

# 127, 17 27, 17 (20) 77, 17 (20









## جامعة عين شمس

التوثيق الالكتروني والميكروفيلم



نقسم بللله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأفلام قد اعدت دون آية تغيرات



## يجب أن

تحفظ هذه الأفلام بعيداً عن الغبار

في درجة حرارة من 15-20 مئوية ورطوبة نسبية من 20-40 %

To be kept away from dust in dry cool place of 15 – 25c and relative humidity 20-40 %



ثبكة المعلومات الجامعية





Information Netw. " Shams Children Sha شبكة المعلومات الجامعية @ ASUNET بالرسالة صفحات لم ترد بالأص

## COMPARATIVE TAXONOMIC STUDIES ON CHAROPHYTES IN RELATION TO OTHER GREEN PLANTS

#### Thesis

Submitted to Faculty of Science Alexandria University
For the Degree of Doctor of Philosophy in Science
(Botany)

By

#### EMAN MOHAMED FAKHRY ABD-EL SALAM

B. Sc. "Hons" (Botany) 1986 M. Sc. (Botany) 1991

Supervised by

#### Dr. ABDEL-FATTAH KHALEAFA

Professor of Phycology Botany Department

#### Dr. SAMI HAMED SHAALAN

Professor of Phycology Botany Department

Faculty of Science Alexandria University 1997 30,90

To my parents, my husband, Ahmed , Khaled & Dahlia With all my love.

### Acknowledgments

First and last, all praise be to Allah, the most high, the beneficent, the merciful, who has taught the use of the pen, has taught man what he did not know.

Deep appreciation and gratitude to Dr. Abd - Elfattah Khaleafa, and Dr. Sami Hamed Shaalan, Professor of Phycology, Botanty Department, Faculty of Science, Alexandria University, for suggesting the problem and program of the work, for invaluable supervisions, guidances, revisions and for continuous serious discussions during the progress of this work.

Special and deep thanks to Dr. Nagwa Gamal El- Din, Lecturer of Botany, Faculty of Science, Alexandria University, for her great help through the analyses of fatty acids and sterols.

I am greatly indebted to Dr. Mohamed Saad, Lecturer of Botany, faculty of Science, Alexandria University, for his magnificent help during the analyses of pigments, protein profile, isozymes and his effective cooperation in photography.

I take the opportunity to thank Dr. Ehab Essa, Lecturer of Dairy Sci. and Tech., Dairy Science and Technology Department, Faculty of Agriculture, Alexandria University, for facilities he kindly put under my disposal.

Acknowledgment is gratefully extended to my lovely mother and my dear father for their continueous kind help and for their endless encouragement & love.

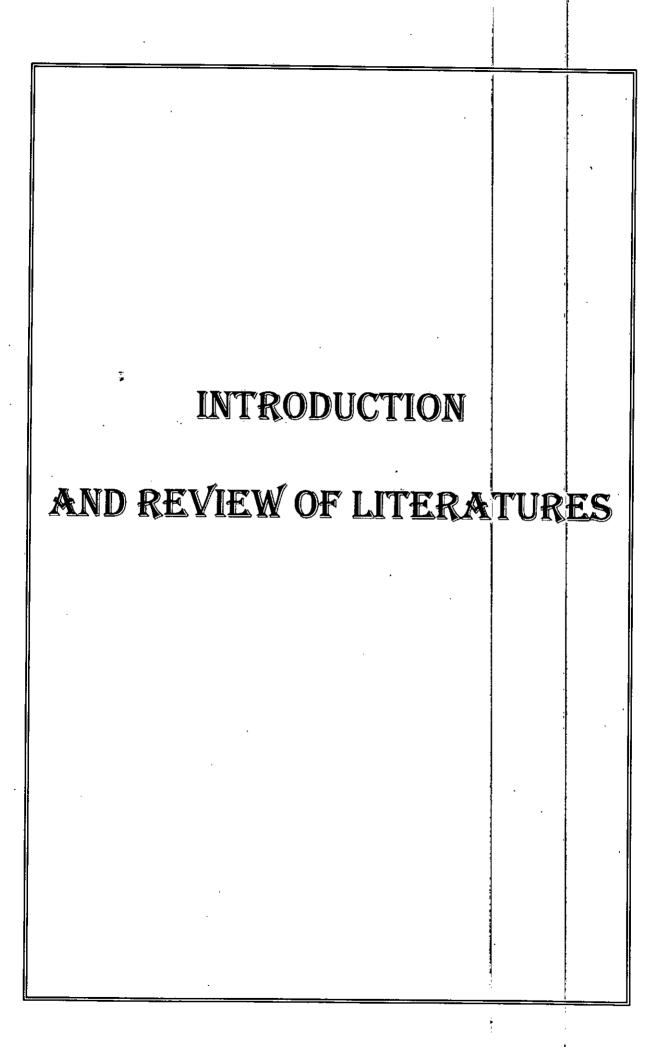
From my deep heart, sincere thanks to my husband Dr. Alaa El-Din Saleh, E.N.T. Surgeon, for his patience and excellent behaviour.

Also, I would like to thank all my collegues in phycology laboratory and all others who supplied me with any kind of help.

## CONTENTS

;		Page
* Introduction and Review of Literature		1
* Historical Review and Aim of Work	I	15
* Materials and Methods		27
I. Collection of biological materials	•	27
II. Experimental uniformity and standarization	•	29
III. Protein profile and isozymal analyses		29
1) Preparation of dialysis tubings for samples dialysi	s	29
2) Extraction of soluble proteins		30
3) Electrophoretic gel preparations		30
4) Electrophoretic runs	1	32
5) gel staining		33
6) Estimation of similarity coefficients	;	35
IV. Determination of sterol and fatty acid fraction		36
1) Extraction	:	36
2) Extraction of unsaponifiable lipids	,	37
3) Isolation of sterols		37
4) Purification and identification of sterols		38
5) Purification and identification of fatty acids	•	39
6) Estimation of individual sterols and fatty acids		40
V. Pigment analyses		41
* Florestic Study		44

* Deculte	62
* Results	62
I. Gel electrophoresis and analysis of data	
1) Total soluble protein profile and zymograms	63
2) Similarity matrices and similarity dendrograms	65
3) Average similarity matrix and average dendrogram	67
II. Fatty acids and sterols	67
Fatty acids composition of the investigated plants	67
2) Similarity matrix and similarity dendrogram	86
3) Sterols composition of the investigated plants	86
4) Similarity matrix and similarity dendrogram	117
5) Average similarity matrix and average dendrogram	117
III. Thin layer chromatography and absorption spectra of	
pigment fractions	146
1) Pigment composition of the investigated taxa	146
2) Similarity matrix	149
* Discussion	160
*Conclusion	182
*Summary	185
*References	190
*Arabic Summary	•
	1
	•
	•
	<u>:</u>
	1



#### INTRODUCTION

#### AND REVIEW OF LITERATURES

microscopy, electron in advances new √hrough electrophoresis, gas chromatography, and other developing techniques, one may well wonder how systematics (which began as a relatively simple activity including only species recognition and are involved into a highly complex science. The classification) incorporation of new data arising from the application of modern techniques, in recent years, allow the detection and botanical identification of extremely minute samples of many compounds that are proving to be as useful systematic tools (Alston and Turner, 1963 a).

The two terms, systematic and taxonomy, are often used synonymously, but more accurately taxonomy is the study of the bases, principles, procedures, and rules of classification (Heywood, 1973) while systematic is the classifactory process itself. Also, careful must be taken to distinguish between the taxonomy of organisms, and the study of their phylogeny, i.e. their origin. Phylogeny can be considered as a taxonomy in which the resulting system is thought to be representative of the historical evolution of the organisms studied. Descent from common ancestors with evolutionary modifications is studied in its various manifestation: morphological, biochemical or otherwise. Consequently a phylogeny, adequately constructed, is a more powerful conceptual framework than is a taxonomy alone.

Why is it useful to construct a phylogeny of the taxa using biochemical data? Much of the promise in chemical data from plants lies in the possibility that certain kinds of chemical evidence may be a reliable guide to phylogenetic relationships of living species.

However, the most objective method for determining phylogeny is to measure homology of the base sequences of comparable nucleic acids or amino acid sequences of comparable proteins (Pigott and Carr, 1972; Dayhoff and Schwartz, 1980; Fox *et al.*, 1980; Doolittle and Bonen, 1981; Cavalier-Smith, 1982; Gray and Doolittle, 1982; Hori *et al.*, 1985 and Lim *et al.*, 1986).

It is not easy to collect the necessary biochemical data, whether reaction pathways, molecular structures, or chemical compositions, there may be problems of the absence of a character due to the repression of a gene, or to a critical mutational step that has occurred recently, or to insensitivity of the analytical method. Also, the culture conditions or an abnormal environment for the organism may cause unnatural change in its biochemistry.

The extent current of interest in biochemical systematic is indicated by the appearance of several books on the topic (Hegnauer, 1962; Swain, 1963 and 1966; Alston and Turner, 1963b; Turner, 1966; Hawkes 1968; Smith 1976; and Ferguson, 1979).

It is important that one does not lose sight of the morphological characters associated with evolution and phylogeny. It is to some degree artificial to speak of morphological phylogenetics and biochemical phylogenetics as if they were independent undertakings. Also, it is important to distinguish between the morphology of the whole organism (macromorphology) and its subcellular structure (micromorphology; e.g. ultrastructural considerations). In algae, the phylogeny (and taxonomy) of the lower ranks "species, genera, and families" is based heavily upon the macromorphological characters, while the conservative micromorphological characters are used more in the erection of the phylogeny of the higher ranks "orders, classes, and divisions" (Ragan and Chapman, 1978).

Old trials for algal classification made by phycologists depended mainly on morphological and anatomical features. However, the prevailing emphasis on morphology has proven unsatisfactory especially in the assemblage of organisms that show polymorphism (algal species exhibit more than one vegetative from when growing in different environments i.e. ecotypes) that might result under different ecological, seasonal and geographical conditions (Dixon, 1973; Sinclair & Whitton, 1977; Cheney & Babbel, 1978; and Allam, 1994) or those in which uniformity of morphology is most noticeable. Therefore many phycologists would agree and acknowledge that algal classification does need new approaches, e.g. biochemical, physiological, cytological, and ultrastructural.

A lot of schemes have been suggested by leading phycologists for algal classification. Non of them could be considered as the best to be accepted and non-of them must not be considered final, (Mohammad, 1981). Authors regard all these systems as tentative and always to be modified in the light of new data (Bold and Wynne, 1978).

Bold (1970) emphasized the difficulties of a taxonomic treatment of the chlorophycean soil algae because of the morphological similarities (coccoid and palmelloid); lack of reproductive structures, and the masking of cellular organization by starch and lipids produced as a result of nutritionally deficient environment. However, Bailey and Samsel (1971) stated that the classification of many species of algae presents the taxonomists with a frustrating paradox.

A number of chemotaxonomic studies have been undertaken for green algae. The extent of these studies has varied considerably depending very much upon the question asked. By far the most extensive studies are those of Kessler's group aimed at establishing a well-substatiated taxonomy for the genera in the Chlorococcales (Kessler's 1974 and 1978; Kessker's and Czygan, 1966; Hellman and Kessler, 1974; Kerfin and Kessler, 1978).

Most of the work that have been done in the field of chemotaxonomy indicated that many biochemical metabolites could have a taxonomic value. The most important and promising of these are: