The large, crustose families such as Thelotremataceae and Graphidaceae are important components of tropical diversity, with many undescribed species. In these families generic concepts may need to be reconsidered in the light of tropical material.

In view of the vast areas, its wide range of altitudinal zones and its considerable climatic variation, the lichen flora of India is very diverse. In contrast to macrolichens, considerable number of the taxa of microlichens, which are ecologically significant as indicators of primary forests from India are yet insufficiently known, revised or even investigated (Awasthi, 1991).

Like many other microlichens (crustose) the family Graphidaceae which is one of the largest and widely distributed groups of lichens in the lichen flora of India are still inadequately studied and the taxa reported till now are mostly in need of revision. Although, several taxa of family Graphidaceae in India have been recorded in many scattered publications, through the preliminary work of Indian lichenologists, there has never been a comprehensive treatment of the members of the family Graphidaceae in India.

The apparent structural simplicity and short taxonomic characters of this family present major taxonomical obstacles in its perusal. Generic concepts in crustose lichens is rapidly changing and the generic and family arrangement of the Graphidaceae is still very incompletely settled.

Also, in view of extinction of several lichen species through man-made atmospheric and terrestrial pollution of the destruction of primary forests in India where the conservation resources are scarest, it is urgently and highly desirable to develop methods for developing lichen thalli in vitro. Such ex-situ preservation of rare lichen germplasm can provide a resource for research as well as for reintroduction if a species is lost in the wild.
The present dissertation is based on the research work done by me during the last two and a half years on the lichen family Graphidaceae in three chapters of the thesis:

**Chapter I.** The comprehensive taxonomic account of the lichen genus *Graphis* (Family *Graphidaceae*) from India.

**Chapter II.** Screening of bioactivities in natural thalli of some taxa of lichen family *Graphidaceae*.

**Chapter III.** Standardization of method of lichen tissue culture *in vitro* for their *ex-situ* conservation.

The first chapter of the thesis deals with the comprehensive taxonomic studies of the lichen genus *Graphis* Adans. ex Müll. Arg. (*Memoir Soc. Phys. et Hist. Nat. Geneve, 29: 38, 1887*) which is the largest cosmopolitan genus in the family *Graphidaceae* claiming over 300 species at world level and previously known by 74 species in India.

The morphological, anatomical, and chemical studies of 1500+ specimens including type/authentic material have resulted into the recognition of as many as 137 species of *Graphis* from India, which includes 11 new records, 8 varieties and as many as 48 new species and 7 unnamed species for India.

The morphotaxonomic account of the 137 species of the lichen genus *Graphis* from India has been prepared and presented in the thesis along with a key for the identification of all species from India. The detail taxonomic descriptions of the species together with the chemistry followed by a short discussion on their geographical distribution in world, their relationship and illustrations have been given.

The second chapter of the thesis deals with the screening of bioactivities in natural thalli of some species (77 species) belonging to the lichen genera namely *Graphina, Graphis, Phaeographina, Phaeographis* and *Phaeographopsis* (Family *Graphidaceae*).
Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. However, very little information is available on the pharmacological properties of lichen metabolites. Lichens have long been recognized as containing bioactive compounds, but little attempts have been made to screen them.

There have been no comprehensive studies of a systematic group of lichens reporting tyrosinase inhibition, superoxide-scavenging activity (SSA) and the inhibition of xanthine oxidase (IXO). With this in mind the study was undertaken. For the studies of these activities dried herbarium material of 77 species of the genera *Graphina, Graphis, Phaeographina, Phaeographis* and *Phaeographopsis* was used.

One of the cause of hyperpigmentation is the over production of dermal melanin pigment within the melanocytes. Tyrosinase plays an important role in the formation of melanin and tyrosinase inhibitors have become increasingly important as cosmetic and medicinal products, primarily to control melanin pigmentation.

Lichens mainly produce phenolics and their oxidative derivatives, therefore they can be thought to be good sources that can supply novel tyrosinase inhibitors. However, it is important to investigate more active and less toxic compounds in natural resources.

It is increasingly being realized that a majority of the diseases/disorders are mainly due to the imbalance between pro-oxidant and anti-oxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to increased generation of free radicals and/or their poor scavenging into the body. Majority of disease conditions like atherosclerosis, hypertension, ischemic diseases, Alzheimer’s disease, Parkinsonism, cancer and inflammatory conditions are being considered to be primarily due to the imbalance between pro-oxidant and anti-oxidant homeostasis.

Therefore, much attention is being directed in harnessing and harvesting antioxidants from natural resources. Biological and chemical pro-oxidants are considered to be important for the provocation of free radical mediated diseases in an individual.
Although free radicals are considered to be important for normal physiology, they cause cellular damage when produced in excess.

Much physiological damage may be directly responsible to the hydroxyl radical produced in vivo would react at or close to its site of formation. Eliminating superoxide would reduce H₂O₂ and there by production of hydroxyl radicals. Xanthine-oxidase catalyses the oxidation of hypoxanthine and xanthine to uric acid.

For the present studies 77 species of the lichen family Graphidaceae have been searched for tyrosinase-inhibitory activity, xanthine oxidase inhibitory activity and superoxide scavenging activity (SSA). Tyrosinase-inhibiting material extracted with MeOH, Acetone, Ethanol, Dimethyl sulphoxide in water and water only. Methanol has been found to be suitable for extracting good amounts of tyrosinase-inhibiting component from the natural thallus. Inhibitory activity of xanthine oxidase was carried out by using enzymatic method with cytochrome c and non enzymatic method with nitro blue tetrazolium (NBT).

The lichen species Graphina glaucorufa (Vainio) Zahlbr., Graphina multistriata Müll. Arg., Graphina salacinilabiata Patw. & Kulk., Graphis assamensis Nagarkar & Patw., Graphis nakanishiana Patw. & Kulk., and Phaeographopsis indica (Patw. & Nagarkar) Sipman & Aptroot, have shown inhibition of tyrosinase over a range of 30-78%.

Half-inhibiting concentration (IC₅₀, µg/ml) has been found to be much lower than the standard tyrosinase-inhibitors like Ascorbic acid, Azelaic acid, Curcumin, Kojic acid, Mimosine and Tropolone, and thus can compete with the other commercially available tyrosinase inhibitors. The extracts of these species have been found to be stable at 4°C.

The potential of the extracts for scavenging of superoxide and inhibition of xanthine oxidase under various physiological conditions have been evaluated. Out of 77 species, 30 species gave extract yields in the range of 7-77.5 µg and were found to show both superoxide scavenging activity (SSA) and inhibition of xanthine oxidase (IXO) activities. The methanolic extracts of the species showed inhibition of xanthine
oxidase remained stable at 4°C and inhibitory properties of xanthine oxidase is found to be IC₅₀ = 2.0 to 5.26 µg/ml and superoxide scavenging capacity as IC₅₀ = 3.63 to 13.88 µg/ml.

The third chapter deals with the standardization of a method of lichen tissue culture in vitro for their ex-situ conservation.

Lichens, as a significant component of the biodiversity in many of the world's ecosystems, with vast array of secondary metabolites known (ca. 700), and with field observations suggesting many are bioactive, they merit particular attention in the current search and should be conserved for naturally occurring agrochemicals, pharmaceuticals and other exploitable compounds.

Lichens are very slow growing organisms. Research in the field of cultivating lichens and their symbionts will open a door to the mass production of lichen substances and their pharmaceutical and technical applications.

Also, with the declining in the area of natural habitats, ex-situ preservation can be used as supplemental tool for maintaining lichen germplasm, offering insurance against the possibility of loss of organisms from the wild. Such ex-situ preservation of rare lichen germplasm can provide a resource for research as well as for reintroduction if a species is lost in the wild.

With this view, in the present studies attempt has been made to develop methods for the ex-situ conservation and the long term preservation of the tissue obtained from the fragments of the natural thallus of the three species namely *Graphis guimarana* Vainio, *Graphis nakanishiana* Patw. & Kulk., and *Graphis schizograpta* Müll. Arg. using the induction method.

All the three species *Graphis guimarana*, *G. nakanishiana*, and *G. schizograpta* have been successfully cultured in vitro. The cultured lichen tissue consists of both symbionts and also the apothecia-like structures. The tissue has been observed to produce the secondary metabolites as in natural thallus.
This was one of the first attempts in India to culture crustose lichens in vitro. It can be undoubtedly said that lichen tissue culture would certainly be highly useful for the industrial production of desired secondary metabolites which have been proved to be of considerable value in their use in medicines, dye extraction and other biotechnological fields. The tissue culture of lichens in laboratory is expected to be great value in areas of experimental lichenology in future. It would also be useful in taxonomic investigations, enabling the role of environmental variables in the determination of thallus form to be investigated and it can provide natural resources to the scientists for biotechnology and to the industries as well.

**Publications**
*(Based On The Present Work)*


Capacity of some Graphidaceous lichens to scavenge superoxide and to inhibit tyrosinase & xanthine oxidase activities. *Curr. Sci.* (communicated).

Tissue culture of selected species of the lichen genus *Graphis* and their biological activities. *Fitoterapia*. Italy, (communicated).