

The chloroform extract of *B. aegyptiaca* seeds caused 100% mortality of *T. castaneum* at 1.131 mg cm⁻² after 12 h of exposure; there were nonsignificant differences ($p = 0.611$) with hexane, which obtained 97.5% at the same dose and reached 100% mortality after 24 h. In this 24-h interval the second dose (0.566 mg cm⁻²) exhibited 90% mortality for the chloroform extract and 87.5% for hexane, with no differences ($p = 0.510$) between them. After 36 h, the chloroform extract recorded 100% mortality and hexane extract 97.5%; hexane achieved 100% mortality after 48 h (Table 1). Our result for the hexane extract, was similar to findings by Nwaogu et al. (2013), who observed 100% mortality of cowpea weevil (*Callosobruchus maculatus* [Fabr.]) after 48 h on cowpea treated with 0.5 mL hexane extract of *B. aegyptiaca* seeds. In addition, various studies have demonstrated the insecticidal activities of chloroform extract against pests from other plants such as shrubby whilevein, *Sanchezia speciosa* Hook. F. (Rafshanjani et al., 2014; Mondal et al., 2014). The ethanol extract showed the lowest mortality at all doses compared with those extracts, and the highest dose (1.131 mg cm⁻²) obtained 40% mortality at the end of the experiment (48 h) (Table 1). This result could imply that bioactive ingredients extracted with this solvent were low compared with other solvents.

In general, the chloroform extract caused 100% mortality at 1.131 and 0.566 mg cm⁻² after 12 and 36 h of exposure, respectively, and the hexane extract after 24 and 48 h at the same doses, respectively (Table 1).

Table 1. Mortality of red flour beetle (*Tribolium castaneum*) adults exposed to oil doses (hexane, chloroform, and ethanol extracts) of desert date (*Balanites aegyptiaca*) seeds in a contact toxicity bioassay after 12, 24, 36, and 48 h.

Treatment	Dose mg cm ⁻²	Mortality ± SE			
		12 h	24 h	36 h	48 h
		%			
<i>Balanites aegyptiaca</i> chloroform	1.131	100.0 ± 0.25a	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a
	0.566	77.5 ± 0.25b	90.0 ± 0.00b	100.0 ± 0.00a	100.0 ± 0.00a
	0.283	37.5 ± 0.48d	45.0 ± 0.29c	52.5 ± 0.48b	57.5 ± 0.25b
	0.142	5.0 ± 0.50fg	12.5 ± 0.48de	12.5 ± 0.48d	15.0 ± 0.29de
<i>B. aegyptiaca</i> hexane	1.131	97.5 ± 0.25a	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a
	0.566	65.0 ± 0.50c	87.5 ± 0.25b	97.5 ± 0.25a	100.0 ± 0.00a
	0.283	27.5 ± 0.48e	37.5 ± 0.48c	50.0 ± 0.41b	52.5 ± 0.25b
	0.142	5.0 ± 0.29ef	7.5 ± 0.25ef	7.5 ± 0.25de	12.5 ± 0.48de
<i>B. aegyptiaca</i> ethanol	1.131	12.5 ± 0.48f	20.0 ± 0.00d	32.5 ± 0.25c	40.0 ± 0.41c
	0.566	5.0 ± 0.29fg	10.0 ± 0.41e	12.5 ± 0.48d	20.0 ± 0.41d
	0.283	2.5 ± 0.25fg	5.0 ± 0.29ef	5.0 ± 0.29de	7.5 ± 0.25ef
	0.142	0.0 ± 0.00g	0.0 ± 0.00f	0.0 ± 0.00e	0.0 ± 0.00f
Control	0.00	0.0 ± 0.00g	0.0 ± 0.00f	0.0 ± 0.00e	0.0 ± 0.00f

Values represent the mean of four replicates each consisting of 10 insects. Means followed by the same letters are not significantly different from each other at $P < 0.05$.

The LD₅₀ of the chloroform extract were 0.347, 0.285, 0.250, and 0.239 mg cm⁻² after 12, 24, 36, and 48 h of exposure, respectively, 0.411, 0.327, 0.271, and 0.251 mg cm⁻² after 12, 24, 36, and 48 h, respectively, for the hexane extract, and 5.564, 3.392, 1.784, and mg cm⁻² after 12, 24, 36, and 48 h, respectively, for the ethanol extract (Table 2). We observed that the LD₅₀ of chloroform after 12, 24, and 36 h was similar to the LD₅₀ of the hexane extract after 24, 36, and 48 h, respectively. The LD₅₀ values of the chloroform extract detected in the present study concur with Khan et al. (2014), who reported that the LD₅₀ of the chloroform fraction of the rhizome of the oakleaf fern (*Drynaria quercifolia* [L.] J. Sm.) against *T. castaneum* was 0.400 and 0.370 mg cm⁻² after 24 and 48 h, respectively. The small differences are related to which part of the plant was evaluated, which means that *B. aegyptiaca* seeds could be more toxic for pests than the rhizome of *D. quercifolia*.

It is suggested that contact toxicity could be attributable to the high quantity and/or good quality of active compounds in *B. aegyptiaca* seeds. According to Kumar et al. (2019), the seeds of this plant contain alkaloids, saponins, steroids, flavonoids, tannins, and phenolic compounds, which have different biological actions such as insecticidal activity. Similarly, the GC-MS analysis detected (9Z,12Z)-octadeca-9,12-dienoic acid, hexadecanoic acid, (Z)-octadec-9-enoic acid, 3,3-dihydroxypropyl hexadecanoate, ethyl hexadecanoate, and methyl hexadecanoate in the plant, which have good insecticidal activity (Hema et al., 2011; Ramos-López et al., 2012; Koul, 2016). Furthermore, Elamin and Satti (2013) demonstrated the insecticidal potential of *B. aegyptiaca* seeds against stored product pest Khapra beetle (*Trogoderma granarium* Everts).

Phytochemical analysis

The major components of *B. aegyptiaca* seed oils (chloroform, hexane, and ethanol extracts) identified in the present study were (9Z,12Z)-octadeca-9,12-dienoic acid (63.08%, 63.66%, and 64.52%), hexadecanoic acid (15.88%, 15.86%, and 10.63%), (Z)-octadec-9-enoic acid (11.35%, 9.01%, and 9.11%), and (E)-octadec-6-enoic acid (4.86%, 3.95%, and 7.65%), respectively (Tables 3, 4, and 5). More main components were found in certain extracts, such as 2,3-dihydroxypropyl(E)-octadec-9-enoate (1.30%) in the chloroform extract (Table 3) and 1,3-dihydroxypropan-2-yl (Z)-octadec-9-enoate (6.66%) and 1-3-hydroxypropan-2-yl (9Z,12Z)-octadeca-9,12-dienoate (2.65%) in the ethanol extract (Table 5). The results were consistent with findings by Bai et al. (2014), who indicated that the GC-MS analysis for the chloroform extract of babul (*Acacia nilotica* [L.] Delile) leaves detected various compounds, including (9Z,12Z)-octadeca-9,12-dienoic acid, hexadecanoic acid, and ethyl hexadecanoate. These findings concur with those mentioned by Kalpana et al. (2012), who detected (9Z,12Z)-octadeca-9,12-dienoic acid, hexadecanoic acid, and (Z)-octadec-9-enoic acid in the ethanol extract of ladynut (*Entada pursaetha* DC.) seeds. Warra and Abubakar (2015) reported that the hexane extract of jatropha (*Jatropha curcas* L.) seeds contained fatty acids such as (Z)-octadec-9-enoic acid, octadecanoic acid, and (E)-octadec-6-enoic acid.

Table 2. Median lethal dose (LD₅₀) values of desert date (*Balanites aegyptiaca*) seed oils (hexane, chloroform, and ethanol extracts) against red flour beetle (*Tribolium castaneum*) adults in a contact toxicity bioassay after 12, 24, 36, and 48 h.

Extract	Exposure time	LD ₅₀	95% Confidence intervals		Chi-square; χ^2 (df)
			Lower limit	Upper limit	
	h	mg cm ⁻²			
Chloroform	12	0.347	0.30	0.41	1.999 (2)
	24	0.285	0.25	0.33	0.807 (2)
	36	0.251	0.22	0.29	2.973 (2)
	48	0.239	0.21	0.27	2.407 (2)
Hexane	12	0.411	0.35	0.48	1.047 (2)
	24	0.327	0.28	0.38	1.593 (2)
	36	0.271	0.24	0.30	0.638 (2)
	48	0.251	0.22	0.29	2.973 (2)
Ethanol	12	5.564	2.01	99292.87	0.348 (2)
	24	3.392	1.64	73.31	0.736 (2)
	36	1.784	1.197	4.837	0.517 (2)
	48	1.398	1.005	2.769	0.803 (2)

df: Degrees of freedom.

Table 3. List of compounds detected in the chloroform extract of *Balanites aegyptiaca* seeds.

Nr	Compound	Molecular formula	Retention time	Peak area
			Min	%
1	Pyridine	C ₅ H ₅ N	4.87	0.03
2	Furan-3-carbaldehyde	C ₅ H ₄ O ₂	6.36	0.03
3	2-Fluoro-2-methylpropane	C ₄ H ₉ F	15.94	0.22
4	Trimethyl-(2-propan-2ylidene-cyclopropyl)silane	C ₉ H ₁₈ Si	30.97	0.07
5	1-Methylcyclohexane-1,2,3,4,5,6-hexol	C ₇ H ₁₄ O ₆	38.90	0.03
6	Methyl hexadecanoate	C ₁₇ H ₃₄ O ₂	41.41	0.31
7	Ethyl hexadecanoate	C ₁₈ H ₃₆ O ₂	43.09	0.31
8	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	43.45	15.88
9	(9Z,12Z)-Octadeca-9,12-dienoic acid	C ₁₈ H ₃₂ O ₂	47.36	63.08
10	(E)-Octadec-6-enoic acid	C ₁₈ H ₃₄ O ₂	47.84	4.86
11	1-3-Hydroxypropan-2-yl-(9Z,12Z)-octadeca-9,12-dienoate	C ₂₁ H ₃₈ O ₄	50.48	0.88
12	2,3-Dihydroxypropyl (E)-octadec-9-enoate	C ₂₁ H ₄₀ O ₄	50.62	1.30
13	(Z)-Octadec-9-enoic acid	C ₁₈ H ₃₄ O ₂	50.75	11.35
14	3,3-Dihydroxypropyl hexadecanoate	C ₁₉ H ₃₈ O ₄	54.16	0.52

Table 4. List of compounds detected in hexane extract of *Balanites aegyptiaca* seeds.

Nr	Compound	Molecular formula	Retention time	Peak area
			Min	%
1	Benzene	C ₆ H ₆	2.58	0.02
2	Methyl hexadecanoate	C ₁₇ H ₃₄ O ₂	41.40	0.02
3	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	43.90	15.86
4	(9Z,12Z)-Octadeca-9,12-dienoic acid	C ₁₈ H ₃₂ O ₂	47.17	63.66
5	(E)-Octadec-6-enoic acid	C ₁₈ H ₃₄ O ₂	47.73	9.01
6	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	49.93	0.83
7	(Z)-Octadec-9-enoic acid	C ₁₈ H ₃₄ O ₂	51.52	3.95
8	1-3-Hydroxypropan-2-yl (9Z,12Z)-octadeca-9,12-dienoate	C ₂₁ H ₃₈ O ₄	52.41	0.05

Table 5. List of compounds detected in ethanol extract of *Balanites aegyptiaca* seeds.

Nr	Compound	Molecular formula	Retention time	Peak area
			Min	%
1	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	43.82	10.63
2	(9Z,12Z)-Octadeca-9,12-dienoic acid	C ₁₈ H ₃₆ O ₂	45.25	64.52
3	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	45.77	0.26
4	(Z)-Octadec-9-enoic acid	C ₁₈ H ₃₄ O ₂	47.55	9.11
5	(E)-Octadec-6-enoic acid	C ₁₈ H ₃₄ O ₂	47.62	7.65
6	1-3-Hydroxypropan-2-yl (9Z,12Z)-octadeca-9,12-dienoate	C ₂₁ H ₃₈ O ₄	50.47	2.65
7	1,3-dihydroxypropan-2-yl (Z)-octadec-9-enoate	C ₂₁ H ₄₀ O ₄	50.73	6.66
8	3,3-Dihydroxypropyl hexadecanoate	C ₁₉ H ₃₈ O ₄	54.12	0.28

CONCLUSIONS

Balanites aegyptiaca seed oils exhibit strong insecticidal activity against *Tribolium castaneum* adults through contact toxicity. The main chemical compounds of the oils are (9Z,12Z)-octadeca-9,12-dienoic acid, hexadecanoic acid, (Z)-octadec-9-enoic acid, and (E)-octadec-6-enoic acid. These findings suggest that *B. aegyptiaca* oils could be useful as a botanical insecticide against the pest. Moreover, our study can be considered as an approach to future studies about this plant to produce a commercial insecticide.

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