BIOCHEMICAL STUDIES ON POLLEN OF SOME PLANTS

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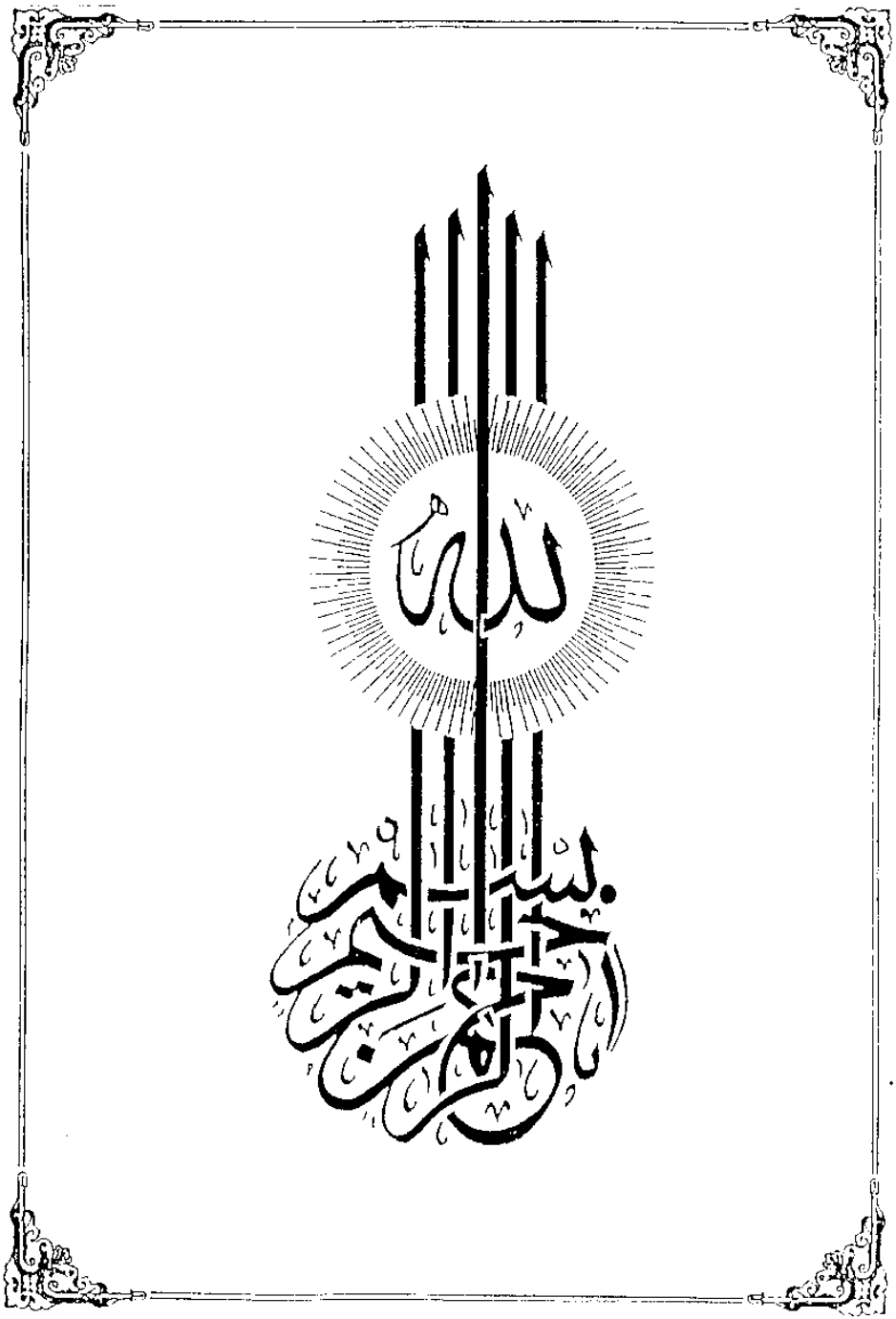

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INTRODUCTION

Pollen is an interesting biological material to use for chemical studies. It is important as a carrier of male genetic materials, as an essential food for honey bees and as a source of many serious allergies of medicinal value. So that, content of lipids, free amino acids, proteins and enzymes in various pollens, has widely interested botanists, entomologists, biochemists and allergologists. However, very little information are available on the construction of such materials in pollen.

On the other hand, taxonomic determination and plant classification based on morphological characteristics together with biochemical techniques seem to be rather interesting during the last two decades. Furthermore, organisms within a species may be distinguished better by differences in both the biochemistry and function of the proteins than by morphological character analysis. There seems to be agreement among some researchers that proteins which have been separated electrophoretically and given a general stain are most useful in differentiating species within a genus or naturally occurring plant populations of a single species. Moreover, some isoenzymes may be used as markers for the identification of genotypes.

It is the aim of this work to execute a comparative qualitative and quantitative biochemical analysis among pollen grains in 8 species of three different families. The fatty acid constituents of four fractions of lipid materials, unsaponifiable matters, free amino acids, di- and tricarboxylic acids, proteins and enzymes were subjected to comparative analysis in order to search for characteristics which might unequivocally identify varieties, species or families.

REVIEW OF LITERATURE

I. Lipid materials in pollen grains:

Scott and Strohl (1962), found that loblolly pine (Pinus taeda) pollen contains about 7.5 to 9 percent lipids, most of which were triglycerides of oleic, linoleic, and palmitic acids. The triglycerides were not easily extracted from pollen unless the grains were fractured. Lipids, which were easily extracted and were propably part of the outer spore coat, comprised 1.6 percent of the pollen. These were mainly octacosanol and hexacosanol with smaller amounts of wax esters and free acids.

Iwanami and Nakamura (1972), soaked pollen grains of Lilium auratum, L.longiflorum, Camellia sasanqua and Impatiens balsamina in various kinds of organic solvents such as acetone, benzene, petroleum benzine, benzyl alcohol, butanol, ethanol, methanol, isopropanol, diethylether, petroleum ether and chloroform, and stored samples at 4-6° C for 24 hrs. Pollen grains showed evidence of viability except for that treated with benzyl alcohol. Grains treated with acetone, benzene, petroleum benzine, diethylether, petroleum ether and chloroform, however, produced longer pollen tubes than fresh pollen grains.

Futhermore, pollen grains could retain their viability after 80 day storage in acetone, benzene, petroleum benzine, diethylether and petroleum ether.

Various studies on lipid composition of pollen grains seemed to be interesting for many investigators:

1. Fatty acids:

Opute (1975, 1978), classified pollen lipids of west African oil palm Elaeis guineensis, Raphia hookeri, R. sudanica, R. vinifera, R. regalis and R. farinifera into neutral lipids (triglycerides, esterified with free sterols and trace amounts of hydrocarbons) and polar lipids (monogalactosyl and digalactosyl diglycerides, phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine). In all Raphia species, the major identified fatty acids were oleic and linoleic. However, in oil palm, linoleic, palmitic and linolenic represented the major fatty acids while oleic, stearic, arachidic, myristic, lauric, palmitoleic and margaric acids were of small to trace amounts. The unsaturated/saturated fatty acid ratio was 3:2. The fatty acid composition of triglycerides were approximately identical to that observed for polar lipids. No significant differences in lipid composition of pollen were also noticed between different varieties of oil palm.

The lipid and fatty acid composition of six species of bee-gathered pollens, representing five plant families were studied by Farag et al. (1978). The total lipid content ranged from 2.24% in citrus to 4.42% in Egyptian clover. Gas liquid chromatographic analysis indicated that linoleic and linolenic were the major acids in citrus and flax pollen, respectively. Broad bean pollen, on the other hand, was high in both palmitic and linolenic acids; myristic acid represented about half of the total fatty acid content of sunflower pollen; the major fatty acid constituents of wild mustard and Egyptian clover pollens were myristic and linolenic. Arachidic acid was noticed in minor amounts in citrus, flax and wild mustard pollens, while decanoic acid was observed in trace amounts in sunflower and wild mustard pollens. No odd-carbon fatty acids were identified in samples under investigation.

Cerri et al. (1979), determined fatty acids in different species of pinus and observed that oleic and linoleic represent the major fatty acids in P. canariensis, P. pinaster and P. pinea species. The unsaturated fatty acids, therefore, predominated over the saturated ones.

Cerri et al. (1982), determined the unsaturated/saturated fatty acid ratio for comparison between different plants. Such a ratio was 1:1 in Cedrus silani

and Cucurbita pepo pollen, whereas the unsaturated fatty acids were predominant in Castanea sativa. The predominant fatty acids in C. sativa, C. pepo and C. silani were linolenic with a 9,12, 15 unsaturated $C_{18:3}$, palmitic and stearic acids, respectively.

Van Der Vorst et al. (1982), compared the chemical composition of lipids in colony and laboratory stored pollen grains gathered mostly from Brassicaceae by bees. Lower concentrations of linoleic and linolenic but higher amounts of palmitic and stearic fatty acids were observed in pollen grains stored in colony compared with that stored in laboratory.

Fatty acid composition was studied in maize pollen by Kirichenko et al.(1984). The amount of palmitic and linolenic acids exceeded 63% of the total fatty acids.

2. Unsaponifiable matters:

Mass spectrometric investigation on sterol pollen fractions for 15 plant species from 11 families was executed by Standifer et al. (1968). In red clover, saguaro cactus, mustard, London-rocket, rye, timothy and sweet corn, 24-methylenecholesterol was found to be the

principal sterol. On the other hand, beta-sitosterol was the predominant sterol in mule fat, juniper, heartsease, waterleaf, Scotch pine, European alder and Lombardy poplar. Cholesterol, however, was the major one in cotton wood.

In a series of studies on constituents of some pollen grains, Ohmoto et al., (1974, 1982) isolated some triterpenoids including alpha and beta amyrin with a few sterols involving campesterol and beta-sitosterol. Two new sterols were identified, by spectroscopic studies and chemical evidence, namely 4α, 14-dimethyl-9, 19-cyclocholestan -3β, 24 ξ -diol and 4 α, 14-dimethyl-9, 19-cyclocholestan - 3β, 24 ξ, 25-triol. Lophenone, Lophenol and cholest-7-en 3B-ol were also detected in pollen grains of Ambrosia elatior.

Studying lipid and sterol constituents of West African oilpalm pollen, by Opute (1975), revealed that 4-desmethyl sterols were the predominant phytosterols in the free form . Low proportions were observed, however, in the esterified state. Furthermore, 28-isofucosterol was found to be the principal one.

Knights and Smith (1976), reported that Zea mays pollen contained 24-methylenecholesterol as a major sterol. Lesser amounts of cholesterol, 24-ethylcholesterol,

(28 Z) -24—ethylidene-cholesterol, 24-methylene-5-alpha-cholest-7-en-3 beta-ol-and 4 alpha-methyl-24 methylene-5 alpha-cholest-7-en-^{3}beta-ol were also observed.

Analytical analysis of sterols in date palm pollen was executed by Mahran et al. (1976). Beta-sitosterol, beta-amyrin and a third unknown crystalline substance were isolated from diethylether unsaponifiable extract. Thin layer chromatographic analysis revealed the presence of oesterone.

Cerri et al. (1979 and 1982), reported that sitosterol was the predominant sterol in pollen grains of Pinus species, Castanea sativa, Cedrus silani and Cucurbita pepo. The most abundant long chain alcohols in Pinus species were n-tetracosanol-1, n-docosanol-1 and n-eicosanol-1.

Chromatographic analysis of pollen unsaponifiable matters in six plants from 5 families; Leguminosae, Cruciferae, Compositae, Linaceae, Rutaceae; revealed the presence of 6-8 sterols, (Farag et al. 1980). Cholesterol, stigmasterol and beta-sitosterol represented the major sterol component, however, beta sitosterol was found to be the predominant one. The same authors stated that unsaponifiable data may be taken as a key for identification of the pollen source.

Analysis of sterols in pollen grains collected from honey bee colonies by Svoboda et al. (1983) revealed the presence of isofucosterol in maple, Δ^{7}-stigmasten-3β-ol in goldenrod, $\Delta^{7,24(28)}$- compestadien-3β-ol in corn, 24-methylenecholesterol in dandelion. β-Sitosterol was found, however, the major one in blackberry, cucumber and honeysuckle colony pollen.

II. Free amino acids in pollen grains:

Many attempts all over the world were done by various scientists to detect free amino acids in pollen grains during the last four decades.

Auclair and Jamieson (1948), investigated the free amino acids in pollen grains of dandelion and willow. Pollen was removed from a brood comb of a colony in the spring and in a mixture of a pollen sample largely composed of dandelion, willow and maple. Pollen grains of different species differed in their constituents of amino acids. Furthermore, some pollen species exhibited variations between the free and conjugated amino acids.

On making comparative chemical analysis of pollen grains of corn (Zea mays) and sunflower (Helianthus annuus) collected by bees at various intervals of time (Cirnu et al. 1969), corn grains were found to be higher