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Contribution to the Habitat and Seed Analysis of *Argemone mexicana* L. Grown in Al-Taif, Saudi Arabia

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ABSTRACT. The soil characters and phytosocial habitat of *Argemone mexicana* L. (Papaveraceae) growing near Al-Taif, Saudi Arabia, is described. The seed oil contains alkaloids and has been analysed for the usual characters. Five fatty acids, with linoleic (61.4%) dominant, have been identified by TLC and GLC.

Argemone mexicana L. belongs to the family Papaveraceae (Täckholm 1974 and Migahid 1978). The plant (Fig. 1) grows in tropics and subtropics of both hemispheres.

There is a vast literature concerning the chemistry of the oil obtained from the seeds of *Argemone mexicana* L. growing in countries other than Saudi Arabia. Bui (1974) studied the oil content from seeds grown in Vietnam, finding that they contained 52.8% oil and 0.43% sanguinarine alkaloid; seeds cultivated in Pyatigorsk (USSR) yielded 35.84% oil, and in addition to sanguinarine contained another alkaloid, allocryptonine.

Bose (1971) studied *Argemone* oil by thin layer chromatography and he was able to separate and identify two alkaloids by using seven solvents.

Several investigations have reported methods for the detection of traces of *Argemone* seeds or oil as contaminants of other materials. Hartman *et al.* (1972) used a paper chromatographic method for the detection of *Argemone* seeds in mustard seeds. Afag *et al.* (1977) studied the market sample of seeds sold in Hyderabad market as Kala Dhatura and revealed that the market sample was the seeds of *Argemone mexicana* and not of *Datura fastuosa*.

Fig. 1. Photo of *Argemone mexicana*.

Bose (1972) developed a colourimetric method for estimation of *Argemone* oil as low as 0.005% in other edible oils. Mohiuddin and Zaidi (1973) used spot and colour tests for detection of *Argemone mexicana* seed oil. They used acidified ceric ammonium nitrate and $NaNO_3$ solution which developed a bright orange colour with *Argemone* oil. The limit of detection was 0.003% with $NaNO_2$. Bose and Roy (1977) developed another method for the detection of oil of *Argemone mexicana* in edible oils by reacting the *Argemone* oil alkaloids with $FeCl_2$ and detecting the resulting crystals microscopically.

Mani and Lakshminarayana (1972) identified the keto, hydroxy, and epoxy fatty acids in *Argemone mexicana* seed oil growing in India; myristic, palmitic, oleic and linoleic acids were also found. Gunstone *et al.* (1977) studied a crystalline material which separated from *Argemone mexicana* seed oil, and it was mixture of 9- and 11-oxo-octacosanoic and 11-oxotriacontanoic acids.

The physiological effects of *Argemone* oil have received some attention. Ranadive *et al.* (1973) studied the carcinogenicity of *Argemone* oil alone or combined with soybean oil; they found no carcinogenicity, but in combination with mustard oil, a slight carcinogenic effect was observed. Ramasastri and Babu (1975) studied the toxicity of *Argemone* oil in experimental animals. They found that daily inges-

tion of 0.025 ml *Argemone* oil/kg body weight by monkeys for 60 days did not produce any toxic symptoms, but some toxic manifestation were observed at a dose of 0.05 ml oil/kg. Bose (1977) removed the toxic alkaloids from the *Argemone* oil resulting in a detoxified oil.

The review of literature shows no work was done on the oil of the Saudi Arabian species *Argemone mexicana*. The present work is an attempt to record the habitat in which *Argemone mexicana* lives. Also the physical and chemical characters of the oil extracted from the seeds were studied. Its constituents were identified by thin and gas-liquid chromatography.

Experimental

Habitat and Source of Material

About one kg of seeds was collected in November, 1980, from plants growing naturally in the desert area near Al-Taif which is located about 100 km from Makkah. These seeds were used for the oil study, and this was the location of the habitat studied. Edaphic factors and vegetation analysis were conducted following usual procedures (Piper 1950, Braun-Blanquet 1932 and Oosting 1956) for soil and vegetation analysis.

Extraction

The pulverised seeds (100 g) were extracted with ethyl ether (free from peroxide) for 24 hr in a soxhlet extractor. The lipids were obtained by distilling off the solvent under reduced pressure (34.22% yield).

Purification of the Oil

The oil gave a positive reaction with Dragendorff's reagent on spot plate, indicating the presence of alkaloids. Accordingly, it was subjected to the following treatment to remove the alkaloids. The oil was shaken several times with 4% HCl, until the acidic extract failed to give a positive reaction with Dragendorff's reagent. The oil, after being free from alkaloids, was washed again with distilled water to remove any traces of the acid. It was then dried over anhydrous sodium sulphate and filtered. This purified, dry, oil was used for the subsequent investigations.

Physical and Chemical Methods

The physical and chemical characters of the extracted oil were studied by the methods of Guenther (1972).

A. *Thin-layer Chromatographic Investigation of Unsaturated Fatty Acids*

The unsaturated fatty acids were detected in the presence of saturated fatty acids by thin layer chromatography. The chromatoplates (20 × 20 cm) were co-

vered with thin layer (250 mm) of Keiselguhr, and after dryness they were impregnated with 5% paraffin in benzene (Shalaby and Steinegger 1964). A sample of fatty acids, prepared by saponification of the purified oil, and references of pure unsaturated fatty acids were spotted on the chromatoplates. The plates were developed in a solvent system of 80% aqueous acetic acid, and the run was continued to a distance of not more than 12 cm. After drying, the spots were revealed by iodine vapour.

B. *Thin-layer Chromatographic Investigation of Saturated Fatty Acids*

The investigation of saturated fatty acids was carried out by two dimensional development using thin layer chromatography (Shalaby and Steinegger 1964). The chromatoplates were spotted in one corner with the extracted fatty acids and developed in a system of aqueous acetic acid (30%) : formic acid : hydrogen peroxide (6:1:1) at 32°C, where the unsaturated fatty acids were oxidized and moved to the front of the chromatoplate leaving behind the saturated fatty acids. After drying at 100°C, the chromatoplate was redeveloped in the second dimension using the system of 90% aqueous acetic acid. After development, the chromatoplates were dried and sprayed with 0.05% Rhodamin-B solution, followed by 10% potassium hydroxide solution.

C. *Gas-Liquid Chromatography*

Gas-liquid chromatographic analyses were conducted on a Pye Unicam dual flame ionization detector, Series 104, under the following conditions; a column package of 10% PEGA (Polyethylene glycol adipate) on chromosorb W, 100-120 mesh; column, 0.5 cm diameter, 6 feet length; column temperature 190°C isothermal; detector temperature 250°C; injector temperature 220°C; carrier gas flow rate (N_2) 45 ml/min; hydrogen flow rate 45 ml/min; air flow rate 450 ml/min; chart speed 5 mm/minute.

For the gas-liquid chromatographic analyses, the purified oil was saponified, and the liberated fatty acids were converted to methyl esters (using HCl-methanol or diazomethane). The methyl esters were chromatographed. The amount of the components were computed by estimation of the area under each peak, by the formula: height × base at half height.

Results

I. Edaphic Factors

The soil at Al-Taif site is yellow-white and sandy. In Table 1 are given the results of its analysis.

Table 1. Analysis of soil supporting *Argemone mexicana*.

Depth (cm)	pH	Total soluble salts (%)	Carbonate (%)	Cl (%)	Organic carbon (%)	Water holding capacity (%)
0-5	7.3	0.091	1.04	0.0036	0.39	26.48
5-10	7.2	0.068	3.08	0.0071	0.27	26.20
10-25	7.2	0.074	3.77	0.0085	0.60	30.90
25-50	7.3	0.075	0.110	0.0053	0.33	33.69

II. Vegetation Analysis

The total plant cover in the area studied at Al-Taif ranged from 20-30% and was mostly contributed by *Lycium shawii* Roem. et Sch. and *Peganum harmala* L. *Argemone mexicana* L. was consistently present in all the represented 20 quadrats (5 × 5 m) studied. There are 18 other associates with low presence estimates and with low values in abundance-dominance scale and sociability (Table 2).

III. Oil Analysis

The mean number of fruits per plant was 160 ± 19, and the mean number of seeds per fruit was 410 ± 32. The mean weight of one seed was 2.1 ± 0.06 mg. Accordingly, the expected amount of seeds to be obtained from one plant was about 137.8 g. The moisture content in seeds used was 12.917%, and the ash content was 29.15%.

Characters and Investigation of Lipids

The lipid fraction extracted with ethyl ether from seeds was orange-yellow liquid having a faint and agreable odour. It stained with fat stains and gave a positive Libermann-Burchard's test for sterols. It was found to be easily soluble in petroleum ether, benzene, chloroform, acetone, carbon tetrachloride, and warm ethanol. The oil gave positive reactions with Dragendorff's, Mayer's, and Wagner's reagents for alkaloids.

The percentage of the lipid was 34.22%. Table 3 summarizes the physical and chemical characters of the oil.

A. *Thin-layer chromatography.* Two unsaturated fatty acids, namely oleic and linoleic acids, were detected by thin layer chromatography.

Table 2. Phytosociological study of the area at Al-Taif dominated by *Argemone mexicana* in November 1982, showing the constituent species. (The first figure represents a combined scale of abundance-dominance and the second represents the sociability (Braun-Blanquet 1932).

Species	Phenology	Ab.-domin./sociability scale
Lycium shawii Roem. et Sch.	Fl., Fr.	4.4
Peganum harmala L.	Fl.	3.4
Argemone mexicana L.	Fl.	2.2
Cynodon dactylon (L.) Pers.	Gr.	–
Panicum turgidum Forssk.	Fr.	–
Tribulus longipetalus Viv.	Fr.	–
Depterygium glaucum Decne	Fl.	–
Cucumis prophetarum Jusl. ap. L.	Fl., Fr.	–
Citrullus colocynthis (L.) Schrad.	Fr.	–
Acacia sp.	Fl.	–
Malva parviflora L.	Fl.	–
Solanum dubium Fres.	Fr.	–
Indifofera spinosa Forssk.	Fl.	–
Aerva javanica (Burm.f.) Spreng.	Fl.	–
Trigonella stellata Forssk.	Gr.	–
Farsetia burtonae Oliv.	Fr.	–
Aizoon hispanicum L.	Gr.	–
Euphorbia granulata Forssk.	Fl.	–
Sonchus oleraceus L.	Fl.	–
Fagonia cretica L.	Fl.	–
Erodium subtrilobum Jord.	Fl.	–

Table 3. Chemical values for *Argemone* seed oil.

Test	Result
Lipid content	34.22%
Specific gravity	0.9292
Refractive index	1.4480
Acid value	2.53
Ester value	203.8
Saponification value	206.43
Iodine value	165.1
Unsaponifiable matter	2.20%
Total fatty acids	91.64%

Only two saturated fatty acids, namely stearic and palmitic acids, were detected.

B. *Gas liquid chromatographic separation of the fatty acids.* The gas-liquid chromatographic analysis, shown in Fig. 2, indicates that the mixed fatty acids are separated into myristic (0.6%), palmitic (17.3%), stearic (0.3%), oleic (20.0%), and linoleic (61.4%).

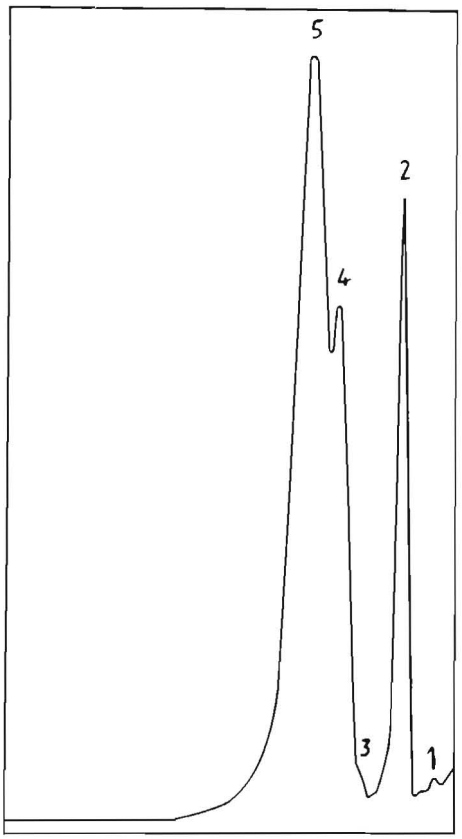

Fig. 2. Gas chromatographic separation of the fatty acids of *Argemone* seeds.
1 = Myristic acid (0.6%), 2 = Palmitic acid (17.3%), 3 = Stearic acid (0.3%), 4 = Oleic acid (20%), 5 = Linoleic acid (61.4%).

Conclusion

The soil supporting *Argemone mexicana* near Al-Taif was studied for various physical and chemical parameters, and the accompanying vegetation in which

Argemone mexicana L. was living, was studied. One plant yielded about 137.8 g seeds, and the seed contained 34.22% of orange-yellow fixed oil which was positive for alkaloids. The physical and chemical characters of the oil were studied by TLC and GLC. The unsaturated fatty acids identified by TLC were oleic and linoleic acids; the saturated fatty acids were stearic and palmitic acids. Gas-liquid chromatography of the methyl esters indicated that the mixed fatty acids were myristic acid (0.6%), palmitic acid (17.3%), stearic acid (0.36%), oleic acid (20%), and linoleic acid (61.4%). This is the first report of the analysis of these seeds as grown in Saudi Arabia.

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إضـــافـــة إلى بيئـــة ومكـــونـــات بذور نبـات الأرجيمون النامى بصحاري المملكة العربية السعودية

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شمل هذا البحث دراسة الظروف البيئية التي يعيش فيها نبات الأرجيمون بمنطقة الطائف، وقد وجد أن متوسط ماينتجه النبات الواحد من البذور حوالي ١٣٧,٨ جم . كما شمل أيضا دراسة الليبيدات الموجودة بهذه البذور حيث تم استخلاصها وتقديرها فكانت ٢٢,٣٤٪ وعينت لها الصفات المختلفة .

وقدرت بعض الثوابت في الزيت بعد تنقيته من المواد القلوانية الذائبة فيه، منها معامل رقم الحموضة، رقم اليود، رقم التصبن. وتم التعرف على الأحماض الدهنية باستخدام كروماتجرافيا الطبقة الرقيقة وكذلك كروماتوجرافيا الغاز .