

CHEMICAL INVESTIGATION OF THE LIPOIDAL MATTER OF *GLOSSOSTEMON BRUGUIERI* AND THE ACARICIDAL ACTIVITY OF ITS UNSAPONIFIABLE FRACTION

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MOGHAT
UNSAPONIFIABLE
SAPONIFIABLE
ACARICIDAL EFFECT

SUMMARY: The unsaponifiable matter (USM) and the total fatty acid methyl ester (FAME) fractions of *Glossostemon bruguieri* (Desf.) growing in Egypt were determined for seeds, leaves and roots. Qualitative and quantitative variations were found by GLC analysis of the "USM" of the three botanical parts. Analysis of FAME revealed that palmitic, stearic, oleic and linoleic were the main fatty acids in the investigated botanical parts. Moreover, acaricidal activity of the unsaponifiable matter of each organ was performed, from which the leaves was the most toxic to both adult and egg stages of *Tetranychus urticae* Koch.

MOGHAT
NON SAPONIFIABLE
SAPONIFIABLE
EFFET ACARICIDE

RÉSUMÉ: Les fractions non saponifiable (USM) et les acides gras méthyl ester totaux (FAME) de la plante égyptienne *Glossostemon bruguieri* (Desf.) sont extraits des graines, feuilles et racines. Des variations qualitatives et quantitatives sont notées par analyse GLC des différentes parties de la plante. L'analyse des FAME révèle que les acides palmitique, stéarique, oléique and linoléique sont les principaux acides gras. L'activité acaricide de la fraction non saponifiable de ces organes est testée, et ceux des feuilles sont les plus toxiques pour les adultes et les œufs de *Tetranychus urticae* Koch.

INTRODUCTION

Glossostemon bruguieri (Desf.) "Moghat" is a plant with nutritional value. The powdered roots with some additives (spices and flavouring agents), sugar and butter are used for preparing a hot drink, specially in winter (IBN SINA, 1923). Ladies, after delivery, used this drink as a general tonic and for increasing uterine contraction and milk secretion. In folk medicine the roots are used as a tonic, for increasing the body weight, also as demulcent and for relief of gout pain (IBN-EL-BITAR, 1871).

Chemical studies on the root reported mucilage (KARAWAYA *et al.*, 1971), proteins and steroidal hormones (AMIN *et al.*, 1969).

No reports were traced in the literature concerning the identification of the unsaponifiable matter of the different plant parts. Accordingly, it was considered interesting to study this subject. The saponifiable fraction, because it is oily in nature, does not penetrate through the leaves treated with it, so its effect will not be clear, and hence the unsaponifiable matter was only the subject of this study. It was noticed that neither the vegetative part nor the roots were infected

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with insects in the field. Scattered individual mites in the field were noticed, but, as the leaves are curled and hairy, the insects and mites are deterred from the plant. The "USM" of the different plant parts were therefore tested biologically for acaricidal activity.

MATERIAL AND METHODS

A. Plant material: *G. bruguieri* (Desf.) seeds were cultivated in the Pharmaceutical Science Department Farm at Giza for two years. The roots were harvested from 1 and 2 year old plants, seeds were collected from the mature fruits, and leaf samples were collected from plants at flowering stage.

B. Apparatus: GLC of the (USM) and (FAME) were carried out under the following conditions:

Condition	Unsap. (USM)	Sap. (FAME)
Column	OV.17 (1%)	PEGA (10%)
Attenuation	5×10^{-2}	5×10^{-2}
Oven Temp.	10°/min. from 70–270° C, then isothermal at 270° C for 25 min.	70–190° C (10°/min.), then isothermal for 25 min.
Det. Temp.	300° C	220° C
Inject. Temp.	280° C	300° C
Flow rate of nitrogen	30 ml/min.	30 ml/min.
Flow rate of air	330 ml/min.	330 ml/min.
Sample size	4 µl	5 µl

C. Rearing of the experimental animals: The mite individuals used were obtained from laboratory culture of the two spotted spider mite (*T. urticae* Koch) on lima bean (*Phaseolus vulgaris* L.) leaves, under $25 \pm 2^\circ \text{C}$ and $70 \pm 5\%$ relative humidity.

Extraction and Fractionation of Lipids

The powdered seeds, leaves and roots (100 g each) were separately extracted to exhaustion with petroleum ether (boiling range $60\text{--}80^\circ \text{C}$) in a soxhlet apparatus. The dry lipid portion of each plant part was saponified (FARAG *et al.*, 1986). The unsaponifiable matter (USM) and the fatty acids methyl esters (FAME) were prepared (JOHNSON & DAVENPART, 1971). The USM of each organ was chromatographed on silica gel plates using benzene-ethyl acetate (86 : 14) and toluene-acetone-acetic acid (10 : 3 : 0.25). GLC fractionation of the USM and FAME

of each plant part was conducted using the aforementioned conditions (BUZAN *et al.*, 1969; LITTLEWOOD, 1970). Peak identification was performed by comparing the relative retention time of the component with those of standard materials. The percentage of each component was calculated using a Pu 4810 computing integrator (Philips).

Toxicity of the Unsaponifiable Matter (USM) on egg stage

Twenty female mites were transferred to the lower surface of raspberry leaf discs and left for oviposition for 24 hours and removed thereafter. The accumulated eggs were sprayed with various concentrations of different USM samples. Each test contained 5 concentrations, each with 5 replicates (20 eggs/replicate), and each assay was replicated twice. In every test, a water control was included. Six days after treatment, the number of unhatched eggs was counted. Mortality counts for eggs were corrected using ABBOTT'S (1925) formula and the mortality data were submitted to probit analysis by the method of FINNEY (1952).

Biological Test

Newly emerged females of *T. urticae* were transferred singly to the lower surface of raspberry leaf discs treated with various concentrations of the different USM samples. The different biological aspects of the females were followed. Another group of mites was placed on untreated discs, which served as a control. Twenty replicates were set up for each concentration. Statistical analyses were carried out using the F-test method.

RESULTS

Chemical results

The percentages of total lipids in plant organs were 23.50, 6.70 and 0.75% for seeds, leaves and roots, respectively.

Data of gas chromatographic fractionation (Table 1) revealed the presence of hydrocarbons

Peak No.	RRt	Relative percentage			Authentic materials
		Seeds	Leaves	Root	
1	0.32	—	—	0.43	n-Tetradecane
2	0.33	—	—	1.83	-Unknown
3	0.36	—	—	5.96	-Unknown
4	0.39	—	—	5.85	-Unknown
5	0.45	—	0.51	7.96	n-Pentadecane
6	0.49	0.76	1.87	5.97	n-Hexadecane
7	0.52	—	2.62	5.76	n-Heptadecane
8	0.55	4.94	2.16	4.91	n-Octadecane
9	0.58	2.25	0.84	4.52	n-Nonadecane
10	0.60	1.72	3.62	2.74	-Unknown
11	0.66	4.68	3.94	5.88	n-Eicosane
12	0.69	1.74	0.56	—	n-Hencosane
13	0.71	0.73	1.57	0.38	β -Amayrin
14	0.77	4.88	18.29	2.50	n-Docosane
15	0.82	15.56	1.95	—	n-Tricosane
16	0.85	2.86	1.49	6.11	n-Tetracosane
17	0.89	4.22	2.77	5.20	n-Pentacosane
18	0.93	3.59	3.47	4.75	n-Hexacosane
19	0.98	—	5.12	—	24-Methylene cycloartenol
20	1.0	40.21	9.12	19.70	n-Octacosane
21	1.06	0.22	10.91	1.18	Squalene
22	1.11	—	3.62	5.80	n-Triacontane
23	1.18	—	3.67	—	n-Untriacontane
24	1.27	—	5.76	—	n-Dotriacontane
25	1.37	—	0.87	—	-Unknown
26	1.41	—	0.96	—	Cholesterol
27	1.52	—	3.24	2.43	Campesterol
28	1.64	—	0.41	0.99	Stigmasterol
29	1.73	10.31	2.69	—	β -Sitosterol
30	1.86	—	6.04	—	-Unknown
31	1.89	—	1.02	—	-Unknown

TABLE 1: GLC fraction of the unsaponifiable fractions of Moghat seeds, leaves and roots. RRt: Retention time relative to n-octacosane.

from C_{14} to C_{32} , with a common occurrence of hydrocarbons from C_{16} to C_{19} , C_{20} to C_{22} and from C_{24} to C_{26} in the three botanical parts with quantitative variations in their percentage. N-Octacosane was the major component in the USM of the three plant parts, while n-tricosane (15.65%) in the seeds and n-docosane (1.9%) in the leaves were major components. However, n-tricosane (3.62%), n-unrtiacontane (3.67%), n-dotriacontane (5.76%), 24-methylene cyclo-artenol (5.12%) and cholesterol (0.96%) were detected in the USM of the leaves only. Squalene commonly occurred in the USM of the three plant parts, with higher amounts being found in the leaves (10.51%). Sterols were present in appreciable quantities in the USM of Moghat leaves.

Peak No.	RRt	Relative percentage				Authentic materials
		Seeds	Leaves	Root 2 year	Root 2 year	
1	0.25	0.97	—	0.90	0.50	Hexanoic
2	0.34	0.73	—	0.210.69	0.21	Heptanoic
3	0.41	0.77	1.22	0.89	0.43	Caprylic
4	0.49	0.27	0.22	0.35	2.27	Nonanoic
5	0.56	0.22	0.66	0.12	2.64	Capric
6	0.64	0.51	—	0.09	1.68	Undecanoic
7	0.72	0.21	1.53	3.90	3.19	Lauric
8	0.81	0.14	0.90	1.12	1.93	Tridecanoic
9	0.86	3.20	0.27	2.78	1.39	Myristic
10	0.94	—	3.50	2.20	1.80	Myristoleic
11	1.00	22.52	29.14	23.24	22.86	Palmitic
12	1.07	5.30	2.46	2.82	1.55	Heptadecanoic
13	1.23	17.55	—	35.6	14.42	Stearic
14	1.28	32.91	18.42	7.66	1.38	Oleic
15	1.31	5.54	25.96	5.89	31.24	Linoleic
16	1.43	2.07	4.18	6.38	4.88	Linolenic
17	1.55	4.66	—	3.37	2.83	Arachidic
18	1.83	2.39	10.49	1.71	4.39	Eicosenoic

TABLE 2: GLC fraction of the fatty acid methyl esters of Moghat seeds, leaves and roots. RRt: Retention time relative to methyl palmitate (19.37).

GLC study of the methylated saponifiable fractions (Table 2) revealed quantitative variation. The analysis showed that saturated fatty acids of the seeds represented about 52.54%. Palmitic (22.52%) and stearic (7.55%) were the major fatty acids. The unsaturated fatty acids amounted to 42.91%, of which oleic acid (32.91%) was the major one. The saponifiable fraction of the leaves was composed mainly of unsaturated fatty acids (about 60.64%), linoleic (20.96%) and oleic (18.42%) are the major acids, accompanied by saturated fatty acids (35.6%), of which palmitic (29.14%) was the major one. The saponifiable fractions of the one-year-old roots, composed mainly of saturated fatty acids (about 66.54%), palmitic and stearic being the major fatty acids. The roots of two-year-old plants resembled those of one-year-old, but with a lower saturated fatty acid content (52.72%).

Biological results

With respect to the biological study, Table 3 represents the data for toxicity of the USM of Moghat plant against egg stage of *T. urticae*. The leaves pro-

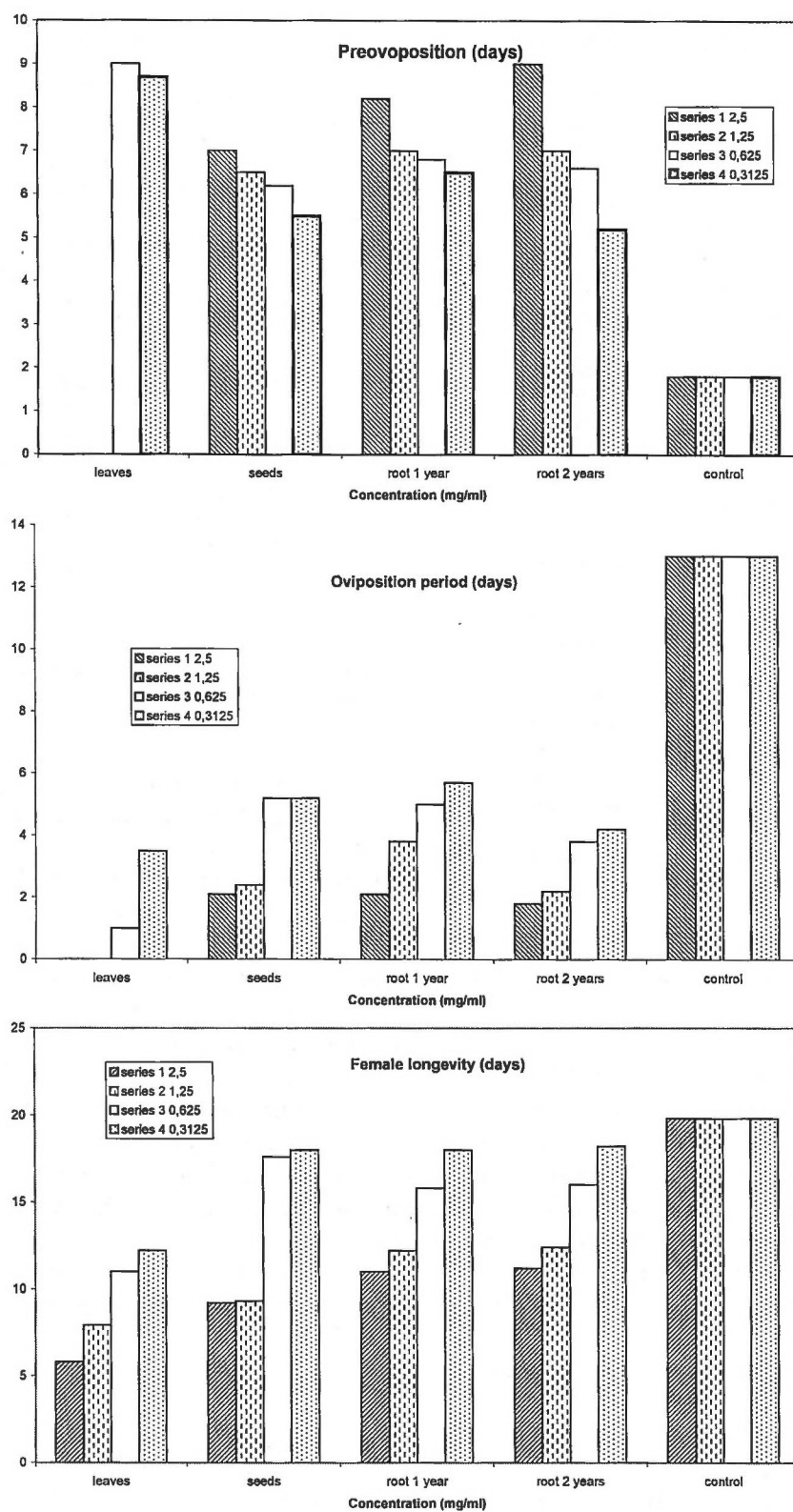


FIG. 1: Effect of the unsaponifiable matter of moghat parts on the different biological aspect of *T. urticae*.

Unsaponifiable matter of	LC ₅₀ mg/ml	LC ₉₀ mg/ml	Slope	Toxicity index at		No. of folds compared with seeds "USM" at	
				LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
Leaves	1.715	8.634	1.82	100	100	14.43	224.81
Roots (1 year old)	2.788	28.49	1.27	61.51	30.31	8.87	68.13
Roots (2 years old)	5.917	31.08	1.78	28.98	27.78	4.18	62.45
Seeds	24.74	1941	0.68	6.93	0.44	1.00	1.00

TABLE 3: Toxicity of the unsaponifiable matter of Moghat on the egg stages of *T. urticae*.

ved to be the most toxic organ, with an LC₅₀ of 100, followed by roots of one-year-old plants.

Leaves of Moghat had the greatest affect on fecundity, as shown in Table 4. No eggs were laid at the higher USM concentrations. All the other organs had an affect on the fecundity.

DISCUSSION

Moghat seeds contains the highest unsaponifiable matter because it is in this organ that oils are accumulate, followed by small amounts in leaves and very little in the roots, which are mainly constituted of starch and mucilage (IBRAHIM, 1997).

The GLC fraction of USM of the different plant organs of Moghat revealed quantitative and qualitative differences. Spot I, having R_f values 0.89 and 0.82, represented the hydrocarbon mixture. GLC fractionation revealed the presence of hydrocarbons from C₁₄ to C₃₂ in the three botanical organs. N-octacosane was the major component in seeds and roots, while n-tricosane (15.56%) was the major in the seeds, and n-docosane (18.29%) dominated in the leaves. The following compounds were detected only in the leaves: n-untriacontane, 24-methylene cycloar-

tenol, cholesterol and two unknown compounds. The highest amount of squalene was present in leaves (10.91%).

Unsaponifiable matter of Moghat leaves was the most toxic fraction to the egg stage of *T. urticae*, while that of the seeds induced the least effect, as indicated by the LC₅₀ values (Table 3).

As seen from Table 4, the unsaponifiable matter affects the fecundity of female. Moghat leaves USM was the most and significantly active organ with all the concentrations used. The eggs laid per female were the lowest for the USM of leaves. The preoviposition period was significantly lengthened by the USM of different organs.

The longevity of females was shortened (Fig. 1) by all the treatments applied. The oviposition period was also sharply decreased by these treatments.

The chemical and biological results obtained suggest that the hydrocarbons of the USM were responsible for the different effects induced. The leaves contained some hydrocarbons that other organs lacked, and these hydrocarbons represent higher values in the leaves, so they may be responsible for the effect itself or their occurrence with other hydrocarbons may cause synergistic effects and hence increased activity. SIDDQUI *et al.* (1988) reported that the hydrocarbon fraction of neem twigs is toxic against mosquitoes (LC₅₀ = 20 ppm) and may act as insect growth regulators. It may be noted in this context that the hydrocarbon fractions isolated from dried neem leaves possessed larvicidal activity against mosquitoes (CHAVAN, 1984).

With respect to the role of sterols, which are present in higher amount in Moghat leaves, BOWERS *et al.* (1966) reported that terpene and sterol derivatives mimic insect hormones, disturbing the life cycle of the pest. This may explain the shortening of larval

Unsaponifiable matter of	Total No. of eggs/female conc. (mg/ml)				L.S.D.	
	2.5	1.25	0.625	0.3125	0.05	0.01
Leaves	0 ± 0	0 ± 0	0.1 ± 0.07	1.03 ± 0.42	2.29	3.03
Roots (2 years old)	0.6 ± 0.41	0.9 ± 0.44	1.9 ± 0.7	2.1 ± 1.07	2.85	3.77
Roots (1 year old)	1.8 ± 0.48	3.8 ± 1.014	8.6 ± 2.07	11.9 ± 2.71	5.47	7.25
Seeds	3.2 ± 1.92	4.4 ± 2.31	8.2 ± 2.02	16.1 ± 2.94	6.26	8.29
L.S.D. (0.05)	3.37	3.97	4.31	7.61		
L.S.D. (0.01)	4.47	5.25	5.71	10.07		

TABLE 4: The effect of the unsaponifiable matter of Moghat on the fecundity of females of *T. urticae*.

period induced by USM. REDFERN *et al.* (1982) attributed the controlling effect of the endocrine hormones, leading to increased time of molting, to the terpenes and sterols. All these conclusions reported by the previous authors are in agreement with the results obtained in this investigation.

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