COMPARATIVE STUDY OF THE COMMERCIAL VARIETIES OF CUCURBITA AND THEIR POSSIBLE RELATION TO THEIR TAENIFUGAL ACTIVITY OF THEIR SEEDS

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Levon M. Karamanukian, B.A., Ph.C.
American University of Beirut
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Approved by

John Mich ij
Advisor

To my parents

for their understanding and

encouragement which made it possible

to complete this work

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TABLE OF CONTENTS

TABLE OF ILLUSTRATIONS

COMPARATIVE STUDY OF THE COMMERCIAL VARIETIES OF CUCURBITA AND THEIR POSSIBLE RELATION TO THEIR TAENIFUGAL ACTIVITY OF THEIR SEEDS

LEVON M. KARAMANUKIAN

B.A., The American University of Beirut, 1944

Ph.C.,The American University of Beirut, 1948

Biology Department,

A.U.B.

(Approved by Dr. J. Mirhij)

To determine the biological origin of 7 commercially available Cucurbita seeds, vegetative parts of planted seeds were studied. All the samples studied were of Cucurbita Pepo L. or its varieties except for one which was from Cucurbita maxima Duchesne.

Histological studies of these Cucurbita seeds showed enough structural variation in the epidermis of seed testa to form a basis of identification of these commercial samples.

The oil content determinations in the samples of seeds showed a great variation in percentage ranging between 33.22 and 47.94, calculated on air-dry basis.

Determinations of the physico-chemical constants of the oils of Cucurbita seeds, indicate that the oil is a semi-drying one. Also, if discoloration and Acid Value increase upon storage can be controlled, the

oils will form a suitable source of vegetable oil or fat fit for human consumption.

Clinical trials based upon the administration of kernels or parts of kernels indicate that there is no correlation between species and taenicidal activities, that a minimum dose of greater than 250 gms. and up to 500 gms. of whole seeds should be administered. It is also demonstrated that the hypocotyl portions of the kernels contain a higher concentration of taenicidal properties than other parts of the kernels.

Petroleum-ether extracts do not have taenicidal properties but aqueous extracts do show this activity, yet to a less extent than whole decorticated seeds.

INTRODUCTION

The gourd family, Cucurbitaceae, includes about 90 genera and about 700 species widely distributed in tropical regions around the world and some extending into temperate zones. Three genera are cultivated for their edible fruits and /or for their seeds namely, Citrullus, the watermelon, Cucumis, melon and cucumber and Cucurbita which includes pumpkins, squashes and gourds.

Wide interest has been shown by numerous investigators in different branches of botany and agriculture in the five cultivated species of Cucurbita. Of these plant science investigators, the systematist, the morphologist, the geneticist and physiologist are but the few. The reason for such a varied interest lies in the fact that: the different species are easy to grow, since they are annuals (except Cucurbita ficifolia Bouche); the flowers and vegetable parts of which are large and show variation in their structure; while the fruit show almost limitless variation in fruit characteristics, hence highly attractive to the geneticist and taxonomist.

A large quantity of "pumpkin seeds" are consumed yearly in Lebanon as in other Middle East countries as salted seeds or as a taenifuge. On the market, a large number of varieties is always available, differing highly morphologically from each other. The different varieities are disgnated by the merchants according to the country of origin, as for example, Roumanian, Hungarian, Sudanese, Turkish, Balady, etc. In certain instances the commercial origin designation is not necessarily

identical with the habitat, as for example the so called "Chinese" commercial samples could have their origin from any of the Far East countries.

The purpose of this investigation is twofold:

I. The Cucurbita seeds constitute a valuable source of proteins and of vegetable oil, but so far the oil from these seeds have not been exploited fully.

II. Cucurbita seeds have been extensively used throughout the world since the Roman times as a taenifuge or a toenicide, but opinions differ regarding its efficiency. A voluminous literature indicates the importance given at various times to the taenifugal activities of the seeds. Often great variations are seen in the reports of authors on the efficiency of the seeds as taenifuge, and sometimes even contradictory reports. The writer of this thesis tried to investigate to find reason or reasons for such varied results.

The approach to this problem was not an easy one since many points had to be investigated at the same time.

A. Usually in literature we find the mention of "pumpkin seeds" or "Cucurbita seeds" as the seeds on which experiments are conducted. Usually no mention is made of the binomial. The term squash and pumpkin has been applied rather indiscriminately by agriculturists, botanists and laymen, resulting in considerable confusion. The seeds of different species or their hybrids may be termed "pumpkin seeds". The writer of this thesis tried to elucidate this by planting seeds and identifying the different commercial samples, with the idea that perhaps there would be a correlation between toenicidal activity of the seeds with different species.

B. The composition of a plant or a plant part is not the same in all circumstances. Seasonal changes, differences in habitat and in the chemical nature of the soil, and artificial interference by man can produce far-reaching changes in the qualitative and quantitative composition of the plant or plant parts.

In literature we come across the opinion that seeds from different countries of origin differ in their potency as taenifugal agents. It is stated that seeds grown in the Middle East, especially Lebanon, are highly active. The writer of this thesis wanted to see if the above opinion was correct by comparing clinically different seeds of different geographical origin.

C. In the investigation of plants with marked physiological action, it is advantageous to carry out biological experiments. In this way it can be determined whether the plant part as well as the extracted substances possess the specific action. Different authors have evaluated the potency of the seeds differently. Usually the methods employed are "in vitro" experiments. Also, clinical investigations have been reported. Marked differences in reported results could rise from the methods used and also from the type of extracts administered. Since only clinical trials are the final step for evaluation of a drug, experiments were conducted by the writer to see if the seeds or extracts by different solvents have any physiological activity.

D. The reports of the different authors have been based on a wide range of doses administered. The range used varies between 30 and 700 gms. Since the range is very wide, differences in reports could be partly due to the administration of seeds with doses below the minimum

one for therapeutic activity. Experiments were conducted to determine the minimum dose to be used as a taenifuge. Since the parasite is the same in children and adults, the same dose was administered to all ages.

E. Since the therapeutic dose of the seeds to be used is large, the writer investigated the possibility of localization of the active constituent or constituents in the different parts of the seeds.

The qualification of prime importance in a taenifuge is of course the safety of the patient. Cucurbita seeds fulfill this qualification. This paper tried to pave partly the way for further investigations, for the extraction, isolation and possible identification of the active constituent or constituents. Also it tries to show that the seeds are a good, potential source of vegetable oil or fat.

REVIEW OF LITERATURE

I. Taxonomy

The cultivated forms of Cucurbita are grouped now under five species. Archeological findings and historical studies indicate that they are all definitely of American origin (45,62). The five cultivated species have never been found in the wild state (22). At least two species were grown in Peru as early as 2000 B.C. The origin of Cucurbita species seems to be tropical America, Cucurbita ficifolia Bouche remaining in the center of origin, C. mixta Pangalo and C. Pepo L. migrating northward, while the remaining two species southward (61).

The five commonly cultivated species of Cucurbita are as follows:

A. Cucurbita Pepo L. includes the field pumpkins used for pies, canning and cattle feed. Different varieties are known under different names (2, 3, 22, 47) some of which are pumpkin, field pumpkin, summer squash, acorn squash, scallop squash, negro squash, pineapple summer squash, custard marrow, cymlings, pattypans, Zucchinis, and some inedible gourds grown for ornamental purposes, as apple -, bell -, bicolor -, egg -, orange -, and pear - gourds. Also the French names citrouille and giraumon refer to fruits of this species (44).

B. Cucurbita moschata Duchesne includes different names as squash, winter squash, sweet potato pumpkins, Quaker pie pumpkin, Japanese pumpkin, Canada crookneck, China crookneck, Japanese crookneck, winter crookneck, Dunkard and autumn and winter varieties of butternut. The seminole pumpkin (13) is a prototype of this species.

C. Cucurbita mixta Pongalo. This species has been recently separated from C. moschata Duchesne (12, 61). Bailey (2) considers it to be

of the same species as C. moschata Duchesne. This species in commerce has fruits sold under the name of cushaw squashes, Pangalo, and a number of gourds.

D. Cucurbita maxima Duchesne. The fruits of this species are variously known as buttercup squash, mammoth Chile squash, Hubbard squash, turban squash, winter squash, marblehead, sibley, etc. In French, potiron refers to fruits of this species.

E. Cucurbita ficifolia Bouche. This is the only perennial species. The Malabar, the figleaf and other ornamental gourds belong to this species.

The terms "gourds", "pumpkin" and "squash" have been used rather indiscriminately and do not have a real taxonomic meaning (3, 61). The term gourd is used to designate fruits that are hard shelled and are used for ornament or for making utensils and containers. The terms "squash" and "pumpkin" are much confused. Their meanings differ in different countries. Usually the term "pumpkin" is applied to the edible fruit of any species of Cucurbita utilized when ripe as table vegetable, in pies, or as cattle feed. The flesh of the fruit is coarse-grained and has a strong flavor. The term "squash" designates a Cucurbita fruit which has a fine-grained flesh and a mild flavor. These fruits are used in the immature state before the shell becomes hard and are then called "summer squash", or in the mature state when they are called "winter squash". The different fruits of Cucurbita species show a pronounced variation in size, shape and color. These variations are not only seen in the different species, but also in the fruits of the same species (3, 21, 61, 62). Fruits of the Cucurbita may be round, flat, scalloped, elongated, crooknecked, club-shaped, turban-shaped, oval, acorn-shaped, etc. Although there is great

variation in shape, the anatomical and histological characters of the fruits are fairly constant (21, 22, 62).

For the identification of species various authors depend on different sets of characteristics comprising whole flowering plants. Whitaker and Bohn (61) make use of seta, stem structure, androecium, peduncle of fruit, fruit flesh, funicular attachment and seed margin as the basis of the identification. Bailey (2) makes use of seed color, leaf margin, peduncle variation and calyx lobes of the plants for the identification of the species. Bailey (3) in his "The Standard Cyclopedia of Horticulture", subdivides the species into annual and perennial, and makes use of variations in leaf margin and peduncle. Mudalliar (36) relies on the characters of the peduncle and of the corolla, but not on those of the leaves. Post (47) relies on the characters and variations of leaf margin, corolla, calyx and peduncle.

II. Histology

Most taxonomic keys make use of the whole plant which normally requires at least one season for growth and fruit formation. Thus the identification of a sample of seeds found in commerce requires about a year. In some instances, these characteristics are impossible to obtain since some samples of seeds are hybrids that do not produce fruits. Histological studies were carried in the Biology department at A.U.B., where histology of seeds was used as a method of identification. A generalized description of seed histology can be obtained from many sources (6, 21, 40, 42, 44, 45, 62). Usually these sources give the histology of one type of seed without describing the variations in different species and varieties.

Barber (4), Yashuda (65) and Russel (50) have studied comparative seed histology and anatomy for identification of the Cucurbita species. Kondo and Fickel cited in Hayword (21) investigated Cucurbita moschata Duchesne and C. Pepo L. seed histology.

The above mentioned studies are not similar to the ones by the writer as the purpose of his histological work was to identify the commercial samples, some of which do have identical biological origin.

III. Oil Content and Oil Analysis

The seeds of Cucurbita species yield an oil called "pumpkin seed oil". The content of the oil in seeds has been reported within ranges of 25% (23) and 42% (1). The physico-chemical constants during different periods have been determined by investigators using different species or commercial samples.

Schattenfroh (52) in 1894 and Poda (46) in 1898 examined certain physico-chemical constants of the oil from Cucurbita Pepo L. seeds. Hooper (23) in 1908 examined two samples of oil, determining certain physico-chemical constants. In one of the samples a very high Acid Value was reported, while in the second a very low Acid Value. Power and Salway (48) made an extended examination of both seed and oil in 1910, determining physico-chemical constants, the percentage of the different glycerides of fatty acids and isolating certain sterols. Albrecht (1) in 1918 studied the method of oil manufacture from the seeds as practiced in Hungary for food and industry use. In 1919, Lindsey et al (31) determined the moisture and oil content in seeds among other constants. In 1920, Baughman et al (5) determined the percentage of oil and physico-chemical constants of cold pressed virgin oil. In 1934, Riebsomer and

Nesty (49), determined the percentages of moisture and ether-soluble extracted oil, and certain physico-chemical constants. In 1939, Kaufman and Fiedler (25) determined the constants of the oil and concluded that it was suitable for human consumption as food, and the cake left after expression of oil, fit for cattle feeding. Chowdhurry et al (10) studied certain fats of the Cucurbitaceae in 1955.

The averages for the various physico-chemical constants are given by Griffin (19), Winton and Winton (62), Woodman (64) and Jamieson (24).

IV. Taenicidal Activities

Giambattista Porta, in his phytognomonica published in 1588, advocated the "doctrine of signatures" or the belief that the form of each plant indicates its medicinal virtue. Paracelsus, a Swiss physician, likewise believed in this doctrine (29). Probably, the origin of the use of Cucurbita seeds rose from this belief, as the seeds resemble somehow crudely, the mature proglottids of certain taperworms. The taenicidal activities were known to the Romans at the time of Pliny and its use was forgotten for centuries till it was rediscovered in 1683 by Tyson (56) and introduced to medicine by Mongemy in 1820 (32).

The 19th century literature abounds with publications on the taenifugal activities of the "pumpkin seeds". According to Lys (32), the various authors reported successful treatment varying between 50 and 90 per cent.

The seeds were official in the British Pharmacopoeia (6), 1914 and in the Pharmacopeia of the United States of America (40) from 1863 to 1936 (rth to 11th Ed.), under the titles of Cucurbitae Semina Praeparata

and Pepo respectively. The B.P. '14 recognized the seeds from Cucurbita maxima Duchesne, while U.S.P. those of Cucurbita Pepo L. Because of the varying results obtained, the drug was deleted from the pharmacopoeias.

The nature of the active ingredient or ingredients is not yet known. Usually contradictory statements are met in the literature. Dorner and Wolkowich (63) in 1862 reported an alkaloid, cucurbitine in "pumpkin seeds". According to Heckel, cited by Lys (32) and Planchon et al (45), the active principle is a resin, peporesin. This is located according to the author in the chlorophyllose covering of the cotyledons to the extent of 1 gm. per 27 gms. of the layer. Power and Salway (48) tested the resin but were unable to find evidence of any vermifuge effect. In 1931, Neely and Davy (37) showed that the active principle was soluble in 75% ethyl alcohol, but insoluble in petroleum ether, was dialysable, and its activity reduced by boiling with dilute sulfuric acid. This extract did not show precipitation with alkaloidal precipitants. In 1931, Pfister (43) by experiments "in vitro" on earthworm showed that the active ingredient was not an alkaloid or a glycoside but believed that it was a substance volatile with steam. In 1937, Krayer (28) states that the active principle is soluble in water and heat resistant. The author states that at least 200-400 gms. of fresh seeds should be administered to a child, and 400-700 gms. to an adult. In 1937, Schubiger (53) states that the active constituent is a heat resistant resin localised in the embryo and the chlorophyllose layer of the tegmen. Freise (16), in 1938 isolated from the embryo of the seeds a crystalline alkaloid to the extent of 0.12 - 0.285%. This alkaloid was soluble in hot water but slightly in cold water, soluble in alcohol and chloroform. In 1949, Veen and Collier (59)

prepared a deproteinized, stable aqueous extract. In 1950, Colorado Iris et al (11) treated 85 cases of tapeworm infection with an aqueous extract of the seed pulp. In 60 cases out of 85, the scolex was recovered, 19 cases passed no more proglottids, and in 6 cases there was no response to the drug. In 1951, Mazzoti et al (35) used an aqueous extract obtained from 500 gms. of decorticated, powdered seeds. In 1956, Chinese researchers like Feng (15) used combined aqueous extracts of Cucurbita seeds and areca nuts. It seems that the combined action is synergistic. For this purpose extracts of 80-125 gms. of "pumpkin seeds" and of 60-100 gms. of areca nuts were used. Also in 1956, Polish researchers like Kuzmicki (30) and Pawlowski (38, 39) investigated the activity of whole seeds. In 1957, Valentin and Brockelt (58) prepared a concentrated extract and isolated an active crystalline substance. Many of the physical constants of this ingredient were determined. Also the authors tabulated the reactions of this principle with many color reagents.

Taenifugal properties are not specific to the seeds of Cucurbita species. Investigators like Fefer (14) proved the vermufugal properties of watermelon seeds. It is a common practice to use as a taenifuge in this country, seeds of Luffa aegyptaca Mill., family Cucurbitaceae.

From the above review, it is seen that the active principle of the seeds is not yet known. Even, if the active principle was identified, biological tests, especially clinical evaluation is essential. "In vitro" experiments on earthworms, planarians, fishes, etc. may not show the effectiveness of an anthelmintic.

EXPERIMENTAL METHODS

I. Cultivation

Due to lack of available space on the campus of A.U.B., the different samples of seeds were grown in Rayak and Anjar, Beka'a district, Lebanon. These sites were chosen as the land has been used for cultivation of Cucurbita species for a long time. In July 1959, samples of seeds were planted in both districts, the plants only flowered but did not fruit.

In May, 1960, 7 commercial samples were planted in both districts on one lot in Rayak, and on three different lots in Anjar. Of these four lots, only two lots produced fruits, the plants on the other two lots died because of drought prevailing in the district. Fruits could therefore be obtained from one lot in Rayak, and from one lot in Anjar.

The different samples of seeds, a total of nine were planted on hills 30 to 50 cm. across on which the plants after growth could stand. On each hill ten to fifteen seeds were dropped and covered with two to three cms. of soil. After germination only two to three plants were left per hill. Every fifteen days the plants were watered, but no fertilizers were used as these would have enhanced the growth of vines and reduce the number of fruits per plant. Each plant produced on the average two or three large fruits or up to ten small fruits. Plant parts as leaves, stems, flowers were collected and pressed regularly. The fruits were harvested in early October, 1960. Flowers, leaves, stems, peduncles of fruits and fruit flesh were preserved in 95% ethyl alcohol for reference.

Seeds of the fruits were dried and stored in tightly closed glass containers. Photographs were taken of the plants at regular intervals of fifteen to twenty days. Also photographs were taken of the fruits and seeds.

II. Histology

Different samples of seeds were soaked for periods of forty eight hours or more in a solution developed in the Biology Department at A.U.B. during the period of this investigation. This solution consists of 50% v/v of ethyl alcohol, 80 parts and glycerin 20 parts by volume. Seeds soaked in this solution can remain indefinitely in excellent condition, ready for immediate hand sectioning and mounting on slides for microscopic examination. The plant parts remain in a condition as suitable for hand sectioning as fresh plant parts.

From such soaked seeds, surface preparations, cross-sections, and longitudinal sections were made by a hand microtome.

The cellular structure of the seed coats were examined by making use of Schultz's Maceration Fluid (26). Microscopic examination, clearing of sections and microchemical tests were performed by standard methods (18, 20, 55, 60) used in pharmacognosy. The following reagents were used respectively for: clearing of sections, chloral hydrate solution; presence of starch, Wagner's reagent; presence of cellulose, Chlor-sinc-iodide solution; staining of aleurone grains, Hager's reagent and oil or fat, Tincture of Alkanna.

Microscopic measurements were made by the use of calibrated ocular micrometer, and histological sketches were mostly done through the help of a Swift-Ives camera-lucida and Abbe drawing apparatus.

III. Oil Content

A. Sampling. The seeds come in jute bags weighing about 60 kgs. each. Gross samples were taken by means of a sampler, two from the bottom and two from the top, these being taken in opposite directions. The total weight of samples taken for an analysis was 500 to 550 gms. These samples were quartered, two of the diagonal quarters were rejected, the rest was carefully mixed and again subjected to a quartering process in the same manner. Two of the diagonal quarters were taken as final samples, weighing between 110-125 gms. (7, 41, 54).

B. Powdering. The samples obtained by the above method were subjected to grinding by means of an electric mixer. The powder obtained could pass through a sieve No. 8, and 40% of which through a sieve No. 20. Finer powdering was avoided to guard against loss of oil and moisture (57) due to heat produced by the electric mixer.

C. Moisture Determination. Each of the 110-125 gms. sample was mixed, quartered repeatedly and 10-12 gms. of aliquots were taken. These samples were placed in previously dried, tared, thin, flat, aluminum dishes, weighed and then heated at 105° C to constant weight. The percentage loss was then calculated. All weighings were done on a torsion balance, correct to the nearest centigram. All analysis of moisture determinations were performed in duplicates.

D. Extraction and Determination of Oil Content. The dried, weighed samples from the above determinations were transferred quantitatively into clean, paper pulp thimblers. These were placed in Soxhlet fat-extraction apparatus. Petroleum-ether, boiling point 30-60° C was used as a solvent for the extraction of the oil. This solvent was preferred over

others because it is cheaper and is not affected by traces of moisture (57, 64). The powdered seeds in the apparatus was left to soak for periods of 3 to 6 hours, and then subjected to heat from a hot plate by continuous extraction with soxhlet for a period of 3 to 4 hours. At the end of this period, the solution of the extracted oil was transferred to a previously tared aluminum dish, the solvent evaporated by means of hot air, and the oil dried at 105° C in an oven to constant weight. All weighings were done on analytical balances, correct to the fourth decimal place. All analysis was performed in duplicate. The percentage of oil in the seeds was expressed as per cent in original seeds and on air-dired basis.

IV. Physico-chemical Constants of the Oils

A. Extraction of Oils. The oils from the different samples were extracted in the cold by maceration with petroleum-ether "Analar", boiling point 30-60° C. The petroleum-ether solution was then subjected to a cold current of air to remove the solvent. The product was then filtered, and stored in tightly closed containers, in a dark place at temperature of 15-20° C. The oils during the period of extraction were never submitted to a temperature above 25° C.

B. Color. The colors were determined by Lovibond Tintometer. Since on standing the oils show discoloration, two different determinations were done: one, immediately after extraction of the oil, and the second after storing the oils in the dark for a period of 68 days. All samples of oils even though stored in the dark, showed deepening in color. Samples of oils left in the laboratory in tightly closed, colorless bottles showed this discoloration much quicker.

C. Refractive Index. To obtain the Refractive Indices of the oils, a Butyro-refractometer was used at a temperature of 20° C. The oils varied in their Refractive Index between 71.5 and 74.5 scale readings, at 20° C, which were converted to Refractive Index readings.

D. Weights Per Milliliter. The weights per milliliter were determined by the use of pycnometers of 10 ml. capacity. These were weighed empty then full. The difference in weight between the 2 weighings, divided by 10 gave the weight per milliliter. All determinations were performed on Sartorius balance and in duplicates.

E. Acid Value. The "Acid Value" is the number of milligrams of KOH required to neutralize the free fatty acids of 1 gm. of oil (7). About 10 gms. of accurately weighed oil was placed in a tared erlenmeyer flask and 50 ml. of a mixture of equal volumes of alcohol 95% and solvent ether which had been previously neutralized after the addition of 1 ml. of phenolphtalein solution were added. The solution was titrated with 0.1N sodium hydroxide, till a pink color persisted for 15 seconds.

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{\text{weight in gms. of oil}}$$

A = Mls. of 0.1N NaOH, aqueous solution used in the titration .

To see the correlation between color development and rancidity, two determinations were performed for each oil, namely, one just after extraction of the oil from the seeds and the other after letting the oil stand for 68 days in the dark in tightly closed light-resistant glass containers. All weighings of oil were done on Sartorius balances. All determinations were performed in duplicates.

F. Iodine Value. Iodine Value is the weight of iodine absorbed by 100 gms. by weight of the oil. The oil, accurately weighed is placed in a dry iodine flask and 10 ml. of carbon tetrachloride is added, and mixed to dissolve the oil. To this solution 20 ml. of Iodine Monochloride solution is added, and the flask is stoppered, the stopper previously moistened with potassium iodide solution. The mixture is let to stand in a dark place at a temperature of about 17° C. for thirty minutes. To this solution 15 ml. of 10% w/v potassium iodide solution is added, followed by 100 ml. of distilled water. This mixture is titrated with 0.1N sodium thiosulfate solution, using starch mucilage as indicator. A blank is performed without using the oil.

$$\text{Iodine Value} = \frac{(b - a) \times 0.01269 \times 100}{\text{weight in gms. of oil}}$$

a = mls. 0.1N Sodium thiosulfate used for the back titration of iodine from the oil determination

b = mls. of 0.1N Sodium thiosulfate used for the titration of iodine in the blank.

In this determination ICI method (Wij's solution) was used instead of IBr method (Hanus solution) because the Iodine Value of the oil is high (64).

Iodine monochloride solution was prepared by passing pure, dry chlorine gas into a solution of iodine in carbon tetrachloride - glacial acetic acid mixture in the absence of moisture. All determinations were done in duplicates.

G. Saponification Value. The Saponification Value is the number of milligrams of KOH required to neutralise the fatty acids resulting from the complete hydrolysis of one gram of oil.

About 2 gms. of the oil was weighed accurately in a tared 200 ml. saponification flask and 50 ml. of alcoholic solution of potassium hydroxide was added, attached to a reflux condenser and boiled for half an hour, frequently rotating the contents of the flask. After cooling the mixture 1 ml. of phenolphthalein solution was added and the excess of alkali in the mixture titrated with 0.5N HCl till the pink color just disappeared. The same procedure was repeated without using the oil. The Saponification Value was calculated from the following formula:

$$\text{Saponification Value} = \frac{(b - a) \times 0.02805 \times 1000}{\text{weight in gms. of oil}}$$

a = mls. of 0.5N HCl used in titration of oil

b = mls. of 0.5N HCl used in titration of blank

All determinations were done in duplicates, while the weighings on analytical balances.

V. Taenicidal Activities

Seven commercial varieties of Cucurbita seeds were examined for their taenicidal activity, known in commerce as "Hungarian", "Sudanese", "Turkish", "Chinese", "Iranian", "Roumanian" and "Lebanese". Four different "in vivo" experiments were conducted simultaneously to determine: the respective efficiency of the commercial samples; the minimum effective dose; the localisation and/or concentration of the active principle or principles; and effectiveness of petroleum-ether and aqueous extracts.

The taenifugal activity was determined clinically, as this is the final test for the efficiency of a vermifuge.

A total of 95-100 kilograms of various commercial samples were decorticated by hand under strict hygienic conditions. The kernel obtained by this method was administered to the patient or the kernel was

separated into hypocotyl and cotyledons by sharp blades and administered separately to different patients.

Each dose was wrapped in wax paper and sealed with tape to prevent undue exposure to air. Each dose in turn was placed in a cardboard box to prevent effect of light on the drug. Also each dose was code-numbered and the description of the contents filed.

The petroleum-ether extracts were prepared by the same method used for the preparation of the oil in the analysis for the physico-chemical constants. Care was taken that no appreciable traces of petroleum-ether had remained in the extract.

The aqueous extracts were prepared by the method described by Veen and Collier (59) except that no vanillin was placed as an aromatic substance in the extract.

A total of 296 doses were distributed to physicians for clinical trials. Most of these trials were performed in Dispensaire N. - D. de la Consolata, Tanail, Beka'a, and some in Zahle, Beirut, Kab-Elias and Chtaura, Lebanon. Only 226 reports could be obtained from the total of 296 samples given out. The reasons for such a loss of data was due partly to the fact that patients treated in Tanail come from districts as far as Merjayoun or Ba'albeck, Lebanon and it was materially impossible to have certain cases followed for full information.

Wtih each dose administered, the physician had to fill a mimeographed questionnaire, a photographic copy of which appears at the end of this section, Fig. 1.

Each dose was administered early in the morning before breakfast without previous fasting or purgation. Though Carman (8) states that food in the stomach materially helps to reduce the efficiency of the drug,

yet Kempt (27) obtained satisfactory results without preliminary fasting or purging in the treatment of 3000 cases of intestinal parasites. This latter method was adopted without any untoward reactions. Within 3 hrs. after the administration of the drug, a purge was taken by the patient. In the majority of the cases treated, a saline purge of 30-40 gms. of sodium sulfate decalydrated, dissolved in a cup of water was used. The use of magnesium sulfate was discouraged as it is reported that it may have a depressant action on the patient. In rare instances, and only for children, castor oil in doses of 15-30 ml. were administered without any ill effects. Most of the infants took as a purge phenolphthalein 0.12 - 0.2 gms. in form of chocolate. Just after the administration of the petroleum-ether extract, usually a carbonated, non-alcoholic soft drink was administered to cover the oily taste in the mouth of the patients.

The stools were examined for the scolex of the parasite, and whenever possible the method recommended by Magath and Brown (33) was applied. A positive result was recorded upon finding the scolex. Failure to identify the scolex was not taken as a negative result as recommended by Sandground (51) and Maplestone et al. (34). Each case was followed for 60-75 days, and if no proglottids appeared in the stools of the the patient within this period, the case was considered as a successful treatment.

In all instances, the tapeworm passed was Taenia saginata. In all cases only one tapeworm came out, except in one woman who passed out 4 tapeworms.

1. Code number of medication (numero du medicament) .....

.....

.....

2. Name of the patient (Nom du malade) .....

3. Age of the patient (Age du malade) .....

4. Sex of the patient (Sexe du malade) .....

5. Date of administration (Date d'emploi du medicament) .....

6. Time of day the drug administred (L'heure d'emploi du medicament) .....

.....

7. Time of day the purgative administered (L'heure d'emploi du purgative)

.....

8. Name of the purgative used (Nature de purgative employee) .....

.....

9. Please state if the patient has been fasting before the administration of drug and how long. (Indiquer, S.V.P. si le malade a jeune avant de prendre le medicament et pour combien de temps) .....

.....

10. Condition of Taenia during expulsion, if any. (Resultat obtenu, et la condition du Taneia apres l'expulsion) .....

.....

.....

.....

11. Please enumerate the names of other Taenifuges, if any, used by the patient before the administration of this drug. (Enumerez, S.V.P., le medicaments Taenifuges que le malade employe pour l'expulsion du Taenia avant ce traitement .....

.....

.....

12. Remarks. (Commentaire) .....

.....

.....

12. Name of the Physician (Nom du Medecin) .....

.....

Fig. 1. Photograph of mimeographed questionnaire.

Photo Steve

RESULTS

I. Taxonomy

Seven commercial varieties of seeds were planted, namely "Hungarian" (Nos. 1-3), "Sudanese" No. 4, "Turkish" No. 5, "Chinese" No. 6, "Iranian" No. 7, "Roumanian" No. 8, "Balady", "Lebanese" or "Syrian" No. 9.

The "Hungarian" samples upon close examination show 3 types of seeds differing in color as well as structurally. A jute bag of "Hungarian" seeds weighing about 60 kgs. was handpicked and separated into 3 parts, cream colored seeds, yellowish-brown seeds, and snow-white seeds. The second and the third type constituted 1.7 and 2.8% respectively of the variety.

These nine samples were planted and the observations of the vegetative parts of the plants appear in Table I. Photographs of leaves, fruits and seeds form (the accompanying) Figures 2-10. A 17 cm. ruler is placed below the seeds and a 40 cm. ruler under the fruits to give an idea about the sizes of these plant parts (to the reader). A magnifying lens of 6-10x shows in the photographs the details of leaf, seed and fruit peduncle necessary for identification of the plants.

From the above data, making use of leaf margin, peduncle of fruit, seed funicular attachment, calyx variations, it was found that samples No. 1, 4, 6, 7 and 8 were Cucurbita Pepo L. seeds, sample No. 5 was Cucurbita Pepo L. variety Ovefera and samples No. 2, 3 and 9 Cucurbita Maxima Duch.

TABLE I

Comparative Study of Vegetative Parts of Cucurbita Plants

Seeds	Life Span	Plant	Stem	Hairs	Leaf Shape and Margin	White Blotches at Angles of Veins
1. "Hungarian" Cream colored	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 5-lobed serrate	Faint
2. "Hungarian" Yellowish-brown	Annual	Long running Vine	Soft, round	Moderately soft	Reniform to orbicular, entire	Absent
3. "Hungarian" Snow-white	Annual	Long running Vine	Soft, round	Soft	Reniform to orbicular, entire	Absent
4. "Sudanese"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 3-lobed, serrulate	Absent
5. "Turkish"	Annual	Bush, semi-erect	Angled, ridged	Harsh	Cordate, deeply 5-lobed, serrulate	Absent
6. "Chinese"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 5-lobed, serrulate	Few
7. "Iranian"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 3-5 lobed, dentate	Prominent
8. "Roumanian"	Annual	Long running Vine	Angled, ridged	Harsh	Deeply 5-lobed, serrate	Prominent
9. "Balady" or	Annual	Long running Vine	Soft, round	Moderately soft	Cordate, faintly lobed, crenate	Absent

TABLE I

Comparative Study of Vegetative Parts of Cucurbita Plants

Seeds	Life Span	Plant	Stem	Hairs	Leaf Shape and Margin	White Blotches at Angles of Veins
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2. "Hungarian" Yellowish-brown	Annual	Long running Vine	Soft, round	Moderately soft	Reniform to orbicular, entire	Absent
3. "Hungarian" Snow-white	Annual	Long running Vine	Soft, round	Soft	Reniform to orbicular, entire	Absent
4. "Sudanese"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 3-lobed, serrulate	Absent
5. "Turkish"	Annual	Bush, semi-erect	Angled, ridged	Harsh	Cordate, deeply 5-lobed, serrulate	Absent
6. "Chinese"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 5-lobed, serrulate	Few
7. "Iranian"	Annual	Long running Vine	Angled, ridged	Harsh	Cordate, 3-5 lobed, dentate	Prominent
8. "Roumanian"	Annual	Long running Vine	Angled, ridged	Harsh	Deeply 5-lobed, serrate	Prominent
9. "Balady" or	Annual	Long running Vine	Soft, round	Moderately soft	Cordate, faintly lobed, crenate	Absent

TABLE I (CONTINUED)

Seeds	Calyx and Corolla	Androcium	Stigma	Peduncle of Fruit	Fruit	Fruit Flesh
1. "Hungarian" Cream-colored	Short sepals, yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular ridged	Large, up to 12 kgs. in weight, 2-3 per plant	Yellow, fine grained
2. "Hungarian" yellowish-brown	Long slender sepals; yellow corolla	Short, thick and columnar	Small, yellowish, smooth	Soft, round and irregularly thickened with soft cork	Large, up to 5 igs. in weight, 2-3 per plant	Yellow, fine grained
3. "Hungarian" Snow-white	Long slender sepals; yellow corolla	Short, thick and columnar	Small, yellowish, smooth	Soft, round and irregularly thickened with soft cork	Large, up to 10 kgs. in weight, 2-3 per plant	Light yellow, fine grained
4. "Sudanese"	Short sepals; yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular and ridged	Small, up to 2 kgs. in weight, 8-10 per plant	Light yellow, fine grained
5. "Turkish"	Short sepals; yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular and ridged	Small, ½-2 kgs. in weight, 8-12 per plant	Dry, hard, yellow, fine grained
6. "Chinese"	Short sepals; yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular and ridged	Small, 1-3 kgs. in weight, 8-10 per plant	Yellow, fibrous
7. "Iranian"	Short sepals; yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular and ridged	Small, 3-4 kgs. in weight, up to 6 per plant	Yellow, fibrous
8. "Roumanian"	Short sepaps; yellow corolla	Short, thick and conical	Small, yellowish, smooth	Hard, angular and ridged	Large, 8-10 kgs. in weight, 2-4 per plant	Yellow fibrous
9. "Balady" or "Lebanese" or "Syrian"	Long slender sepals; yellow corolla	Short, thick and columnar	Small, yellowish, smooth	Soft, round and irregularly thickened with soft cork	Large, 6-17 kgs. in weight, 1-2 per plant	Yellow, fine grained

TABLE I (CONTINUED)

Seeds	Placenta	Seeds	Funicular attachment	Seed margin
1. "Hungarian" Cream-colored	Collapsing, dry seeds separating easily from the placenta	Cream colored, ovate to elliptical, slightly wrinkled on the surface	Obtuse	Enlarged, obtuse, smooth
2. "Hungarian" yellowish-brown	Collapsing, moist seeds separating with difficulty	Yellowish-brown, ovate to elliptical, smooth surface	Acute	Obtuse, smooth
3. "Hungarian" snow-white	Collapsing, moist seeds separating with difficulty	Snow-white, elliptical, smooth surface	Acute	Obtuse, smooth
4. "Sudanese"	Collapsing, dry seeds separating easily	Cream colored, elliptical, heavy wrinkles on the surface	Obtuse	Obtuse, very thin, smooth
5. "Turkish"	Collapsing, dry seeds separating easily	Cream colored, ovate to elliptical smooth surface	Obtuse	Obtuse, thin, smooth
6. "Chinese"	Collapsing, dry seeds separating easily	Cream colored, ovate to elliptical smooth surface	Obtuse	Obtuse, thin, smooth
7. "Iranian"	Collapsing, dry seeds separating easily	Cream colored, elliptical smooth surface	Obtuse	Obtuse, thin, smooth
8. "Roumanian"	Collapsing, dry seeds separating easily	Cream colored, elliptical to ovate smooth surface	Obtuse	Enlarged, smooth
9. "Balady" or "Lebanese" or "Syrian"	Collapsing, moist, seeds separating easily	Snow-white to yellowish	Acute	Very thin to thin, obtuse smooth

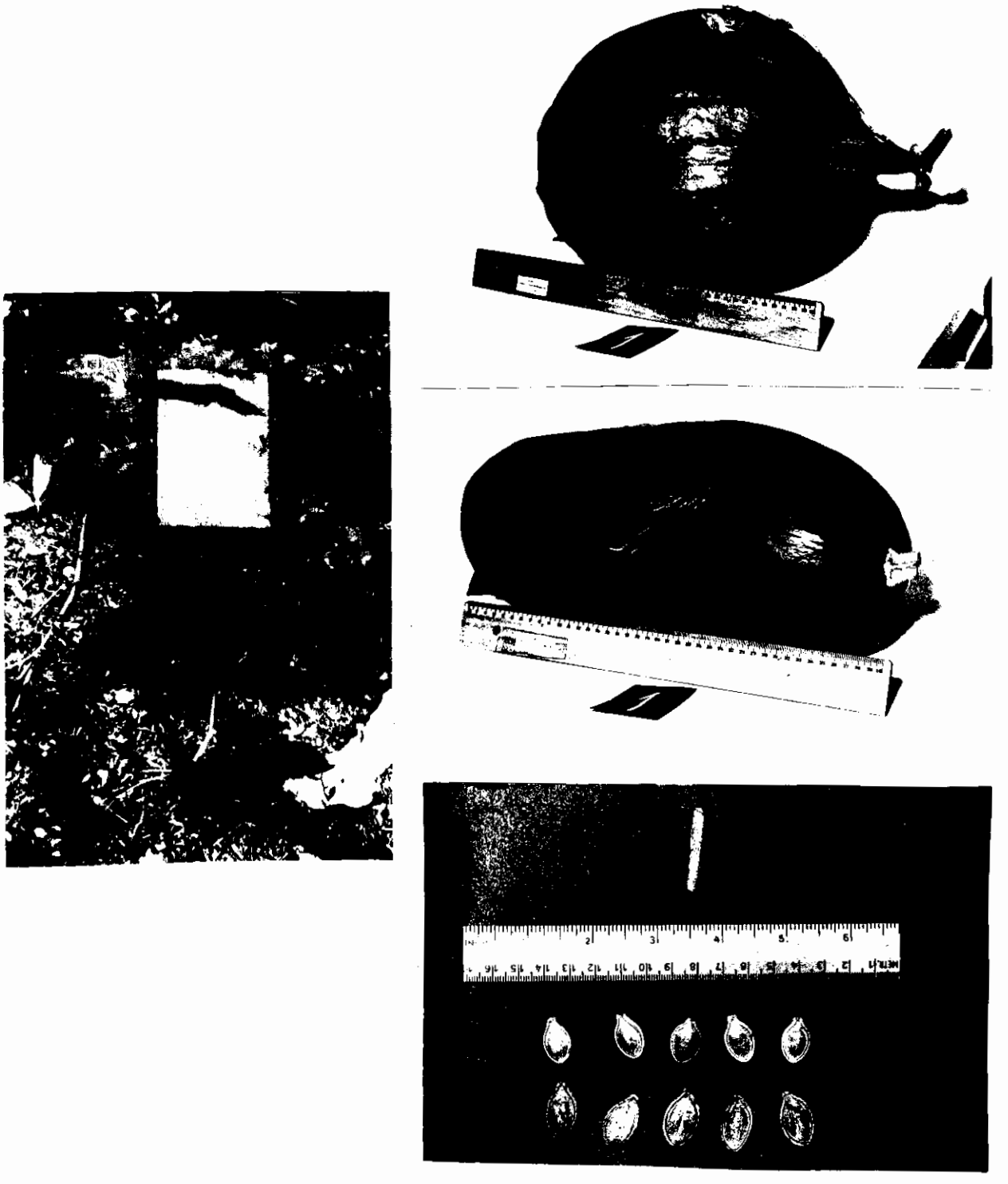
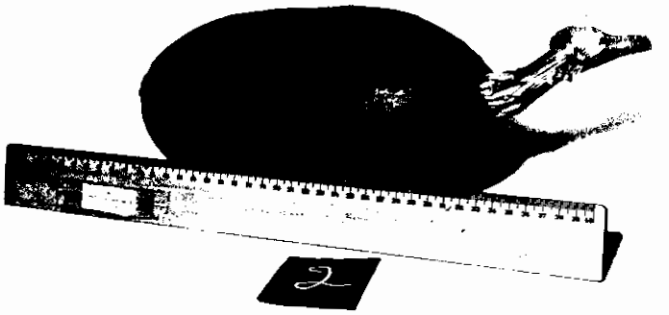


Fig. 2. Leaves, fruits and seeds of "Hungarian" cream colored seeds.

Photo Steve and L. Karamanukian



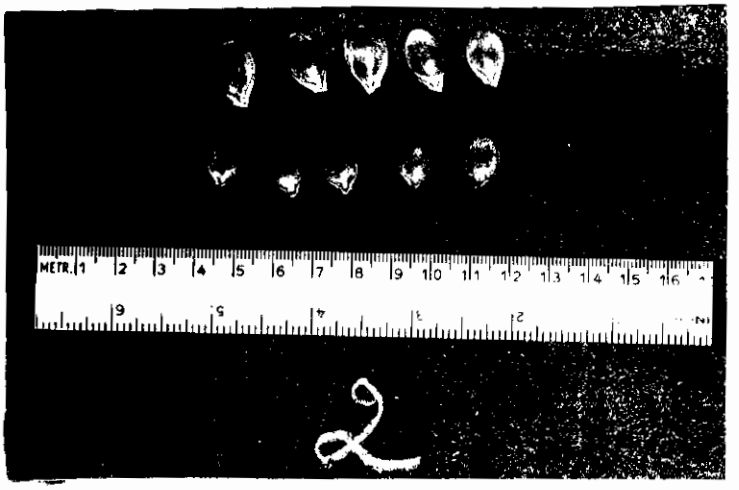
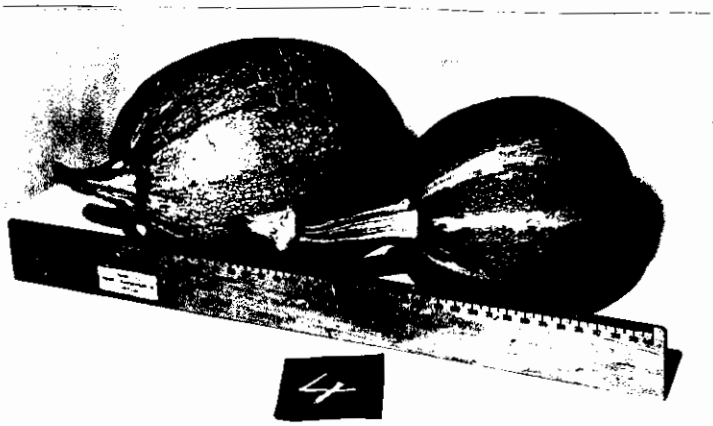


Fig. 3. Leaves, fruit and seeds of "Hungarian" yellowish-brown seeds.

Photo Steve and L. Karamanukian

Fig. 4. Leaves, fruits and seeds of "Hungarian" snow-white seeds.

Photo Steve and L. Karamanukian



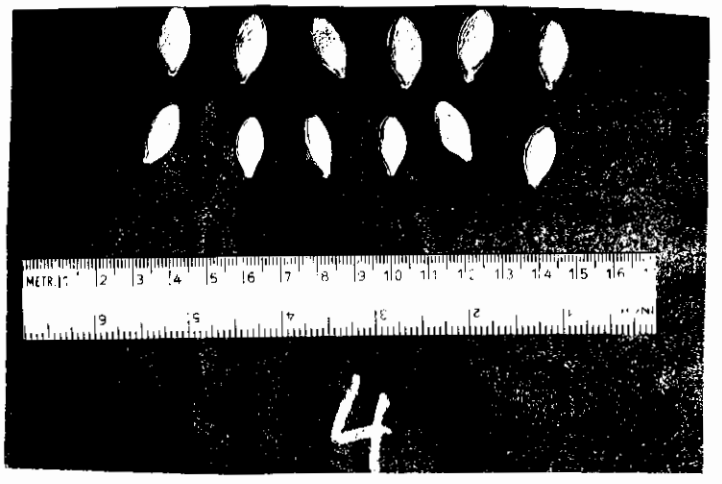
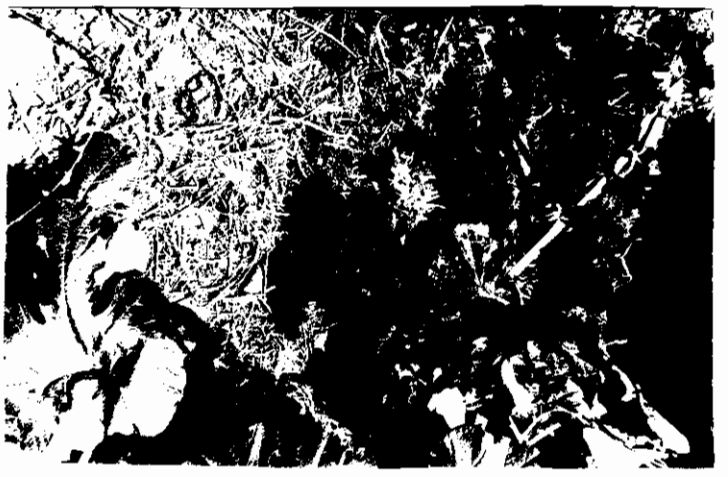


Fig. 5. Leaves, fruit and seeds of "Sudanese" variety.

Photo Steve and S. Ayvazian



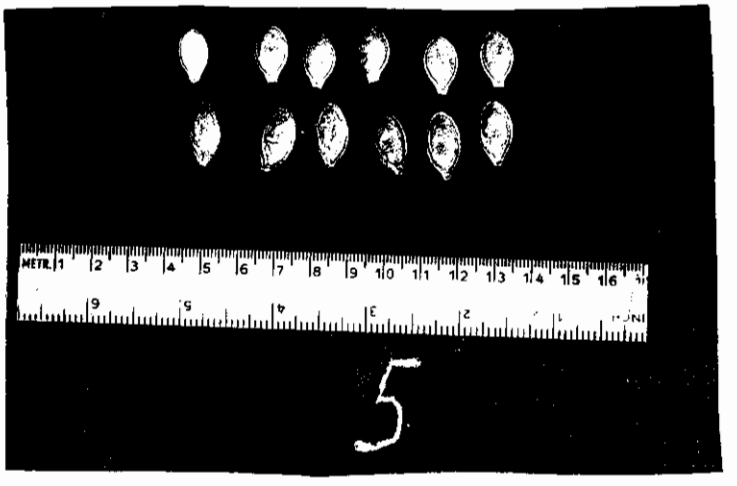
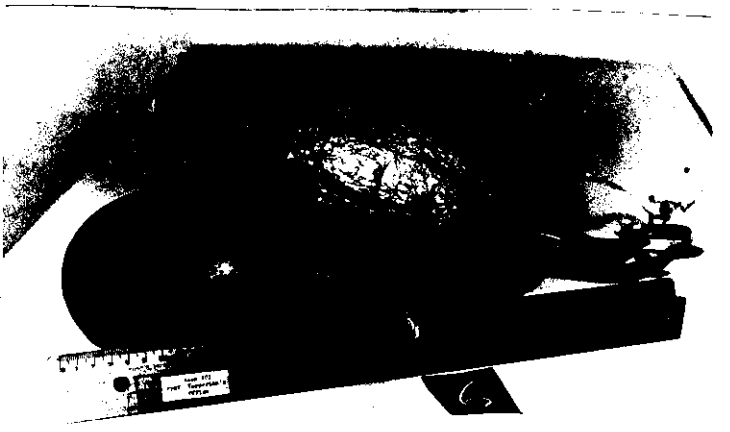


Fig. 6. Leaves, fruits and seeds of "Turkish" variety.

photo Steve and S. Ayvazian



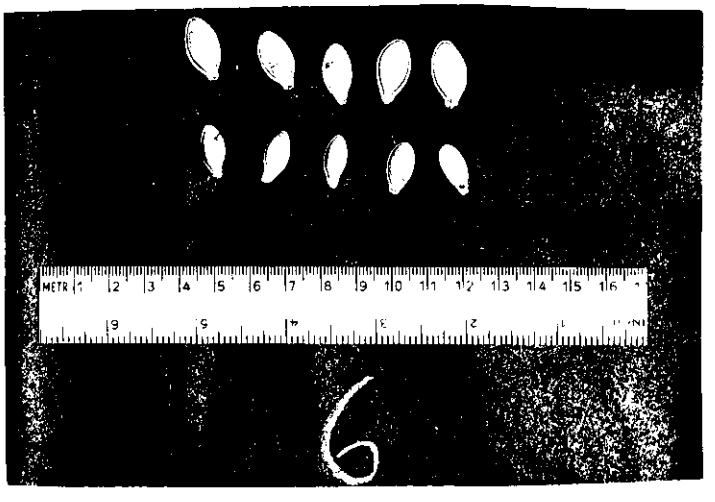
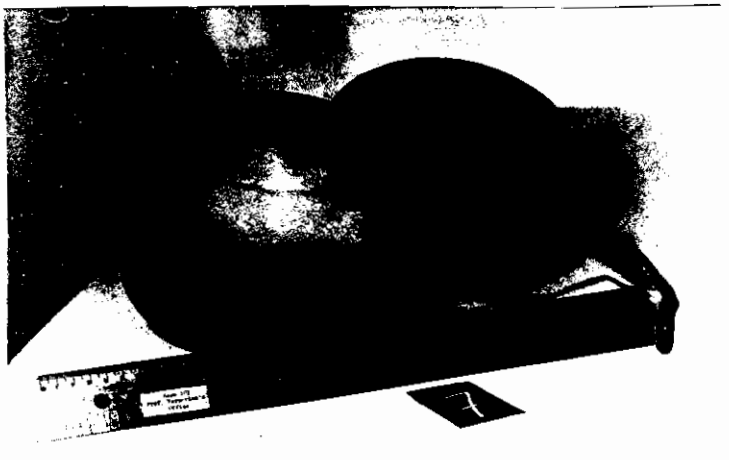


Fig. 7. Leaves, fruits and seeds of "Chinese" variety.

Photo Steve and S. Ayvazian



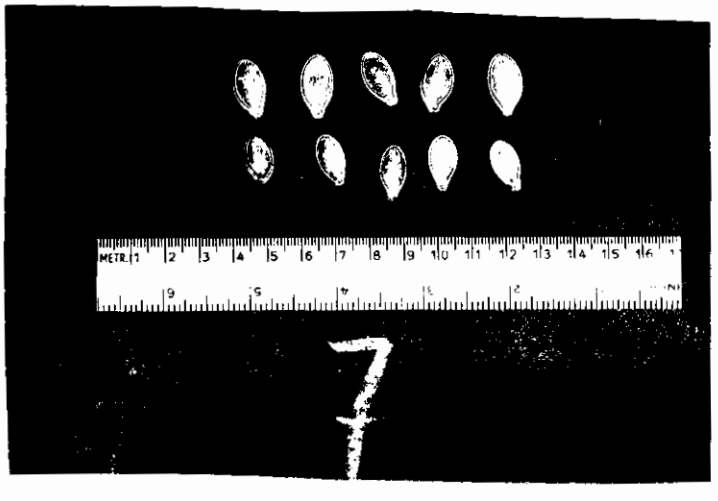
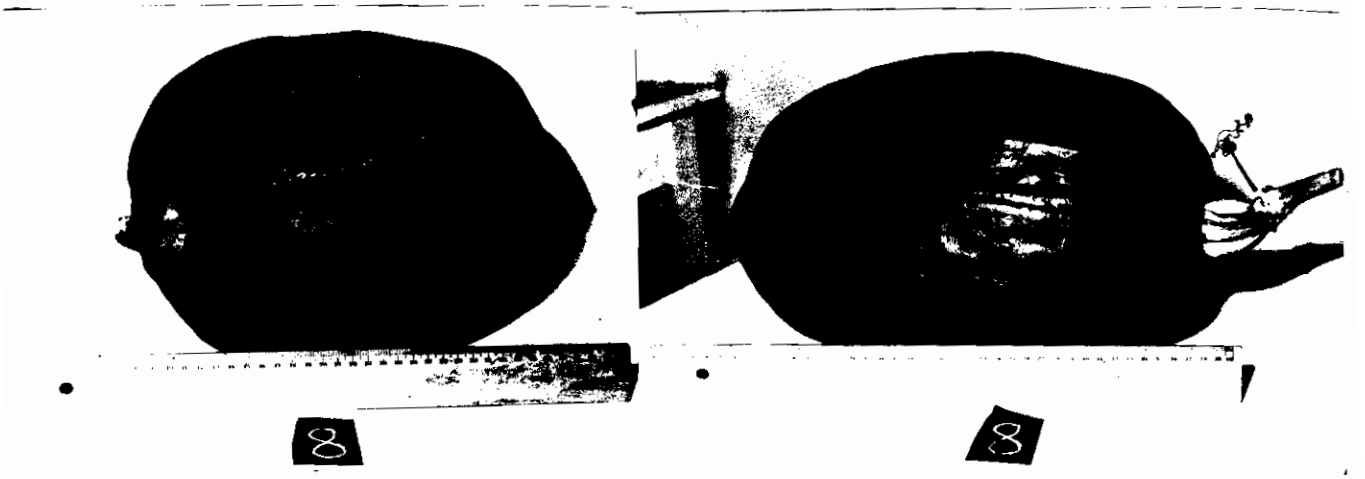


Fig. 8. Leaves, fruits and seeds of "Iranian" variety.

Photo Steve and L. Karamanukian



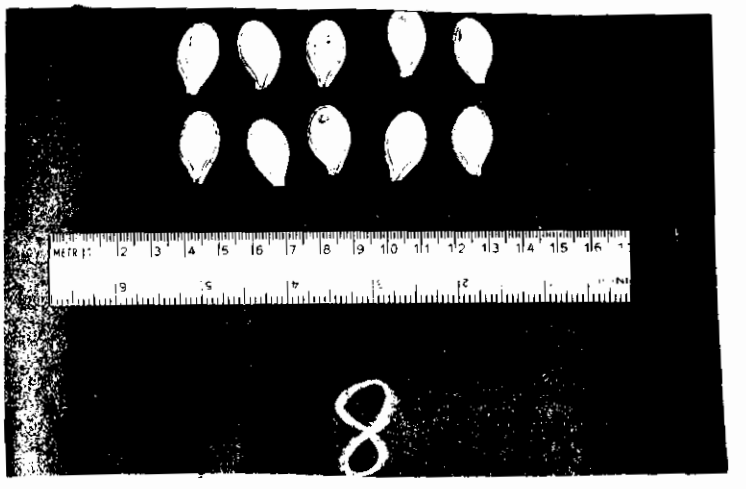


Fig. 9. Leaves, fruits and seeds of "Roumanian" variety.

Photo Steve and L. Karamanukian

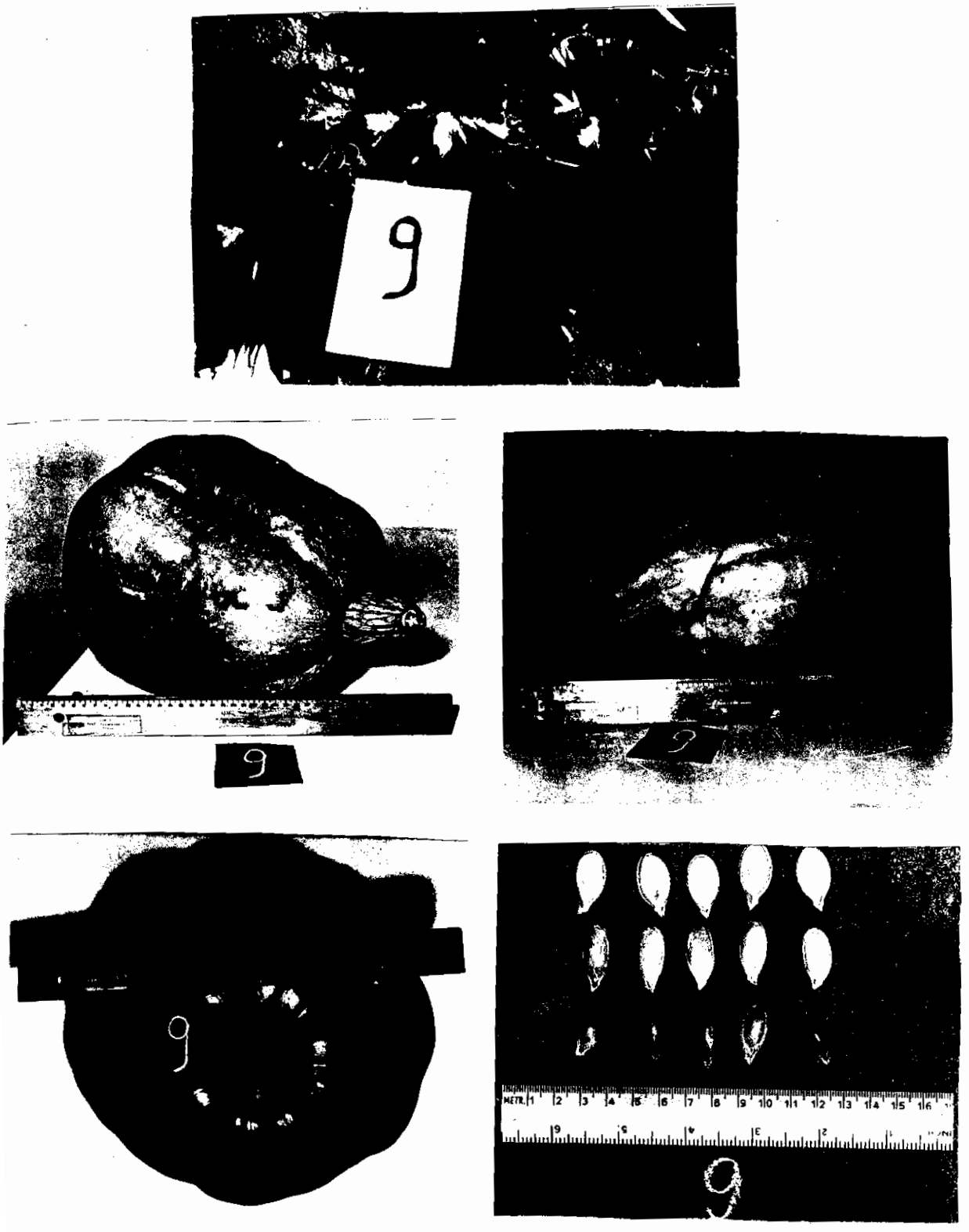


Fig. 10. Leaves, fruits and seeds of "Balady" variety.

Photo Steve and L. Karamanukian

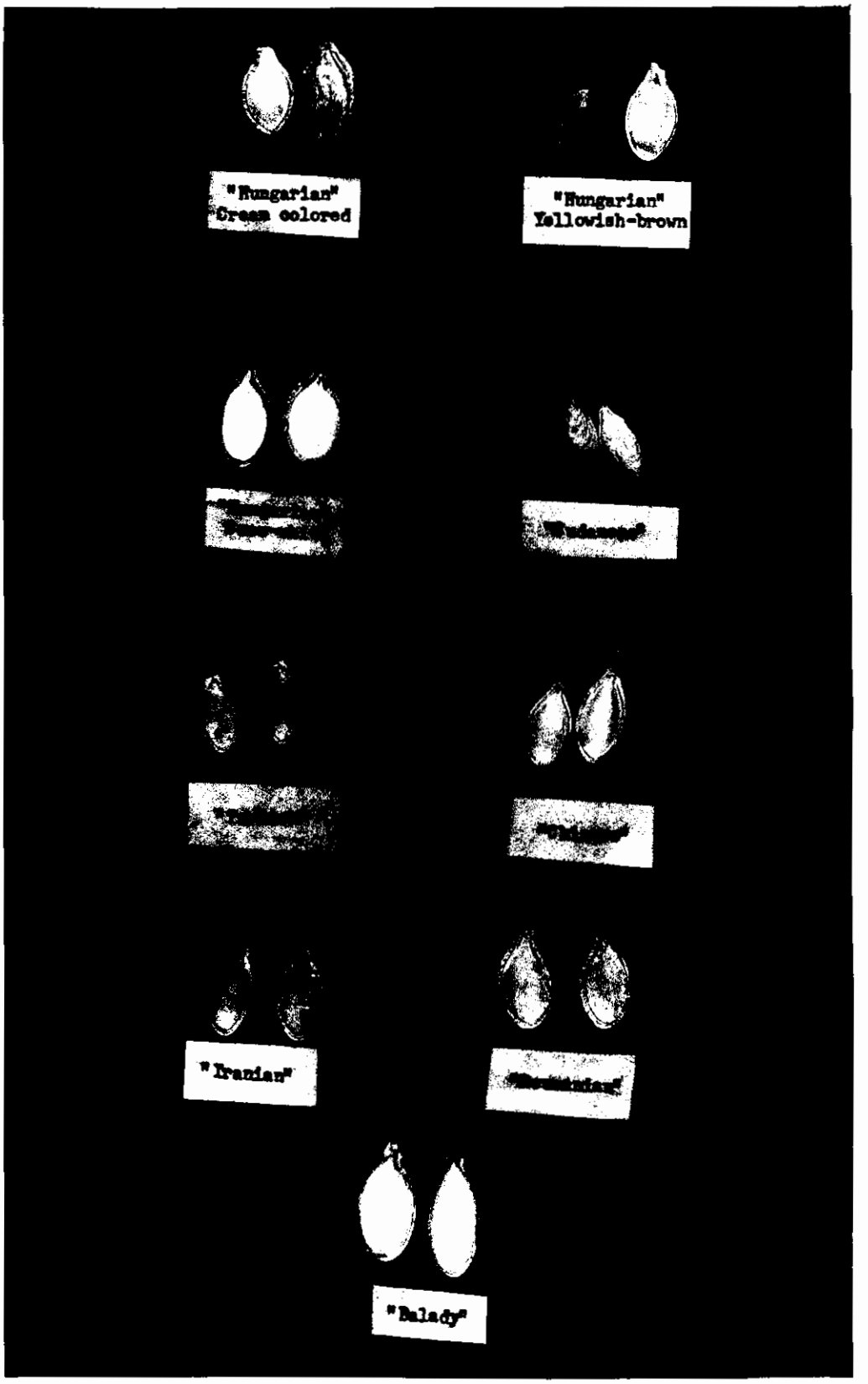


Fig. 11. Comparative study of seeds

Photo H. Chohmelian

II. Histology

Usually the descriptions obtained about the histology of Cucurbita seeds from standard scources are based on studies of a single variety of seeds. The histology of the various types of seeds show structural differences. A description of the microscopical structure of the cross-section of seeds based upon studies of 9 varieties follows: A cross-section of a seed shows 6 different layers. An outer (a) epidermis consisting of palisade-like cells, having thickenings which may be straight and branching, spirally curved or wavy, beaded slightly or heavily. The cell walls may be pitted or not, or may show slit like pits. They may show lignification or the thickenings may show only slight lignification. Usually very small starch grains may be found in these cells. Certain of the commercial varieties, during handling, that is removal from the fruits and drying, have their epidermal cell walls destroyed. When such seeds are examined microscopically, the epidermis appears to have abundant non-glandular hairs, which in reality are thickenings of the epidermal cells. The lengths of the epidermal cells vary, some reaching more than 2 mm. in length. This layer is followed by a (b) 4-5 layered polygonal or rounded, pitted or reticulately thickened cells. They are all lignified in all the varieties of seeds examined. As we go inwards, these cellas become larger. They are connected to each other by means of large circular or elliptical pores. On the margins of the seeds, this layer of cells may be 10-12 cells thick. Just below this layer, a (c) one-cell thick stone cells are found, which show heavy, thick striated walls, highly lignified in all samples of the seeds examined. The lumen of these cells are circular or elliptical or irregular

in outline. The cells are 75-100 microns in thickness. Next a (d) parenchymatous layer is found. This layer is really of three distinct and different layers. The outer one of these consists of small pitted cells, very similar in structure to the subepidermal cells seen above. They are pitted or show reticulate thickenings. In this area no intercellular air spaces are to be found. These cells are highly lignified. The second layer of this parenchymatous cells consists of large, irregular, pitted or with slit-like pits, reticulately thickened cells. They anastomose with large, circular, elliptical holes. They show in between them large intercellular air spaces. This layer of parenchyma is heavily lignified. The different samples studied showed variations in the size and shape of these cells. But, these variations are not large enough to differentiate easily the species and the hybrids of Cucurbita. The third layer of parenchyma cells, consists of very thin walled, cellulosoic cells. These are usually collapsed. Inside the seed coat is found the (e) perisperm or the tegmen. This layer is parenchymatous and chlorophyllose, that is greenish in color. Inside the perisperm is found the cotyledon (f), which consists of isodiametric, elongated or palisade like cells, containing fixed oil or aleurone grains.

TABLE II

Comparative Histology of Seed Coats in Various Seeds

Sample	Epidermis thickness in microns	Rest of seed coat thickness in micr.	Lignification of epidermal layer	Outline of epidermal layer seen in cross-section	Types of thickenings in epidermal cells seen in cross-section	Pores in epidermal cells
1. "Hungarian" cream colored	75	375	None	Not seen	Straight, not wavy, often bending and branching	None
2. "Hungarian" yellowish-brown	1000	450	In cell walls and in the thickenings	Thick and well defined outline	Band-like thickenings showing reticulations	Simple pits
3. "Hungarian" snow-white	225	600	None	Well defined outline	Wavy and thick	Slit-like pits
4. "Sudanese	75	375	None	Well defined outline	Short and undulating	None
5. "Turkish"	75	300	None	Well defined outline	Wavy, more or less spiral	None
6. "Chinese"	150	600	None	Light outline of cell wall	Very wavy, more or less spiral thickening	None
7. "Iranian"	85	300	None	Well defined outline	Wavy and beaded thickenings	None
8. "Roumanian"	75	260	None in cell wall, slight in the thickenings	Very light outline of cell wall	Wavy and beaded thickening	None
9. "Balady" or or "Lebanese" or "Syrian2	250	650	In cell walls and in the thickenings	Well defined outline	Wavy and thick	slit-like pits

From the above description it is seen that one can depend mainly on the structure of the epidermal layer of seed coats. The main differential points are tabulated and are seen in Table II. The Cucurbita maxima Duch. seeds show a thick epidermis varying between 225-1000 microns in diameter, while the Cucurbita Pepo L. seeds varying between 75-150 microns in diameter. Making use of lignification of epidermal layer, outline of epidermal layer in cross-section and types of thickenings in the epidermal layer, the different varieties can be identified microscopically.

III. Oil Content

Table III shows the analysis of oil content of the 9 varieties of seeds examined. The moisture content of the seeds are fairly constant, varying between 6.94 and 7.93%. The oil contents of the different varieties show appreciable differences, with a percentage ranging from 30.61 to 44.56% which if calculated on air-dry basis range between 33.22 and 47.94%.

TABLE III

Comparative Study of Oil Content of the Various Seeds

Seeds	Percentage Moisture	Percentage Oil in Original Sample	Percentage Oil Calculated on Air-dry basis
1. "Hungarian" cream colored seeds	7.54	37.02	40.04
2. "Hungarian" yellowish-brown	7.85	30.61	33.22
3. "Hungarian" snow-white	7.25	37.54	40.48
4. "Sudanese"	7.60	38.96	42.16
5. "Turkish"	7.93	30.99	33.66
6. "Chinese"	7.05	44.56	47.94
7. "Iranian"	7.26	37.54	40.48
8. "Roumanian"	7.09	40.58	43.68
9. "Balady" or "Lebanese" or "Syrian"	6.94	36.36	39.06

IV. Oil Analysis

The physico-chemical constants of the oils analysed are compared in Table IV.

Table V compares the physico-chemical constants of olive oil, cottonseed oil and the constants obtained from the oil analysis of Cucurbita seed varieties. This table shows that the oils examined can be classified as semi-drying oils, more or less similar to cottonseed, soya bean or sesame oils.

The increase in color intensity and the increase in Acid Value on storage show a definite correlation and that color intensity as well as Acid Value can be increased by subjecting the oils to high temperatures, light or to storage.

TABLE IV

Comparative Study of the Physico-chemical Constants of the Different Seeds

Seeds	Color by Lovibond Tintometer just after extraction	Color by Lovibond Tintometer 68 days after storage in the dark	Refractive Index at 20°C.	Weight per milliliter	Acid Value just after extraction	Acid Value after storage of 68 days in dark	Iodine Value	Saponification Value
1. "Hungarian"	8.0 Red 15.0 Yellow 1.2 Blue	29.9 Red 7.4 Yellow 1.2 Blue	1.4738	0.9126	12.0	15.2	108.3	197.2
2. "Hungarian" yellowish-brown	3.2 Red 20.0 Yellow 1.0 Blue	13.3 Red 9.4 Yellow 2.6 Blue	1.4747	0.9068	3.5	8.5	126.6	184.7
3. "Hungarian" snow-white	2.2 Red 20.0 Yellow 0.9 Blue	18.1 Red 9.9 Yellow 1.2 Blue	1.4750	0.9059	1.3	2.9	124.9	194.2
4. "Sudanese"	22.0 Red 9.0 Yellow 2.0 Blue	29.9 Red 0.8 Yellow 1.9 Blue	1.4738	0.9179	3.8	4.8	120.6	193.8
5. "Turkish"	8.5 Red 8.0 Yellow 1.3 Blue	28.0 Red 4.5 Yellow 1.8 Blue	1.4735	0.9186	2.5	7.8	119.2	191.2
6. "Chinese"	9.0 Red 20.0 Yellow 1.0 Blue	29.9 Red 3.5 Yello 1.9 Blue	1.4741	0.9201	1.6	2.4	119.5	192.1
7. "Iranian"	5.4 Red 5.0 Yellow 1.6 Blue	29.9 Red 5.4 Yellow 2.9 Blue	1.4741	0.9213	2.5	8.8	123. 9	187.1
8. "Roumanian"	9.9 Red 20.0 Yellow 1.3 Blue	28.7 Red 6.9 Yellow 1.8 Blue	1.4731	0.9126	6.7	11.8	119.8	191.0
9. "Balady" or "Lebanese" or "Syrian"	4.7 Red 25.0 Yellow 2.2 Blue	7.0 Red 22.0 Yellow 4.4 Blue	1.4734	0.9210	1.2	1.9	118.9	184.1

TABLE V

Comparative Study of Cottonseed, Olive and "Pumpkin Seed" Oils

	"Pumpkin Seed" Oil	Olive Oil	Cottonseed Oil
Refractive Index	1.4731-1.4750	1.467	1.471
Weight per Milliliter	0.9059-0.9213	0.914-0.920	0.9130-0.930
Acid Value	1.2-15.2	2 or more	2 or more
Iodine Value	108.3-126.6	77-95	104-116
Saponification Value	184.1-197.2	185-196	191-196

V. Taenicidal Activities

Out of 296 samples distributed, results from 226 cases could be obtained. The other 70 cases could not be followed. Of these 226 cases treated 28 were males and 128 females. The ages varied between one and sixty nine. The number of cases treated in each age group is given in Table VI. Out of 226 cases treated with different seed parts or extracts, 104 gave positive response and 122 negative response.

89 doses were administered in form of decorticated whole seeds i.e. kernel and tegmen from 500 gms. of seeds. From this study it is seen that the "Hungarian" variety possesses the best taenicidal properties with a 100% cure. The "Balady" variety showed a cure of 60% while the poorest in taenicidal properties are the "Iranian" and "Roumanian" varieties showing a 25 and 27.3 percent elimination of taenia respectively. These results are compared in Table VII.

The "Hungarian" and the "Balady" seed varieties were subjected to further experiments to determine minimum effective dose. Kernels and tegmen obtained by decortication of 250 gms. of seeds were administered to 19 patients. The "Hungarian" variety showed an 80% cure while the "Balady" variety 55.5% cure.

9 cases were treated with kernels and tegmen of 125 gms. of Hungarian seeds. A 22.2% of elimination of Taenia was observed.

18 cases were treated with the cotyledons and tegmen of 500 gms. of seeds, i.e. decorticated seeds from which the hypocotyls were removed. The percentage cure was 80 and 50 respectively for the "Hungarian" and "Balady" varieties.

20 cases were treated with the cotyledons and tegmen of 250 gms. of seeds. The percentage cure was 70 and 40 respectively for "Hungarian" and "Balady" varieties.

22 patients were administered the hypocotyl from 500 gms. of decorticated seeds. A cure of 50 and 33 percent was obtained in the "Hungarian" and "Balady" varieties respectively.

To 9 cases the hypocotyl of 250 gms. of seeds was administered. No positive response was obtained.

To 20 patients 150 gms. of petroleum-ether extracts were administered representing 500 gms. of seeds. These extracts were prepared from "Hungarian" variety of seeds. No elimination of taenia was observed in all cases treated.

To 20 patients, aqueous extracts prepared by Veen and Collier (59) method was administered. A 70% cure in the patients resulted. The above data is given in Table VIII.

The "Hungarian" seeds consist mainly (95.5%) of Cucurbita Pepo L. seeds, the "Balady" variety are from C. maxima Duchesne seeds and the rest are obtained from C. Pepo L. Table VI data show that there is no correlation between the taenicidal properties and botanical origin, since the seeds showing the best (100%) efficiency and the poorest (25 and 27.3) efficiency belong to Cucurbita Pepo L. varieties.

Though the amount of whole seeds taken is the same as well as the moisture contents, yet the kernel-tegmen weights obtained from the samples of different varieties did not come out to be of the same weight. The small differences between kernel-tegmen weights cannot possibly

explain the percentage efficiency differences between the "Hungarian" and "Iranian" or "Roumanian" varieties. It is true that there is an appreciable difference in weights of kernel-tegmen of "Hungarian" and "Balady" varieties, still the difference in weights cannot be equal to the 20% difference in efficiency. This difference in efficiency between the "Hungarian" and "Balady" seed parts do come out consistently as shown in Table VII.

The three experiments performed to determine the minimum effective dose indicate that it should be more than 250 gms. and nearer to 500 gms. of whole seeds.

The experiments performed on kernel parts indicate that the hypocotyl contains a higher percentage of active principles than the rest of the kernel-tegmen portion. This is seen when the hypocotyl of 500 gms. of seeds weighing on the average 23.75 gms. gave a better percentage cure (50%) than when 125 gm. of whole decorticated seeds (22.2%) were. Also, when we compare the results of kernel-tegmen of 500 gms. of seeds a decrease in weights of 16 gms. in the case of the "Hungarian" seeds due to removal of hypocotyls, an efficiency decrease of 20% is observed. While in case of "Balady" seeds a decrease in weight of 5.5 gms. due to removal of hypocotyls, shows an efficiency decrease of 10%.

The results obtained of hypocotyl administration of 250 gms. of "Hungarian" seeds, indicate that the active principle or principles found in the amount is below the minimum effective dose.

The results from petroleum-ether extracts indicate that the oil is devoid of active taenicidal principles.

The results of aqueous extracts indicate that the active principle or principles are soluble in water, but the efficiency is slightly decreased during the process of extraction. This could be due to the treatment of the extract with acid or due to absorption of the active principle during deproteinisation or due to decomposition partly of the active principle on standing as for example due to hydrolysis.

TABLE VI

Number of Patients Treated and Their Age Groups

Age Groups	Number of Cases Treated
1-2	4
2-9	22
10-19	61
20-29	48
30-39	33
40-49	26
50-59	24
60-69	8

TABLE VII

Comparative Study of Efficiency of Seeds as a Taenifuge

Seeds	Weight of kernel and tegmen from 500 gms. of seeds	Number of cases treated	Number of positive response	Number of negative response	Percent successful treatment
1. "Hungarian"	341	10	10	0	100%
2. "Sudanese"	334	10	4	6	40%
3. "Turkish"	298	23	11	12	47.8
4. "Chinese"	332	13	6	7	46.2%
5. "Iranian"	329.5	12	3	9	25%
6. "Roumanian"	331.5	11	3	8	27.3
7. "Balady" or "Lebanese" or "Syrian"	280.6	10	6	4	60%

TABLE VIII

Comparative Study of Efficiency of Various Seed and Seed Parts

Seeds	Weight in gms.	Number of cases treated	Number of Positive response	Number of Negative response	Percent successful treatment
"Hungarian" kernel and tegmen from 250 gms.	162	10	8	2	80%
"Hungarian" kernel and tegmen from 132 gms.	125	9	2	7	22.2%
"Hungarian" cotyledons with tegmen from 500 gms.	325	10	8	2	80%
"Hungarian" cotyledons with tegmen from 250 gms.	159	10	7	3	70.0%
"Hungarian" from 500 gms. hypocotyls	23.75	10	5	5	50%
"Hungarian from 250 gms. hypocotyls	11.4 gms.	9	0	9	0%
"Balady" kernel with tegmen from 250 gms.	138.0	9	5	4	55.5%
"Balady" cotyledons with tegmen from 500 gms.	276	8	4	4	50%
"Balady" cotyledons with tegmen from 250 gms.	133.8	10	4	6	40%
"Balady" from 500 gms. hypocotyls	6.7	12	4	8	33.3%
"Hungarian" petroleum-ether extract from 500 gms.	150 gms.	20	0	20	0%
"Hungarian" aqueous extract from 500 gms.	100 ml.	20	14	6	70%

SUMMARY AND CONCLUSIONS

Seven commercially available Cucurbita seeds were planted to identify their biological origin. The variety known as "Balady" was found to be Cucurbita maxima Duchesne, the "Hungarian" variety consisted mainly of Cucurbita Pepo L. seeds and a small percentage of Cucurbita maxima Duchesne seeds. The rest of the commercial varieties, "Sudanese", "Chinese", "Turkish", "Iranian", and "Roumanian" were found to be seeds of Cucurbita Pepo L. and its varieties. For the identification, fruit peduncle, leaf margin, funicular attachment of seeds, and sepal and androecium characteristics were made use of.

The 7 commercially available samples were histologically examined, and structural differences were seen. These differences were mainly in the epidermis of the testa. By making use of these differences the commercially available samples could be identified, hence their botanical origin.

The different samples were subjected to moisture and oil content analysis and were shown to contain approximately the same percentage of moisture while the oil content analysis showed a variation of 30.61 and 44.56 percent calculated on original sample equivalent to 33.22 and 47.94% calculated on air-dry basis.

The examination of the physico-chemical constants of the oil indicate that it is a semi-drying oil. The discoloration of the oil upon storage and the increase in Acid Value prohibits the use of this oil as a vegetable oil or fat. This discoloration and increase in Acid

Value could not be due to enzymatic activity as the oils were subjected to heat for long periods during the process of extraction in the soxhlet apparatus. Unless means are found to check these deteiorations, the oil cannot be used except for industrial purposes.

Investigations on the taenicidal properties indicate that the "Hungarian" variety of seeds is the most efficient and next in efficiency being the "Balady" variety. No correlation was observed between species and taenicidal activity. It was observed that the hypocotyl portion of the kernel of seeds contains a higher proportion of taenicidal principle or principles, than the cotyledon and tegmen portion of dicorticated seeds.

It was also demonstrated that a minimum effective dose is much greater than 250 gms. of whole seeds, it is nearer to 500 gms.

It was also demonstrated that petroleum-ether extracts were devoid of taenicidal principles, while the aqueous extracts though effective yet not as effective as whole seeds.

BIBLIOGRAPHY

1. Albrecht, E., The Manufacture of Pumpkin Seed Oil. Z. Angew. Chem., 31(1), 132 (1918). Through Chemical Abstracts, 12, 2453 (1918).

2. Bailey, L.H., Manual of Cultivated Plants, The Macmillan Co., New York, 1944, p. 733.

3. Bailey, L.H., The Standard Cyclopedia of Horticulture, The Macmillan Co., New York, Vol. I, 1944, P. 909, Vol. II, 1944, p. 1361, Vol. III, 1944, p. 2859.

4. Barber, K., Comparative Histology of Fruits and Seeds of Certain Species of Cucurbitaceae. Bot. Gaz., 47, 263 (1909).

5. Baughman, W., D. Brauns and G. Jamieson, Cantaloupe-Seed Oil. J. Am. Chem. Soc., 42, 2398 (1920).

6. The British Pharmacopoeia, Constable & Co., London (1914).

7. The British Pharmacopoeia, The Pharmaceutical Press, London (1958).

8. Carman, J., A Note on the Clinical Aspect of the Treatment of Taeniasis with Carbon Tetrachloride. Trans. Roy. Soc. Trop. Med. Hyg., 25, 187 (1931).

9. Cavier, R., Therapeutiques des Affections Determinees par les Plathelminthes. Prod. Pharm., 8 (8), 407 (1953).

10. Chowdhury, D., M. Chakarbarty and B. Muhherji, Studies on some Fats of Cucurbitaceae Family. Jour. Amer. Oil Chemist's Soc., 32 (7), 384 (1955).

11. Colorada, I., A. Trevino, R. Dominguez and L. Mazzotti, La Semilla de Calabaza en el Tratamient de las Teniasis. Rev. Inst. Salubrdad y Enferm. Trop., 11 (1), 57 (1950). Through Biol. Abstr., 25, 12298 (1951).

12. Cutler, H. and Th. Whitaker, Cucurbita mixta Pang., its Classification and Relationship. Bul. Torrey Bot. Club, 83 (4), 253 (1956). Through Biol. Abstr., 31, 11730 (1957).

13. Erwin, A. and E. Lana, The Seminole Pumpkin. Econ. Bot., 10 (1), 33 (1956).

14. Fefer, I., M. Mindlein and N. Prokopovich, Anthelmintic Properties of Watermelon Seeds. Farmakol. i Toksikol., 17 (5), 50 (1954). Through Biol. Abstr., 31, 11501 (1957).

15. Feng, L., The Combined Use of Pumpkin Seed and Areca Nuts in the Treatment of Tapeworm Infection. Chinese Med. J., 74 (1), 17 (1956). Through Trop. Dis. Bull., 53, 1025 (1956).

16. Freise, F., Pharm. Zentralhalle, 79, 97 (1938). Through (9) and (32).

17. Golse, J., Precis de Matiere Medicale, G. Doing & Co., Paris, 1955, p. 841.

18. Greenish, H., Microscopical Examination of Foods and Drugs, P. Blackston's Son and Co., Philadelphia, 1923, p. 368.

19. Griffin, R., Technical Methods of Analysis, McGraw-Hill Book Co., Inc., 2nd Ed., 1927, p. 304.

20. Gurr, E., Microbiological Staining Techniques. The Export Review, 13 (1), 55 (1952).

21. Hayward, H., The Structure of Economic Plants, The Macmillan Co., New York, 1948, p. 580.

22. Hill, A., Economic Botany, McGraw-Hill Book Co., Inc., N.Y., 2nd Ed. 1952, p. 382.

23. Hooper, D., Characters of Certain Fixed Indian Oils. J. Soc. Chem. Ind., 27, 906 (1908).

24. Jamieson, G., Vegetable Fats and Oils, Reinhold Publishing Corporation, N.Y., 2nd Ed., 1943, p. 248.

25. Kaufman, H. and H. Fiedler, The Field Fats LXVII. Pumpkin Seed Oil. Fette u. Seifen, 46, 125 (1939). Through Chemical Abstracts, 33, 4445 (1939).

26. Kay, L., The Microscopical Study of Drugs, The Williams and Wilkins Co., Baltimore, 1939, p. 17.

27. Kemp, A., Intestinal Parasites: A Suggestion in the Method of Treatment. J.A.M.A., 96, 1948 (1931).

28. Krayer, O., Klin. Wschr. 16, 1651 (1937). Through (17) and (32).

29. Kremers, E. and G. Urdang, History of Pharmacy, J.B. Lippincott Co., Philadelphia, 2nd Ed., 1940, p. 54.

30. Kuzmicki, R., Investigations upon Efficiency of Pumpkin Seed in the Treatment of Taenia Saginata Infection. Wiadomosci Parazytologiczme, 2 (2), 85 (1956). Through Trop. Dis. Bull., 53, 903 (1956).

31. Lindsey, Smith and Beals, Massachusetts Agr. Exp. Sta., Spec. Bul. (1919). Through (62).

32. Lys, P., Les Medicaments Taenifuges. The Lebanese Pharmaceutical Journal, 4 (2 & 3), 148 (1956).

33. Magath, T. and P. Brown, Standardized Method of Treating Tapeworm Infestations in Man to Recover the Head. J.A.M.A. 88, 1548 (1927).

34. Maplestone, P. and A. Makerji, Treatment of Taenia Infections. Ind. Med. Gaz., 66, 667 (1931).

35. Mazzoti, L. and A. Trevino, La Semence de Courge dans le Traitement de Taenia. Sem. Hop. Paris, 27 (80), 3184 (1951).

36. Mudalliar, C., Studies in the Nomenclature of South Indian Cucurbits. South Indian Hort., 1, 13 (1953). Through Biol. Abstr., 27, 32811 (1953).

37. J. Amer. Pharm. Ass., 20, 555 (1931).

38. Pawlowski, Z., Results of Treatment with Pumpkin Seed. Wiadomasci Parazytologiczne, 2 (2), 93 (1956). Through Trop. Dis. Bull., 53, 902 (1956).

39. Pawlowski, Z., Wyniki Leczenia Taeniarchynchosis Nasionami dyni Semina Cucurbitae. Wiadomosci Parazytologiczne Warsaw, 2 (2), 93 (1956). Through Helminth. Abstr., 25, 151e (1956).

40. The Pharmacopoeia of the United States of America, Lippincott Co., 5th - 11th Ed., (1863-1936).

41. The Pharmacopoeia of the United States of America, Lippincott Co., Philadelphia, 15th Ed. (1955).

42. Perrot, E., Matieres Premieres Usuelles du Regne Vegetal, Masson et Cie., Paris, Vol. II, 1943-44, p. 2055.

43. Pfister, A., Vermifugal Action of Pumpkin Seed. Pharm. Ztg., 80, 394 (1935). Through Chemical Abstracts, 29, 3775 (5) (1935).

44. Planchon, L., Ph. Bretin and P. Manceau, Precis de Matieres Medicale, Libraire Maloine, Paris, Vol. II, 1946, p. 1432.

45. Planchon, G. and E. Collin, Drogues Simples D'Origine Vegetale, Octave Doin, Paris, Vol. II, 1896, p. 297.

46. Poda, Zeitsch. Nahr. Genussm, 1, 625 (1898). Through Allen's Commercial Organic Analysis, J. and A. Churchill, London, 5th Ed. Vol. II, 1924, p. 186.

47. Post, G., Flora of Syria, Palestine and Sinai, American Press, Beirut, Vol. I, 1932, p. 483.

48. Power, F. and A. Salway, Cucurbita Pepo Seed, Chemical Examination. J. Amer. Chem. Soc. 32, 346 (1910).

49. Riebsomer, J. and G. Nesty, Examination of the Fatty Acids from Pumpkin Seed. The Constitution of Linoleic Acid. J. Am. Chem. Soc., 56, 1784 (1934).

50. Russel, P., Identification of the Commonly Cultivated Species of Cucurbita by Means of Seed Characters. Jour. Wash. Acad. Sci. 14, 265 (1924).

51. Sandground, J., Newer Drugs for the Treatment of Tapeworm Infestations. New Eng. J. Med. 218, 298 (1938).

52. Schattenfroh, Z. Nahr. Unters. Hyg. Waarenk. 8, 202 (1894). Through(62).

53. Schubiger, E., Pharm. Arch., 8, 49 and 65 (1937). Through (9).

54. Scott, W., Standard Methods of Chemical Analysis, D. Van Norstand Co., Inc., 5th Ed., Vol. II, p. 1301.

55. Trease, G., A Textbook of Pharmacognosy, Balliere, Tindall and Cox, London, 7th Ed., 1957, p. 659.

56. Tyson, E., Physiological Transaction, (1683). Through (32).

57. Union Internationale de Chimie, Methodes Unifiees pour l'Analyse de Matieres Grasses, Troisieme rapport de la commission internationale pour l'etude des matieres grasses, Maison de la Chimie, Paris (1948).

58. Valentin, J. and G. Brockelt, Pumpkin Seed as a Taenifuge. Pharmazie, 11 (6), 412 (1956).

59. Veen, A. and Collier, W., Stable, Palatable Preparation of Laboe Marah (Cucurbita moschata). Dutch, 63, 33 (1949). Through Chemical Abstracts, 43, 7198 (1949).

60. Wallis, T., Practical Pharmacognosy, J. and A. Churchill, Ltd., 6th Ed., 1953, p. 227.

61. Whitaker, T. and G. Bohn, The Taxonomy, Genetics, Production and Uses of the Cultivated Species of Cucurbita. Econ. Bot. 4 (1), 52 (1950).

62. Winton, A. L. and K.B. Winton, The Structure and Composition of Foods, John Wiley and Sons, Inc., New York, Vol. II, 1935, p. 429.

63. Wood, H. and Ch. LaWall, The Dispensatory of the United States of America, J.P. Lippincott Co., Philadelphia, 21st Ed., 1926, p. 820.

64. Woodman, A., Food Analysis, McGraw-Hill Book Co., Inc., 4th Ed., 1941, p. 170.

65. Yashuda, A., On the Comparative Anatomy of the Cucurbitaceae, Wild and Cultivated, in Japan. Jour. Coll. Sci. Imp. Univ. Tokyo, Japan, 18, 1 (1903). Through (21).