

**INVESTIGATIONS ON ECO-ENVIRONMENTAL STATUS
OF BRYOPHYTES IN MELGHAT REGION WITH
REFERENCE TO SOIL MICRO FLORAL ASSOCIATION**

Thesis submitted for the degree of
Doctor of Philosophy in Botany

In the
**Faculty of Science
Sant Gadge Baba Amravati University
Amravati**

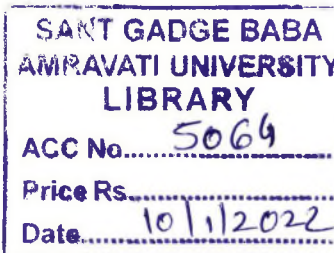


By
TUSHAR BHIMRAO WANKHEDE

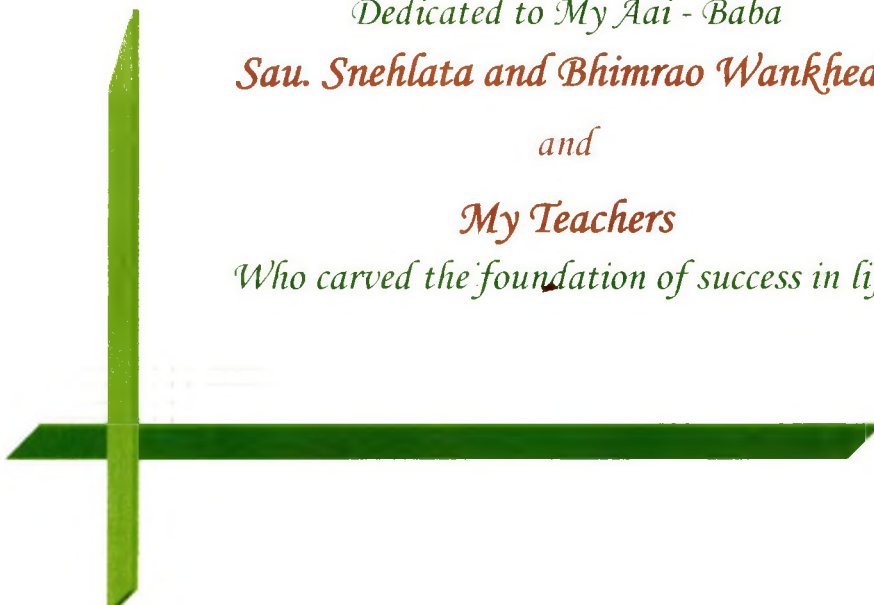
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Guide

Dr. S. R. MANIK
Professor and Head
Post Graduate Department Of Botany
Sant Gadge Baba Amravati University, Amravati – 444 602



MAY 2014



Dedicated to My Aai - Baba
Sau. Snehlata and Bhimrao Wankhede
and
My Teachers
Who carved the foundation of success in life



Dr. S. R. Manik
Professor and Head
Department of Botany
Sant Gadge Baba Amravati
University Amravati - 444 602
Maharashtra, India


CERTIFICATE

This is to certify that **Mr. Tushar Bhimrao Wankhede M.Sc.**, (FDP fellow) Department of Botany, Sant Gadge Baba Amravati University, Amravati has completed his research work entitled *Investigations on Eco-Environmental Status of Bryophytes in Melghat Region with reference to Soil Micro Floral Association* under my supervision.

It is further certified that the thesis submitted by him embodies his original work, planned and carried out under my guidance. The work carried out by the candidate is comprehensive and has not been submitted elsewhere for any other degree.



Signature of Supervisor


(S. R. Manik)

Date: 05/05/2014

Professor and Head
Department of Botany
Sant Gadge Baba Amravati University
Amravati

DECLARATION

I hereby declare that the work presented in this thesis entitled *Investigations on Eco-Environmental Status of Bryophytes in Melghat Region with reference to Soil Micro Floral Association* submitted for the degree of Doctor of Philosophy in Botany, was carried out by me under the guidance of Dr. S.R. Manik, Professor and Head, Department of Botany, Sant Gadge Baba Amravati University, Amravati and is approved by the Research and Recognition Committee.

The matter embodied in this thesis submitted by me is original, genuine and is not substantially the same as one already submitted for the degree or any other academic qualification at any other University or examining body.



Tushar Bhimrao Wankhede

Place - Amravati

Date - 05.05.2014

Guide




Dr. S. R. Manik

Professor and Head,
Department of Botany,
Sant Gadge Baba Amravati University,
Amravati



Forwarded

Signature of Head of the Department



Professor and Head,
Department of Botany,
Sant Gadge Baba Amravati University,
Amravati

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Dreams ! Dreams !! Dreams !!!

Convert your dreams into thoughts

And then transform those into actions

- Dr. A.P.J. Abdul Kalam



(Tushar Bhimrao Wankhede)

Amravati

03/05/2014

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ABBREVIATIONS

GPS	:	Global Positioning System
MSL	:	Mean Sea Level
TDS	:	Total Dissolved Solids
EC	:	Electric Conductivity
% C	:	Organic Carbon
µm	:	Micrometer
µg	:	Microgram
°C	:	Degree Celsius
<i>et al.</i>	:	et alia and associates
GOI	:	Government of India
Km	:	Kilometer
m	:	Meter
cm	:	Centimeter
ha	:	Hectares
mg	:	Milligram
g	:	Grams
ml	:	Milliliter
mm	:	Millimeter
nm	:	nanometer
ppm	:	Parts Per Million
MTR	:	Melghat Tiger Reserve
KMnO ₄	:	Potassium per magnet
H ₂ SO ₄	:	Sulphuric Acid
HCl	:	Hydrochloric Acid
VAM	:	Vesicular Arbuscular Mycorrhiza
Min.	:	Minutes

ABBREVIATIONS

hrs.	:	Hours
Std.	:	Standard
NaHCO ₃	:	Sodium Bi-carbonate
P ₂ O ₅	:	Phosporous pentaoxide
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
FeCl ₃	:	Ferric Chloride
PVLG	:	Polyvinyl Alcohol Lacto-Glycerol
FAA	:	Formalin: Acetic Acid: Alcohol
v/v	:	Volume/Volume
w/v	:	Weight/Volume
N	:	Nitrogen
P	:	Phosphorus
K	:	Potassium
EC	:	<i>Escherichia coli</i>
PV	:	<i>Proteus vulgaris</i>
KP	:	<i>Klebsiella pneumoniae</i>
SF	:	<i>Shigella flexneri</i>
SA	:	<i>Staphylococcus aureus</i>
PA	:	<i>Pseudomonas aeruginosa</i>
SA	:	<i>Salmonella typhimurium</i>
AN	:	<i>Aspergillus niger</i>
CA	:	<i>Candida albicans</i>
RA	:	<i>Rhizopus oryzae</i>
GC- MS	:	Gas Chromatography- Mass Spectroscopy
RT	:	Retention time

ABBREVIATIONS

- T. hypophylla* : *Targionia hypophylla* Linn.
- C. tuberosum* : *Cyathodium tuberosum* Kash.
- C. cavernarum* : *Cyathodium cavernarum* Kunze.
- A. angusta* : *Asterella angusta* (Steph.) Kachroo.
- R. hemisphaerica* : *Reboulia hemisphaerica* (Linn.) Raddi.
- P. appendiculatum* : *Plagiochasma appendiculatum* Lehm.et. Lindenb.
- P. intermedium* : *Plagiochasma intermedium* Lindenb.et .Gott.
- P. rupestre* : *Plagiochasma rupestre* (Forst.) Steph.
- R. gangetica* : *Riccia gangetica* Ahmad.
- R. discolor* : *Riccia discolor* Lehm.et. Lindenb.
- A. erectus* : *Anthoceros erectus* Kash.
- F. udarii* : *Folioceros udarii* Asthana.et.Srivastava.
- N. indica* : *Notothylas indica* Kash.
- P. laevis* : *Phaeoceros laevis* (Linn.) Prosk.
- F. hygrometrica* : *Funaria hygrometrica* Hedw.
- B. turgidum* : *Brachymenium turgidum* Broth. ex. Dix.
- B. coronatum* : *Bryum coronatum* Schwaegr.
- S. decorum* : *Stereophyllum decorum* (Mitt.) Wijk. et.Marg.
- H. involuta* : *Hyophila involuta* (Hook) Jaeg.
- H. recurvirostre* : *Hymenostylium recurvirostre* (Hedw.) Dix.

ABBREVIATIONS

Abbreviations	Common Name and Author Index (As per Schenck & Perez Manual, 1990)	Code
<i>A. delicata</i>	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	ADLC
<i>A. denticulata</i>	<i>Acaulospora denticulata</i> Sieverding & Toro.	ADTC
<i>A. mellea</i>	<i>Acaulospora mellea</i> Spain & Schenck	AMLL
<i>A. myriocarpa</i>	<i>Acaulospora myriocarpa</i> Spain, Sieverding & Schenck.	AMYC
<i>A. nicolsonii</i>	<i>Acaulospora nicolsonii</i> Walker, Reed & Sanders	ANCS
<i>A. rehmi</i>	<i>Acaulospora rehmi</i> Sieverding & Toro	ARHM
<i>A. rugosa</i>	<i>Acaulospora rugosa</i> Morton	ARGS
<i>A. scorbiculata</i>	<i>Acaulospora scorbiculata</i> Trappe	ASCB
<i>G. albida</i>	<i>Gigaspora albida</i> Schenck and Smith	GABD
<i>G. gigantea</i>	<i>Gigaspora gigantea</i> (Nicolson & Gerdemann) Gerdemann & Trappe	GGGT
<i>G. rosea</i>	<i>Gigaspora rosea</i> Nicolson & Schenck	GRSA
<i>G. aggregatum</i>	<i>Glomus aggregatum</i> Schenck & Smith emend. Koske	LAGR
<i>G. albidum</i>	<i>Glomus albidum</i> Walker & Rhodes	LABD
<i>G. citricola</i>	<i>Glomus citricola</i> Tang & Zang	LCTC
<i>F. constrictum</i>	<i>Funneliformis constrictum</i> (Trappe) Walker & Schüßler	LCST
<i>R. diaphanum</i>	<i>Rhizophagus diaphanum</i> (Morton & Walker) Walker & Schüßler	LDPH
<i>C. etunicatum</i>	<i>Claroideoglomus etunicatum</i> (Becker & Gerdemann) Walker & Schüßler	LACT
<i>R. fasciculatum</i>	<i>Rhizophagus fasciculatum</i> (Thaxter) Walker & Schüßler	LFSC
<i>F. fragilistratum</i>	<i>Funneliformis fragilistratum</i> (Skou & Jakobsen) Walker & Schüßler	LFGS
<i>F. geosporum</i>	<i>Funneliformis geosporum</i> (Nicolson & Gerdemann) Walker & Schüßler	LGSP
<i>G. glomerulatum</i>	<i>Glomus glomerulatum</i> Sieverding	LGML
<i>G. rubiformis</i>	<i>Glomus rubiformis</i> (Gerdemann & Trappe) Almeida & Schenck	LRBF
<i>G. tenerum</i>	<i>Glomus tenerum</i> Tandy emend. McGee	LTNR
<i>S. auriglobosa</i>	<i>Scutellospora auriglobosa</i> (Hall) Walker & Sanders	CARG
<i>S. pellucida</i>	<i>Scutellospora pellucida</i> (Nicolson & Schenck) Walker & Sanders	CPLC
<i>S. persica</i>	<i>Scutellospora persica</i> (Koske & Walker) Walker & Sanders	CPRS
<i>S. tricalypta</i>	<i>Scutellospora tricalypta</i> (Herrera & Ferrer) Walker & Sanders	CTRC
<i>S. weresubi</i>	<i>Scutellospora weresubi</i> Koske & Walker	CWRS

PROLOGUE

The present thesis embodies the results of investigations carried out on the bryophytes of Melghat forest with reference to its distribution, soil characteristics, and mutualistic association with mycorrhizal fungus, antimicrobial potentials and the phytochemical aspects. The presentation of research work consists of six major chapters followed by the seventh chapter comprises of alphabetically arranged bibliography cited by author in each chapter.

The first chapter deals with introductory general information of the Melghat forest and bryophytes distribution in the world and within Indian subcontinent. The works by the legends of bryologists were also reviewed with the present age scenario. The origins of bryophytes, their soil relation, the mutualism with mycorrhizal fungus, antimicrobial potential of bryophytes along with phytochemical information also includes in the chapter.

The second chapter comprises with the review of literature, which covers the information of past classical works in the subject as well as the present status of the subject. The review also comprises of bryophytes classical literature and their probable relations with soil and VAM fungi, phenomenon of antibiosis and phytochemical analysis.

The third chapter deals with the material and methods used to perform various experiments as well as field works cited in the thesis. The objectives set up and framed for the said research work were carried out using standard protocols and are illustrated in a very lucid way.

The fourth chapter consists of heavy collection of data and findings of the experiments performed and results obtained with morpho-taxonomic illustrations, tables, graphs, statistical analysis and the photo plates. Comparative analysis of the various findings were represented and interpreted in this chapter and covers the most comprehensive part of the thesis.

The fifth chapter consists of fruitful discussion of the subject based on the findings and interpretation of the results. This part comprises with the new insight to the findings of the present research work. Moreover, the last sixth chapter is of a conclusion which reflects the sound basis of the work, and the results interpretation with potential opportunities and prospects in the subject. Bryophytes are the pioneers in the process of succession hence their conservation is a key attribute of this thesis.



CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

"Bryophytes, the small Lilliputians of the plant kingdom" are non-vascular, herbaceous beguilingly simple, often diminutive and primitive land plants found in close vicinity of water bodies. Bryophytes share several ecological traits that make their joint assessment of climate impacts as an efficient starting point.

Bryophytes have traditionally been grouped under the umbrella term "Cryptogams" which indicate that the sexually derived reproductive propagules involved in their dispersal are microscopic haploid spores rather than seeds (Goffinet *et al.*, 2008). A second important trait is of their "Poikilohydric" nature i.e. they do not have active mechanism to prevent desiccation (Nakanishi, 1999). Bryophytes lack a specialized system for water and nutrient transport as that found in 'vascular plants' or "tracheophyte." Instead, the water status of bryophytes is highly responsive to ambient environmental conditions, with the organism tending to be turgid and photosynthetically active when the day time environment is wet, and in a state of desiccation when the environment is dry (Glime, 2006). Within these constraints, bryophytes species are differently adapted to a spectrum of moisture regimes with some species associated with constant wetness or humidity while others demonstrate an extreme tolerance of prolonged desiccation (Choudhary *et al.*, 2008).

The apparent simplicity of bryophyte-water relations belies a delicate equilibrium between their anatomical and physiological traits and small-scale environmental variation. This equilibrium has been shown to control the species realized niche for a long range of contrasting factors like biotic and abiotic components in system (Hebrard and Liosel, 1994). They are considered as pioneers that colonize terrestrial habitats from an aquatic environment. Though terrestrial, there are few aquatic forms such as *Riccia fluitens*, *Ricciocarpus natans* and *Riella* sp. *Cryptothallus* and *Buxbaumia* sp. are saprophytic genera of liverworts and mosses (Andrew *et al.*, 2003). Bryophytes are more common in humid areas and during rainy seasons, but usually show a preference for microclimatic niches such as crevices of rocks and trees and the vicinity of small shady springs (Shaw and Renzaglia, 2004). However, they can grow on wide range of substratum. Interestingly they may found on old discarded abandoned leather goods, rubber tires, wooden articles, tiled and asbestos roofs and mortar of stone and mud walls. They can grow as epiphytes on bark of trees (Corticolous), leaves (Folicolous), rocks (Rupicolous) on stones and pebbles (Saxicolous), on fallen logs (Lignicolous), riverbanks and roadside cuts

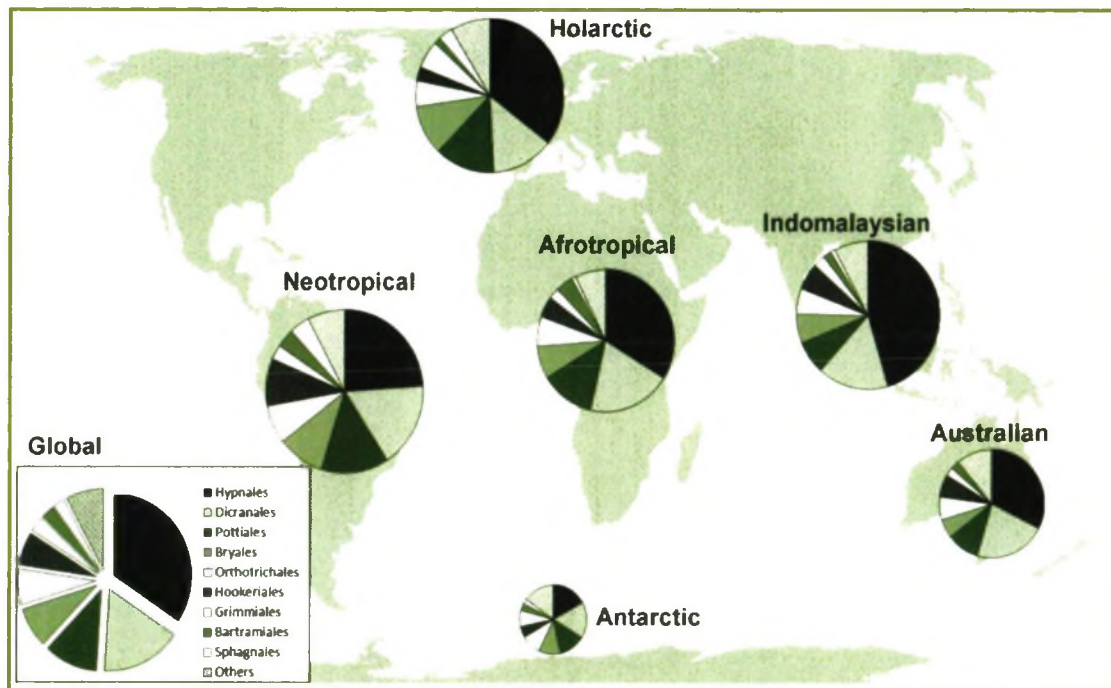
(Terricolous). Since water is inevitable for completing their life cycle, they are known as the “amphibians” of the plant kingdom (Daniels and Kariyappa, 2007). However, many are drought tolerant and are secondary colonizers on barren rocks in a xerosere after lichens. With a remarkable capacity to absorb water they turn fresh in no time and hence are known as "resurrection plants."

1.1 Biodiversity of bryophytes

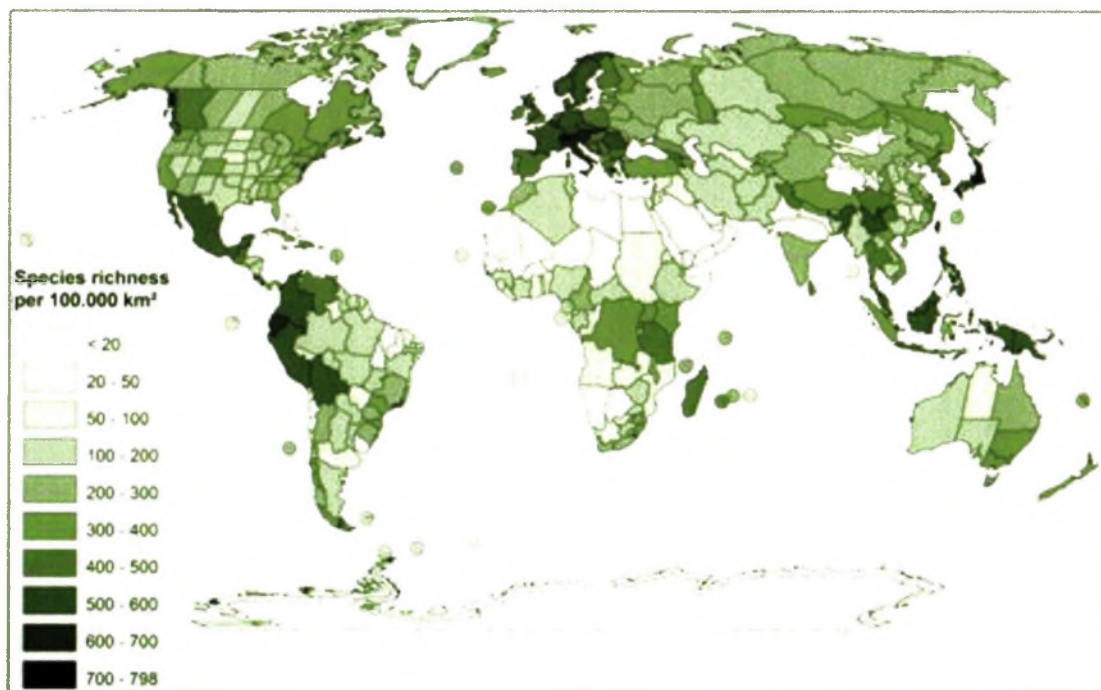
Bryophytes make a significant contribution to the floral diversity of this "watery planet" and since its inception constitute an important component of the forest ecosystem being the first colonizers on variety of habitats. They are highly specific group of plants with about 25,000 species distributed the world over, making it the second largest group of land plants after angiosperms (Alam *et al.*, 2011). Norse and McManus (1980) defined the term "Biodiversity" as the variability among living organisms from all sources and ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems. The taxic diversity measures for the position of taxon, where the species is considered as a basic unit. Genetic diversity is the diversity of the sets of genes carried by different organisms, which occurs among organisms of same population or different populations of the same species, those in different families, orders, kingdoms and domains. A large area and variety of phytoclimatic conditions with specific topography interacts with one another (both biotic and abiotic component) makes the ecosystem diversity for bryophytes and biogeochemical cycles (Porada *et al.*, 2014).

Geffert *et al.*, (2013) divided all the global mosses diversity into six major regions viz. Antarctic, Australian, Indomalaysian, Afrotropical, Holarctic, and Neotropical region (Fig: 1.1 A-B). The Centres of moss diversity includes the northern Andes, Southeast Asia, Mexico, and Japan, as well as the Himalayan region, Madagascar, the East African Highlands, central Europe, Scandinavia, and British Columbia. This analysis of the global patterns of moss species diversity based on a dataset created from checklists, online databases records for over 400 different geographical units and standardized species taxonomy using the TROPICOS - named database of the Missouri Botanical Garden at United States of America.

Fig: 1.1 Global Moss Diversity: Spatial and Taxonomic Patterns of Species Richness (Geffert *et al.*, 2013)



A) Major orders of mosses for the main floristic kingdoms. The size of the pie charts indicates the total species richness for the respective region.

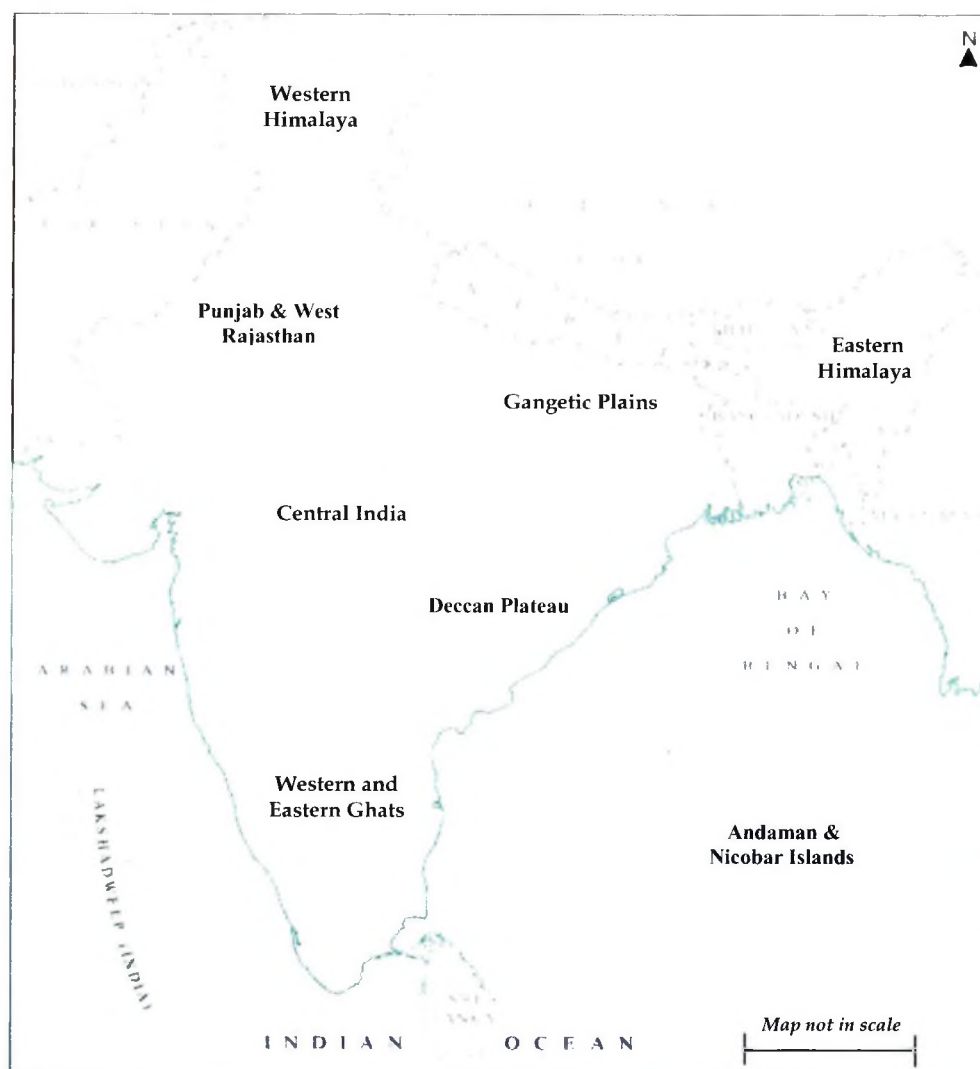


B) Patterns of global species richness in mosses. Species numbers have been standardized for an area of 100 000 km²

Pande (1958) divided India into six bryogeographical regions, namely the Western and Eastern Himalayas, Punjab and West Rajasthan, Gangetic Plains, Central India, Deccan Plateau and the Western and Eastern Ghats. However, Singh (2001) expanded the list into eight regions by adding Andaman and Nicobar Islands of the Indian subcontinent with reports based on the exploration (Fig: 1.2).

The conservation of natural ecosystems and nurturing biodiversity is critical for the survival of the human race. Hence, proper understanding of biodiversity (GOI, Ministry of Environment, and Forests, 2001) can evolve sound management strategies for sustainable development of ecosystem. The bryophytes constitute an important component of the flora and ecosystem almost throughout the world with vital role in moist-forest, wetland, mountain and Tundra ecosystem.

Fig: 1.2 Bryo-ecological regions of India by Pande (1958) and Singh (2001)



1.2 Origin of bryophytes

1.2.1 Algal origin

Lignier (1903) suggested a hypothetical landform "Prohepatics" arose from algal ancestors, which in turn gave rise to bryophytes. Bower (1908) suggested algal origin of bryophytes as they are more akin to Chlorophyceae due to similarity in presence of chloroplast, reserved food material and cell wall.

Zimmermann (1932) stated that primitive bryophytes might be having sporophytes more or less independent of gametophytes and rather similar to each other. Campbell (1940) and Smith (1955) strongly supported the view that the bryophytes evolved from algal ancestors monophyletically, whereas, Eames (1936) regarded them polyphyletic and to have progressed from simple to more complex forms. Lam (1952) considered that mosses were derived from Chlorophyceae and liverworts from the Phaeophyceae. Jeffery (1962) expressed that origin through a fresh water transition stage represents the most probable course of evolution of bryophytes from algae. Plumstead (1966) also postulated that land plants along with bryophytes originated independently from a group of Precambrian archegoniates, which in turn derived from different algal sources. Mehra (1967, 1969) proposed diphyletic origin of vascular plants but believed in origin of bryophytes from members of Chlorophyceae with similarities in photosynthates and type of ciliate cells at reproductive phase. Doyle (1970) expressed that embryophyta either evolved from green algal ancestor or had common ancestry with them.

1.2.2 Pteridophytic origin

This view is based upon the morphological complexity of *Anthoceros* sporogonium and holds psilophytalean ancestry from the bryophytes. Anthocerotales sporogonium showed similar evolutionary derivatives of the primitive plants like cylindrical sporophyte, devoid of leaves, terminal columellate sporangia, pigments, cell wall, food reserves, the sex organ and mode of reproduction.

Kidston and Lang (1917) shown that the simple vascular plants Psilophytales and their sporophytes bear striking resemblance with those of *Anthoceros*. Kashyap (1919) stated few forms of algae like *Coleochaete*, *Chara* and *Ectocarpus* do not show resemblance with Hepaticae but may have arisen from forms similar to Equisetales. Haskell (1949) postulated the origin of bryophytes from algae through Psilophytales and are reduced group having similarity of sporogonium structure of

Anthoceros and *Horneophyton*. Takhtajan (1953) also suggested that the bryophytes might be reduced descendants of the Psilophytales with similarity between sporophyte of the Devonian *Sporogonites* and *Horneophyton*. Christensen (1954) believed the pteridophytic origin of bryophytes from a leafless Rhyniaceae with polyphyletic origin from different types of pteridophytes. Steere (1958) suggested that bryophytes and pteridophytes might have been derived from a common and more primitive stock, long back before extinction and it might be polyphyletic.

Stebbins (1960) and Proskauer (1961) suggested that Anthocerotalean sporophyte is derived by reduction and specialization from the sporophyte of *Horneophyton*. Khanna (1965) expressed the resemblances of certain bryophytes like Anthocerotales to Psilotales with sporophytic characteristics, represent nothing more than a case of homoplastic evolution in two different lines. Cronquist *et al.*, (1966) suggested bryophytes have originated by reduction from higher plants and should be placed between Psilophytales and Psilotales. Fulford (1965) and Campbell (1971) considered the origin of bryophytes as quite debatable and mystery but conclusively considered as polyphyletic. Miller (1974) stated that bryophytes arose in the Early to Middle Devonian from Rhyniophytina and Zosterophytina.

1.3 Legacy in Indian bryology

The initiative of classical study of mosses was undertaken by Dellenius (1741) with six moss genera and Linnaeus (1753) with eight genera while Hedwig (1801) published "*Species Muscorum*" presented 35 genera. Hooker (1820) described several Indo-Nepalian species for first time in "*Musci Nepalensis*". Mitten (1860) published "*Hepaticae Indiae Orientalis*" which is the first comprehensive work embodying all the Indian species known at that time in subcontinent, and listed 39 genera with 205 species of hepatics. Schiffner (1899) described 35 sp. of liverworts from Indo-Bhutan region. Stephani (1900-1924) in his six volumes of "*Species Hepaticarum*" described hepatic flora of the world in which many species included from India and Andaman and Nicobar, Burma, Ceylon and Nepal.

Goebel (1910), Muller (1901) and Gola (1914) contributed in hepatics in post-Mitten period. Bruhl (1931) in his "*Census of Indian Mosses*" reported 2,471 species belonging to 371 genera. Kashyap (1929 and 1932) was the first Indian who made noteworthy and monumental work dealing with an illustrated account of "Liverworts of Western Himalayas and Punjab Plains" with two parts, (second part with Chopra)

describing 53 genera with 161 species and many were new to the scientific world. Chopra (1943) made exhaustive census of hepaticae of India and in 1975, he has dealt with 2000 species of Indian mosses belonging to 329 genera and 56 families. In post Kashyap era, studies on hepaticae remain confined to Lucknow under benevolent guidance of S. K. Pande (1960) followed by his students. Bartram (1960) listed moss flora of North Western Himalayas in India with 2,100 species.

Bharadwaj (1965, 1981), Udar (1959) and Kachroo (1954) contributed substantially for the hepatics from Western Himalaya, Assam and West Bengal. Eastern and Western Himalayas exploration of bryoflora was carried out by Pande and Ahmad (1957), Naguchi (1958), Udar (1959, 1965), Gangulee (1969, 85) and many others. In Central India, Parihar (1961), Bhargava and Thampi (1960), Bapna (1969), Handoo and Shrivastava (1963) worked on bryoflora of the region. Hattori and Mizutani (1968) also worked on Hepatics of Himalayan Region. Bapna and Kachroo (2000) published two volumes of 'Hepaticology in India', as a most comprehensive flora.

After 1980's, there has been hiatus in Indian bryology and considered as neglected area than more flashy and exciting angiosperms. Joshi *et al.*, (1992) have studied the bryoflora of Andaman and Nicobar Island. Asthana and Nath (1993) have discussed the distribution pattern of hornwort *Phaeoceros* Prosk. Chaudhary *et al.*, (1993) extensively worked on Bryoflora of Rajasthan and bryoflora of Gujrat state (2006) with 67 species. Nair and Madhusoodhanan (2002) contributed to the bryophytic flora of Kerala state in the Southern India. Madhusoodhanan *et al.*, (2007) studied the diversity in the bryoflora of Eravikulam National Park, Kerala. Verma and Srivastava (2011) reviewed the endemism in liverworts of Western Ghats and their present status of 68 genera, 34 families and 319 species. Alam (2011) studied bryoflora of Ranthambor National Park, Rajasthan and liverworts flora of Parson's Valley Nilgiri (2012) hills in South India. Recently, Singh and Singh (2013) presented an exhaustive appraisal of the genus *Marchantia* in Indian region with new findings.

1.4 Bryology in mega state Maharashtra

A complete survey of Bryophytes in Maharashtra has not been made. Stray references to them are found in literature and the papers by Mahabale (1987) and his associates. Blatter (1929) published Mosses of Bombay Presidency while Bruhl (1931) made census of Indian mosses. Mahabale *et al.*, (1941, 42) extensively

reported the liverworts of Maharashtra state like *Riccia*, *Targionia*, *Leptocolea*, *Marchantia*, *Plagiochasma* and other Metzgeriales. Apte and Sane (1942) reported new species of *Anthoceros dixitti*, *Anthoceros sahyadrensis*, *Aspiromitus khandalensis* and *Aspiromitus fergusonii* from Western Ghats. Mahabale and Bhate (1942); Chavan and Mahabale (1945); Mahabale and Deshpande (1947) recorded historical liverworts like *Asterella*, *Plagiochasma*, *Aspiromitus* and *Notothylas* species from Maharashtra.

Joshi and Biradar (1984) studied the liverworts flora of Western Ghats with special reference to Maharashtra region. Palav (1994) and Patil (1996) have undertaken the palynological studies among mosses of Khandala and Mahabaleshwar of Western Ghats. Dabhade (1998) published the first extensive handbook of mosses of Khandala and Mahabaleshwar with masterpiece work of 65 new species recorded from Western Ghats. Shirke and Biradar (2002) provided the monographic account on the bryophytes distribution of Western Ghats in Maharashtra. Choudhary *et al.*, (2008) published the book “Bryophyte flora of North Konkan, Maharashtra” incorporating the information about hundreds of bryophytic species from the region as a first comprehensive collection of records. Bagwan and Kore (2012) reported 20 species of bryophytes from the world famous UNESCO (United Nations Environment Science and Cultural Organization) heritage site “Kas Plateau” at Satara District. Gaikwad *et al.*, (2012) studied the recent distribution of bryophytes in Bhor and Velhe region of Pune district Maharashtra.

Most of the time attention was attracted towards Sahyadri ranges of Maharashtra rather than Satpura ranges of Maharashtra because of more humid ecological conditions of Sahyadri than that of Satpura.

1.5 Importance of bryophytes

Certain bryophyte occurs at specific pH range and their presence can be used as indicator of soil pH. Aquatic mosses can be used as indicator of calcium and nutrient content in water. Bryophytes envelops the forest cover with moisture, temperature, prevents soil erosion (*Atriam*, *Pogonatum*, *Blasia* etc.) and provides seed beds. The *Merceya* sp. is indicator of copper in copper rich soil. Species like *Fontinalis* can be used to monitor water pollution while many mosses are good indicators of air pollution (Saxena and Harinder, 2004).

Sphagnum moss used in horticulture due to its high water retention capacity. Moss peats are used as fuel in many countries as low sulphur contents. Bryophytes also used for house construction and beautification. Medicinally, bryophytes used extensively in Chinese, Native Americans and Indian sub continents. *Philontis*, *Bryum*, *Sphagnum* used on burns and wounds, *Marchantia* and *Plagiochasma* used on boils, burns and abscess. *Physcomitrilla* has unique blood clotting factor IX to cure "Haemophillia" B. The plant like *Plagiochasma* and *Reboulia* possesses strong antibacterial and antifungal properties (Banerjee and Sen, 1972).

1.6 Bryophytes and substrate chemistry

Soil is a vibrant ever-changing element. It is characterized not only by chemical and geological properties but also physical and biological characteristics. The quality of soil is rather dynamic and is the end product of soil degradative or conservative processes. It is controlled by chemical, physical and biological components of the soil and their interactions (Kennedy and Papendick, 1995).

Bryophytic vegetation responds to soil texture, soil pH, and soil nutrients and also exhibits specificity to electrical conductivity, organic matter and soil depth. They are confined to slight acidic, moderately neutral and medium basic soil depending upon geographical distribution and also signifying specificity with the environmental conditions. The terricolous habitat of the bryophyte gets more nutritional stability as compared to saxicolous and corticolous habitat. Hence, it plays a major role in development and functioning of terrestrial ecosystem. Bryophytes provide shelter to various invertebrates present in the soil and also microbial biomass contributing the maintenance of soil fertility or soil quality (Smith *et al.*, 1993).

Certain bryophytes and their occurrence are good biological indicators as they are confined to represent specific soil and its pH.

1.7 Bryophytes and soil microflora association

Certain fungi are peculiar of a particular vegetation type or geographical area and sometimes act as indicator of the population in temperate, tropics or savannas region (Christensen, 1981). The microbial biomass in the soil is made up of bacteria, fungi, algae, protozoan, and nematodes contributing 25% of the total biomass on the earth. It is a complex mixture of living, dead, decomposing material and inorganic compounds (Gupta, 1998).

Vesicular Arbuscular Mycorrhiza (VAM) fungi are naturally occurring fungal component of soil biota in most terrestrial ecosystem. These fungi are ubiquitous in distribution and unique as they are partly inside and partly outside the root. The vesicles, arbuscules and hyphae that are formed inside the host root does not encounter competition and antagonism from soil microorganisms to host rhizospheric, soil conditions and host genotype (Manoharachary *et al.*, 2002). The fossil evidences clearly indicates that the invasion of land by the early land plants like bryophytes clearly seems to have been facilitated by the origin of symbiotic association between the plants and mycorrhizal fungi at early Devonian period (Remy *et al.*, 1994). Hence, fungal symbioses are one of the key attributes of present day land plants.

Nebel *et al.*, (2004) reported the well establishment of members of Glomeromycota, Ascomycota and Basidiomycota from symbiotic association with liverworts. The glomeromycota are obligate mycorrhiza partners with 80% of land plants and the specific fungal structures are the same in tracheophytes and liverworts. This symbiotic fungal association of liverworts with AM fungi might be considered as the ancestors of true mycorrhizae. Selosse (2005) reaffirms the concept of liverworts imitating mycorrhizas due to their interaction with symbiotic fungi. Kottke and Nebel (2005) indicated strong congruency between the evolution of liverworts with their specific symbiotic fungi and suggested their association as the possible ancestors of mycorrhizae.

Although, liverworts do not have roots, but a similar organ of attachment like rhizoids are responsible for fixation and absorption of water or nutrients from soil. The cellular structures of association and the fungi involved with mycorrhiza like association is reported in *Marchantia foliacea* using scanning or transmission electron microscopy (SEM or TEM). Using molecular techniques, it was found that the pattern of DNA variation is seen in r-DNA sequences of *M. foliacea* endophytes was consistent with previous studies of glomeromycotan fungi (Russell and Bulman, 2004). On recent studies, Lingrone *et al.*, (2007) strongly supports that the topology of liverwort phylogenies shows symbioses with glomeromycete fungi as a basal trait of liverworts that long predates Vesicular Arbuscular Mycorrhiza in other plants.

Bidartondo and Duckett (2009) studied the conservative ecological and evolutionary patterns in liverworts with fungal symbioses and confirmed that simple thalloid liverworts associate with glomeromycete fungi in a way similar to most vascular plants.

Pressel *et al.*, (2010) provided new insight in the twenty first century about fungal symbioses in bryophytes. Isolation, re-synthesis and sequencing experiments have shed considerable light on functional relationship of bryophyte as host and fungi as symbionts. Here *Treubia* and *Haplomitrium* were currently considered as bryophytes of very long history in the land plant tree of life showing presence of *Glomus* i.e. mycorrhizal endophytes. Liepina, (2012) studied 43 bryophyte species and reported Glomalian fungi in most of liverworts and hornworts but less in mosses. More axenic cultures of bryophytes are needed to understand further interactions among bryophytes and VAM fungi.

1.8 Antimicrobial potential of bryophytes

It is noteworthy that besides presence of bryophytes vegetation in close vicinity of water bodies, moist, humid and wet infectious conditions, they are free from attack of pathogens due to their unique pungent chemical nature. The occurrence of antimicrobial substances in thalli of several bryophytes has been reported. Madsen and Pates (1952) reported first time antibiosis in *Sphagnum strictum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Belkin *et al.*, (1952) found that ethanolic extract of *Polytrichum juniperum* possess antitumorigenic activity. Banerjee and Sen (1979) examined 52 species (40 genera) of the bryophyte for their antimicrobial activity. Out of those species 29 were active against at least one of the test bacteria. The liverwort *Asterella sanguinea* and *Marchantia paleacea* and the moss *Brachythecium procumbens* showed broad spectrum of antimicrobial activity

1.9 Chemistry of bryophytes

Most of the bryophytes used as medicinally important plants due to characteristics pharmacognostic compounds present in the thalli. Using advanced Gas Chromatography and Mass Spectroscopy (GC-MS) techniques compounds like monoterpenoids, sesquiterpenoids, diterpenoids, bicarbocyclic diterpenoids, triterpenoids, sterols, flavonoids, phenolic compounds and fatty acids can be found out (Banerjee, 2001). The characteristic adour, earthy and pungent smell of bryophytes is due to presence of various chemical constituents present in the thalli. Asakawa *et al.*, (2013) suggested that Bryophytes emit volatile terpenoids of simple

aromatic compounds responsible for intense terpenic, mushroomy, sweet woody, sweet mossy, seaweed like or carrot like odour.

1.10 Melghat as a virgin area of bryophytes

Melghat is a prime biodiversity repository of Maharashtra state enriched with diverse and luxuriant growth of lower plants like bryophytes. Present study is the first attempt to explore the bryophytic flora of Melghat region and to assess Bryophyte association with VAM fungi and its antimicrobial, *in vitro* screening with chemical analysis. This may lead to open new avenues to study mycorrhizal association with lower plants. The phylogeny or taxonomic review and interpretation of such association may lead towards the evolutionary significance among these life forms. The aromatic and pungent chemical habitats of bryophytes make them anti-feedant for animals and also provide shelter to lower invertebrates. Hence the phyto-constituents or chemical compositions of bryophytes are also explored during course of work.

This work is the classical exploration of Botanical field tending towards the possible applied approach

1.11 Melghat at a glance

a) Physiographic

Melghat i.e. “a meeting place of ghats” is the north western compact block of forest with a canopy spreading over 3,075 sq. km. in Amravati district of Maharashtra (old C.P. and Berar). It extends about 65 km from north to south between latitude 21⁰ 46' and 20⁰ 11' north and about 95 km from east to west between longitude 77⁰ 34' and 76⁰ 38' east (Central Province District Gazetteer, 1911). The total area under MTR is around 2027.39 sq. km. The core area of the reserve is named as Gugamal National Park with an area of 361.28 sq. km and the buffer area of about 1666.11 sq. km. The other regions includes Melghat Sanctuary of 788.75 sq.km, Multiple use area of 526.90 sq.km, Project Tiger Melghat of 1676.93 sq.km, Narnala Sanctuary of 12.35 sq.km, Wan Sanctuary of 210.00 sq.km, Ambabarwa Sanctuary of 127.11 sq.km (GOI, Forest Tiger Status Report 2001).

b) Geology

The region consists of a succession of hills, valleys and variation in altitude and gradient situating on the branch of Satpura or Gawilgarh ranges to south of Tapi river (Dhore, 2002). The crests of the range attain an average height of elevation of altitude 350 m- 1178 m above mean sea level (MSL) and composed of Deccan trap of the upper Cretaceous or lower Eocene group. The highest spot in the track is Vairat hill which is about 1178 meter. The only geological formation represented in the Melghat Tiger Reserve area is the Deccan trap and underlying rock is basalt in one form or another. The most common form is a hard dark coloured rock, compact or fine grained, but occasionally with numerous phenocrysts (GOI, Forest Tiger Status Report 2001).

c) The riverine plains

The area is in catchment to the five major rivers viz. Khandu, Khapra, Sipna, Gadga and Dolar, all of which are tributaries of the river Tapti. The North-Eastern boundary of the reserve is marked by river Tapti. The Chandrabhaga river which originates from Chikhaldara has its watershed in the reserve. Water is most prevalent limiting factor in MTR and at present there are 132 water holes both natural and artificial one.

d) Natural vegetation

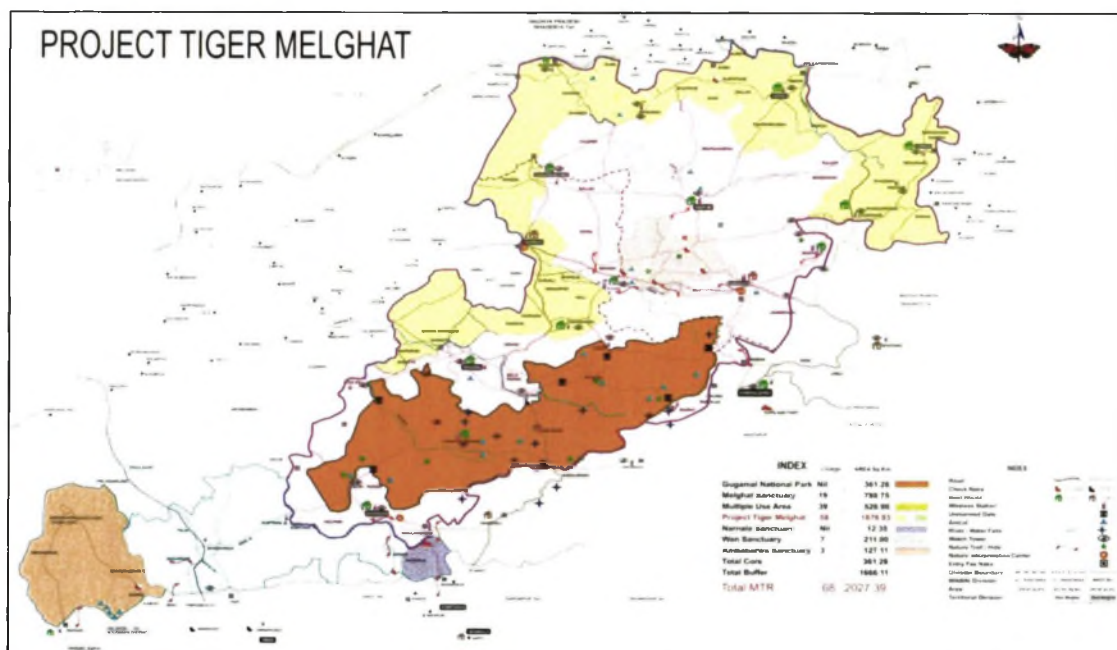
The entire area of the Melghat Tiger Reserve is under the natural cover of forest. *Tectona grandis* (teak) is the most dominant species. The associates of *Tectona grandis* differ depending upon latitude, gradient and other physiographic feature of the habitat. However, its most common associates in almost all localities are *Lagerstroemia parviflora*, *Lannea coromandelica*, *Emblica officinalis*, *Terminalia tomentosa*, *Anogeissus latifolia*, and *Ougenia oojeinensis*. At lower elevations, other associates of teak are *Boswellia serrata*, *Wrightia tinctoria*, *Cassia fistula*, *Miliusa tomentosa*, *Bauhinia raemosa*, *Butea monosperma* etc. The higher elevation and in more moist localities other associates are mainly *Mitragyna parviflora*, *Adina cardifolia*, *Schleichera oleosa*, *Albizzia procera* etc. Till date 772 plant species have been enlisted in the Flora of Melghat (Dhore and Joshi, 1988 ; Bhogaonkar and Devarkar, 1999). These species belong to about 400 genera representing as many as 97 families. There are 91 tree species, 109 shrubs, 450 herbs, 38 climbers and 84 grasses (GOI, Forest Tiger Status Report 2001).

Fig: 1.3 Melghat map

A) Physiographic representation (2007)



B) Administrative regions (2013)



Courtesy :- Chief Conservator of Forest and Field Director, Melghat Tiger Project, Amravati

e) Climate

The forest is southern tropical and dry deciduous but seasonal green during monsoon. The monsoon season extended from mid-June to September, winter is from December to February and summer from March to mid June.

Climate of MTR is varying due to variation in altitude, aspect and distinct seasons viz Monsoon or rainy, winter and summer seasons. The area experiences a good rainfall during monsoon which varies from time to time and regions to regions with average 65 to 60 numbers of rainy days. Temperature varies considerably with altitude. The high hills, plateau and valleys to the North of Gawilgarh ridge are cooler in summer than the southern foothills. The plateau and high hills enjoy almost equitable pleasant climate throughout the year. The maximum temperature of 44⁰C was recorded in summer and the average minimum temperature was recorded 6⁰C at different sites in the forest depending upon the regions and of course seasons.

f) Soil

The edaphic factors also show variation in soil composition like clay, alluvium, lateritic or gritty loam depending upon plain or plateau (Patel, 1968).

The Bouldery soil type covers the greater part of the reserve. This is mostly confined to slopes. The Lateritic loam generally occurs on hill tops and plateau and is noticed around Chikhaldara, Vairat and other parts of the reserve. While the Clay soils generally occurs in depression and on level areas. These soils are very fertile but have poor drainage status. The combinations of red, brown and black colour of soils is a common characteristics of the regions.

g) Fauna mammals

The phytogeographic region of Melghat is known for the rich heritage of flora and fauna. The Govt. of India resolution 1974 segregates the area by establishing (MTR) Melghat Tiger Reserve (revised 1983-85, 1994) i.e. a highly protected forest of ecological conservation. The wild animals like Tiger, Leopard, Sloth bear, Wild dog, Jackal, Sambar, Gaur, Barking deer, Nilgai, Cheetal, Chousinga, Ratel, Flying Squirrel, Wild boar, Langur, Rhesus monkey, Porcupine, Pangolin, Mouse deer, Python, Otter, Caracal and Black napped hare are commonly located in the forest.

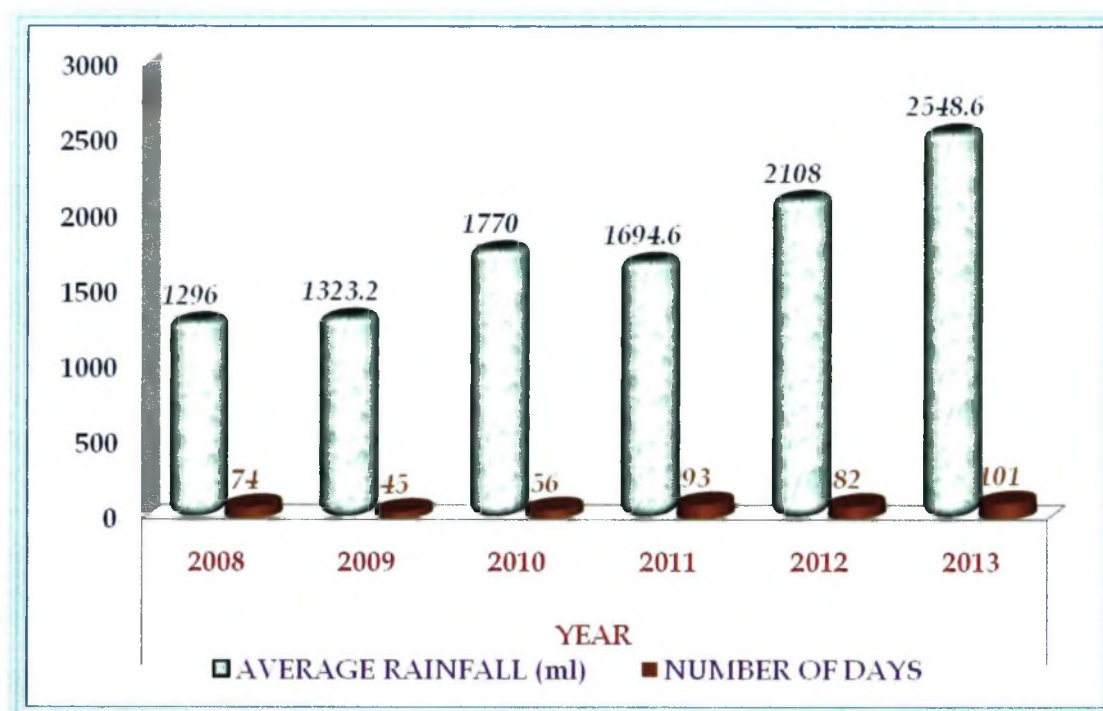
h) Rainfall

During the study period, about six years rainfall data was collected by the author and compared with the data available at Sipna Weather Observatory, Chikhaldara (Table 1.1). The annual rainfall ranges between 1296 – 2548.6 mm. The least rainfall of 1296 mm was recorded in year 2008 with 74 rainy days while maximum rainfall data of 2548.6 mm with 101 rainy days was recorded in the year 2013 and author found the region covered with more lush green carpet (Fig 1.4).

Table: 1.1 Melghat Rainfall data collected during 2008-2013

Sr. No.	Year	Average Rainfall in mm	No. of days in whole year
1.	2008	1296	74
2.	2009	1323.2	45
3.	2010	1770.3	56
4.	2011	1694.6	93
5.	2012	2108.0	82
6.	2013	2548.6	101

Fig: 1.4 Average rainfall data 2008-2013



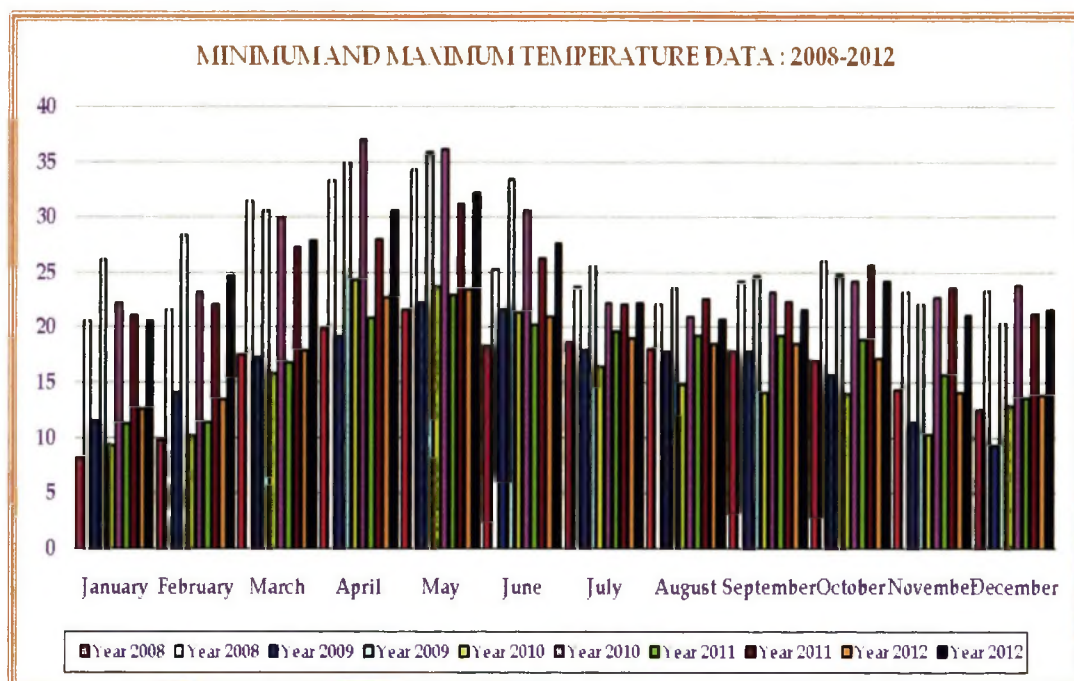
i) Temperature

Survey and recordings of five years temperature data was carried out at morning and evening time considering maximum and minimum temperature at two constant times. The average temperature of the Melghat forest ranges between 8.3^oC during winter season and maximum of 37^oC during summer (Table: 1.2). However, at some instances variable temperature were also found and recorded at different parts of the Melghat forest (Fig: 1.4).

Table: 1.2 Melghat temperature data collected from 2008-2012

Month	Year 2008		Year 2009		Year 2010		Year 2011		Year 2012	
	Temp ^o C Min 8.30 AM	Temp ^o C Max 5.00 PM	Temp ^o C Min 8.30 AM	Temp ^o C Max 5.00 PM	Temp ^o C Min 8.30 AM	Temp ^o C Max 5.00 PM	Temp ^o C Min 8.30 AM	Temp ^o C Max 5.00 PM	Temp ^o C Min 8.30 AM	Temp ^o C Max 5.00 PM
January	8.3	20.7	11.6	26.2	9.4	22.3	11.3	21.2	12.7	20.7
February	9.9	21.7	14.1	28.4	10.2	23.3	11.4	22.1	13.6	24.8
March	17.6	31.5	17.3	30.6	15.9	30.0	16.9	27.3	18.0	27.9
April	19.9	33.3	19.2	34.9	24.4	37.0	20.9	28.1	22.8	30.6
May	21.6	34.3	22.3	35.9	23.7	36.2	23.0	31.2	23.5	32.3
June	18.4	25.3	21.7	33.5	21.4	30.7	20.3	26.3	21.1	27.7
July	18.7	23.7	18.0	25.6	16.5	22.3	19.7	22.1	19.1	22.3
August	18.1	22.1	17.8	23.6	14.9	21.0	19.3	22.6	18.6	20.8
September	17.9	24.3	17.8	24.7	14.1	23.3	19.3	22.4	18.6	21.7
October	17.0	26.1	15.7	24.9	14.0	24.2	18.9	25.7	17.2	24.2
November	14.4	23.3	11.4	22.2	10.3	22.8	15.7	23.6	14.1	21.2
December	12.5	23.4	9.3	20.4	12.9	23.9	13.7	21.3	13.9	21.7

Fig: 1.5 Average analysis of temperature data (2008-2013)



j) Humidity

Sampling and data recorded from last five years and its ranges found in between 50 % to 87 % as it depending upon the season-to-season (Table 1.3). However, during monsoon season humidity attains to maximum during June to September as monsoon prevail the region and decreases considerably during hot and dry summer season (Fig: 1.4)

Table: 1.3 Average humidity data collected from 2008-2012

Month	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
	H %	H %	H %	H %	H %
January	63	67	65	64	79
February	61	58	62	68	72
March	58	51	53	59	63
April	52	50	57	63	66
May	54	57	56	69	61
June	73	81	71	80	79
July	84	82	85	84	85
August	86	79	83	85	87
September	70	78	80	84	84
October	64	51	73	75	82
November	66	71	78	73	81
December	62	68	74	74	77

Fig: 1.6 Average rain fall 2008-2013

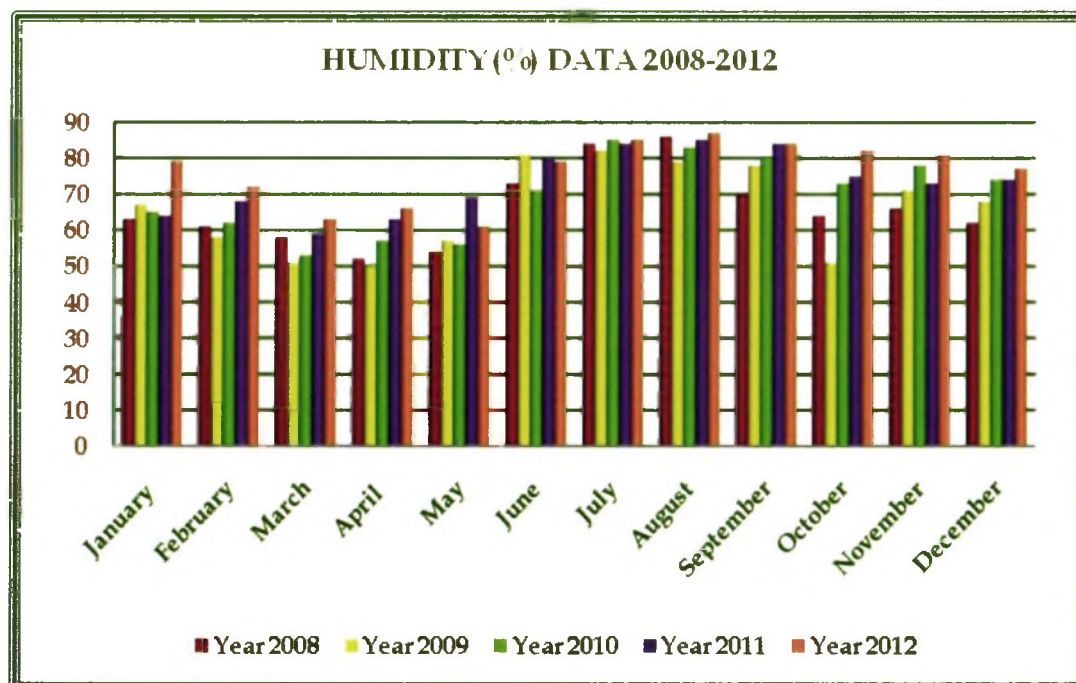


Table: 1.4 Survey and selection of sites of Melghat forest for bryophytes study

Sr. No.	Name of the site	GPS Coordinates	Elevation (Meter)	Accuracy (Meter)	Conversion in Degree, Minutes, Second	Forest Compartment Number
1	Ghatang	N = 21.4439 E = 77.4177	732	3.0	N 21°24'52" E 77°20'24"	38
2	Semadoh	N = 21.5034 E = 77.3362	506	7.2	N 21°30'13" E 77°20'10"	148
3	Makhala	N = 21.5354 E = 77.3841	678	4.7	N 21° 26' 38 E 77° 25' 4"	35, 182
4	Raipur	N = 21.5332 E = 77.2718	540	5.2	N 21° 31' 59" E 77° 16' 18"	248, 249, 251
5	Kolkhas	N = 21.4996 E = 77.1869	492	3.9	N 21° 29' 58 E 77° 11' 9"	157, 149
6	Tarubanda-Koha	N = 21.3465 E = 77.1360	640	7.0	N 21°20'47" E 77°8'9"	764,765
7	Belkund-Koha	N = 21.3518 E = 77.1312	679	5.8	N 21°21'6" E 77°7'52"	809 , 814
8	Khongada-Parsapur	N = 21.3765 E = 77.1289	590	7.0	N 21°22'6" E 77°7'52"	868, 950
9	Gugamal	N = 21.4259 E = 77.3220	945.9	4.0	N 21°25'33" E 77°19'19"	42
10	Memna	N = 21.4233 E = 77.3255	933.2	3.9	N 21°25'24" E 77°19'31"	41
11	Chikhaldara Plateau	N = 21.4050 E = 77.3457	1040	3.4	N 21°24'18" E 77°20'44"	25
12	Gawilgarh Fort	N = 21.3829 E = 77.3358	1044	4.5	N 21°22'58" E 77°20'8"	26
13	Vairat	N = 21.3830 E = 77.2530	1140	4.5	N 21°22'58" E 77°15'11"	38
14	Churani	N = 21.3974 E = 77.3298	1070	4.5	N 21°21'58" E 77°12'9"	39
15	Bhimkund	N = 21.3941 E = 77.3468	1011	3.4	N 21°23'39" E 77°20'48"	25
16	Devipoint	N = 21.3940 E = 77.3296	1074	6.7	N 21°23'38" E 77°19'46"	25
17	Amazari	N = 21.4439 E = 77.4177	862.8	3.9	N 21°26'38" E 77°25'4"	23
18	Bori	N = 21.4224 E = 77.3758	856.9	7.5	N 21°25'20" E 77°22'32"	49
19	Salona	N = 21.4335 E = 77.4097	851.3	3.7	N 21°26'0" E 77°24'35"	10
20	Madaki	N = 21.3832 E = 77.4044	749	3.7	N 21°22'59" E 77°24'16"	15
21	Chikhaldara Garden	N = 21.4014 E = 77.3174	1116	4.1	N 21°24'5" E 77°19'2"	25
22	Chikhaldara- Paratwada (3 km)	N = 21.4055 E = 77.3499	1029	3.1	N 21°24'20" E 77°20'59"	27
23	Semadoh - Chikhaldara (11 km)	N = 21.4407 E = 77.2978	683.3	4.9	N 21°26'26" E 77°17'52"	148
24	Ghatang - Semadoh (15 km)	N = 21.4694 E = 77.4145	743.6	4.9	N 21°28'9" E 77°24'52"	38
25	Ghatang - Chikhaldara (06 km)	N = 21.4439 E = 77.4177	862.8	3.9	N 21°26'38" E 77°25'4"	23

Fig: 1.7 Satellite Picture Melghat Tiger Reserve (2014)

Courtesy: <https://maps.google.co.in/?mid=1398287881>

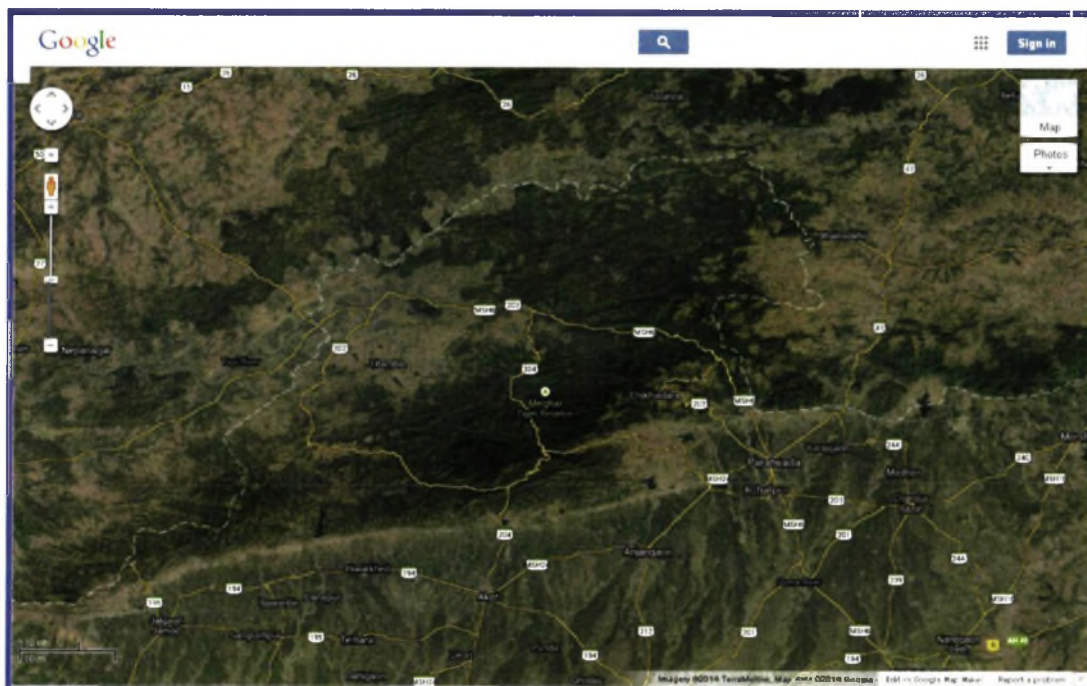


Fig: 1.8 Locations and coordinates confirmation by using GPS data

Courtesy: <http://www.gps-coordinates.net>

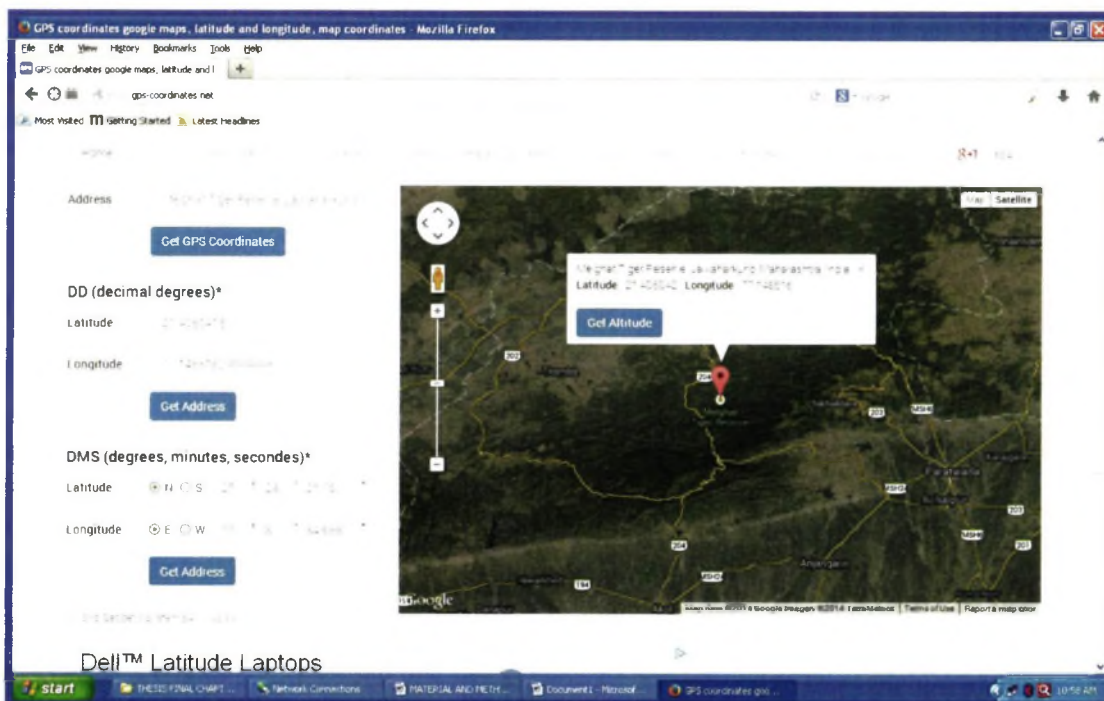


PLATE – 1
Panoramic view of Melghat forest



A) Bandarkahu valley in monsoon



B) Bhimkund roaring waterfall



C) Deep valley at Chikhaldara



D) View of Chati Bilta valley



E) Gawilgarh fort adjacent view



F) Fog cover and misty wrapping



G) Sipna river scenario at Semadoh



H) Green carpet at Kolkhas

1.12 Aims and objectives of the work

The present study is the first attempt to explore the virgin bryophytic flora in the highly conserved Melghat region with special ecological considerations. Different localities were screened for the analysis of bryoflora distribution. The bryophytic plant occurs with different soil and habitats hence the soil physico-chemical analysis was framed to know their nutrient status under different parameters.

VAM fungi are mostly associated with higher vascular plants as they bear a developed root configuration. However, the thallophyte as bryophytes are the first land plants of the earth and mycorrhizal association is of natural occurrence among them. In the evolution of the plant kingdom, Bryophytes played a pivotal role in conquering the terrestrial habitat and making soil conducive for the establishment of land vascular plants. Hence, along with bryophytes the fungal association also occurred during geological past during Ordovician period of Paleozoic era (Remy *et al.*, 1994). The co-evolution of bryophytes and VAM fungi helped in enriching the soil micro-biota subsequently making it fertile for the introduction and sustenance of newly evolved forms Selosse, (2005); Lingrone *et al.*, (2007); Arora, (2008) and Liepina, (2012) reported the occurrence and distribution of VAM fungi in bryophytes.

Hence, investigations has been undertaken on the exploration of the ubiquitous existence of soil borne VAM fungi with bryophytes along with assessment of inter and intra species diversity occurring in situ in bryophytes.s

Bryophytes are the natural reservoirs of antimicrobial substances showing the phenomenon of antibiosis (Banerjee and Sen, 1979). As the antimicrobial potentials are natural inheritance in the bryophytes, their antimicrobial sensitivity was also decided to be studied using selected human pathogenic gram positive and gram-negative bacterial and fungal strains.

To elicit out their chemical nature, preliminary phytochemical analysis, and GC-MS (Gas Chromatography and Mass spectroscopy) analysis was also set to find out novel chemical compounds (Asakawa, 2004) from the experimental bryophytic plants. The landmark inkling or clues for further characterization and elucidation of chemical structure and composition for novel drugs can be possible in further post research studies on the bryophytes.



CHAPTER TWO
REVIEW OF
LITERATURE

2. REVIEW OF LITERATURE

2.1 Soil rhizosphere biology

Soil is the upper portion of the earth's crust and acts as a medium for the sustenance of forest flora and fauna. Plant growth and development are controlled largely by the soil environment in the root region. Availability of nutrients in the rhizosphere is governed by the combined effect of soil properties, plant characteristics and the interaction of plant roots with microorganisms and the surrounding. Different plant species as well as genotype within a species differently influence the quantitative and qualitative composition of microbial population in the rhizosphere. Hence, plant growth is influenced by the microorganisms present in the root zone i.e. inside the root, on the surface and around the root.

Starkey (1929) proposed that, the 'rhizosphere' generally refers to the zone of soil surrounding the roots with higher microbial activity than in soil remote from roots. He also postulates that, the zone where soil microflora interacts with roots exudes or release organic substrate is referred as 'histosphere' or 'endosphere'. Starkey (1938) also suggested that the "endorhizosphere" or inner rhizosphere is formed by the invasion and colonization of root cortical tissues by soil microorganisms.

Clark (1949) used the term "rhizoplane" for the root surface and demonstrated that soil pH, and soil moisture affect the count of microbes in rhizosphere soil. Katznelson (1965) supported the significance of rhizosphere microflora in relation to soil microflora. Timonin (1966) emphasized that number of microorganisms was higher in root zone of the diseased than resistant plant varieties. Gerhardson and Clorholtu (1986) suggested that the high population of bacteria in rhizosphere favour the increase in number of protozoa which feeds on these bacteria.

Ganopadhyay (1992) showed that edaphic factors like organic matter, EC, temperature and N, P, K; ratio has a significant effect on vegetation as well as soil microflora. Bowen and Rovira (1999) believed that, the rhizosphere is a partnership between the plant, soil and soil organisms. Plant provide carbon and food source for soil organisms that bind the soil particles into aggregates and recycle soil nutrients while the soil provides the habitat, water and mineral nutrients for both soil organisms and plants.

Dezzeo *et al.*, (2004) studied the different soil properties like pH, EC, TDS, organic carbon and vegetation characteristics in southern Venezuela and showed the difference among vegetation type based on the existence of organic matter in soil.

2.1.1 Mycorrhizae and mycorrhizosphere

In nature, most of the actively absorbing rootlets form symbiotic associations with Mycorrhizal fungi which are major inhabitants of soil (Linderman, 1988). Mycorrhizae formation significantly alters the physiology or morphology of roots and plants in general leading to altered root exudation (Bansal and Mukerji, 1994).

The changes in root exudates affect the microbial communities around the roots and so the rhizosphere microflora also show a drastic change (Bansal and Mukerji, 1996). This changed situation around mycorrhizal roots is known as "Mycorrhizosphere". The Mycorrhizosphere is the zone of soil where the physical, chemical and microbiological processes are influenced by plant roots and their associated mycorrhizal fungi (Bansal *et al.*, 2000).

In mycorrhizosphere, extra material hyphae of mycorrhizal fungi are also present around roots and it extends well beyond the roots into the black soil with important source of carbon to the soil organisms (Foster, 1981). Mycorrhizal hyphae increases the soil aggregation and in root exudation favouring microbial growth (Yang *et al.*, 2000). Hence, as a consequence, the microbial community in a mycorrhizosphere differs from that in a non- mycorrhizal rhizospheric soil (Fitter and Garbaye, 1994). It is noteworthy that the soil biota influences the growth and development of vegetation in relation to richness of nutrients. Mycorrhization in plants is a mutualistic symbiotic association of mycotrophic nature (Manoharachary *et al.*, 2002). This association might have arisen in early land plants like bryophytes as an early establishment on the earth soil surface.

So, it is apparent to undertake quantitative analysis of soil samples from various sites of Melghat forest.

2.2 Perspectives of Indian bryology

The Indian subcontinent has a rich heritage of Bryoflora with wide range of diversity and distribution. Mitten (1860) reported 290 species of liverworts in his '*Hepaticae Indiae Orientalis*'. The bryologists like Stephani (1900-1924) published comprehensive six volumes of '*Species Hepaticarum*' on the bryophytes. Benedix

(1953), Kashyap (1929), Chopra (1938), Bharadwaj (1950), Kachroo *et al.*, (1977), Udar (1976) and Srivastava (1979), Joshi (1984) have contributed extensively towards the exploration of bryoflora of India. Bapna and Kachroo (2000) and Asthana *et al.*, (2008) reviewed the contributions of bryologists and worked on bryophytic flora.

Kachroo (1954) provided taxonomic account of species like *Notothylas*, *Anthoceros* and *Riccia* from Gauhati, Assam. Pande and Srivastava (1954) described three species of *Asterella* and reported the occurrence of 24 species of the genus in Eastern India. Chopra *et al.*, (1956) published the preliminary list of the mosses of Mussoorie including 143 species, 77 genera belonging to 27 families. Pande and Ahmad (1957) listed 40 species of epiphyllous liverworts from India and Ceylon. Pande and Udar (1957) worked comprehensively on morphotaxonomy and distribution of *Riccia* in India like *R. aravalliensis*, *R. tuberculata*, *R. attenuata* and discussed synonymy of *R. discolor*, *R. frostii* and *R. billardieri*. Further Udar also investigated *Riccia pandei*, *R. reticulata* and considered *R. tuberculata* as a synonym of *R. grollei*.

Noguchi (1958) studied a collection of mosses from regions of South India like Kodaikanal, Coonoor and Ootacamundalam and enumerated 27 taxa including a new variety in his revision of family Trachypodaceae of India. Chopra (1960) listed 68 genera and 158 species of mosses from Nainital (Himalayas). In his preliminary list, terrestrial and epiphytic moss species were included. Pande (1960) discussed the morphological details of thallus, antheridial chamber and sperms of Anthocerotales concluding that the genera fall into 3 groups in which *Phaeoceros* Prosk. was separated from *Anthoceros* complex and placed with *Megaceros* Campb. and favoured the segregation of *Notothylas* into a distinct family Notothylaceae. Foreau (1961) summarized the work done on the moss flora of the Palani hills, in South India and listed 368 species of mosses.

Chavan *et al.*, (1961) reported eight mosses from Pavagarh, Gujarat viz. *Fissidens obscurus* Jaeg., *Merceya geddeana* (Lac.) Fleisch., *Hyophila involuta* Brid., *Hyophila rosea* Williams. *Funaria hygrometrica*, *Bryum coronatum*, *Philonotis hastata* (Dub.) Wijk. & Marg., under the name *P. imbriculata* Mitt, and *Stereophyllum tavoyense* (Hook.) Jaeg. Bapna (1962) gave an account of liverworts of Mount Abu and reported 28 species belonging to order Anthocerotales, Metzgeriales and most dominant order Marchantiales. Handoo and Srivastava (1964) reported 11 mosses

from Bhopal including *Nanomitrium ienerum* (Bruch.) Lindb. this is a new record for India. Udar and Chandra (1965) investigated life history of *Plagiochasma intermedium* Lindb. et Gott. Dabhade (1966) collected some Indian mosses from Western India, these are *Hyophila involuta* (Hook.) Jaeg., *Octoblepharum albidum* (L.) Hedwig, (Epiphytic), *Calymperes thwaitesii* Besch. (Epiphytic) and on stones, *Gymnostomiella vernicosa* (Hook.) Fleischer. (On old temple wall), *Funaria hygrometrica* Hedw. (On burnt ground and on waste lands).

Gangulee (1969-85) made valuable contributions in his work, 'Mosses of Eastern India and Adjacent Regions - A Monograph' which is provided with a well illustrated, detailed taxonomic account, and the distribution map of each species. This is the first of its kind in India and certainly forms the basic reference manual to study the Indian mosses. Hoffman and Kazmierski (1969) noted a difference between tree base bryophytes and those found higher up the trunk and noted that bryophyte communities on tree trunks were displaced upward as moisture increased and described an ecological study of epiphytic bryophytes and lichens on conifer like *Pseudotsuga menziesii*.

Bharadwaj (1971) segregated a new genus *Folioceros* Bharad. from *Anthoceros* complex on the basis of thick-walled elaters and spinose or baculate spores and gave a detailed taxonomic account of seven species of *Folioceros* from Asia and Africa. Mizutani (1971) made a critical review of 12 species of *Lejeunea* from the Himalayan region collected by Hara Tuyama, Kanai Kurosawa, Murata and Togoshi during University of Tokyo Botanical expedition to Eastern Himalayas during the study period of the year 1960. Vohra (1972) reported mosses from hills and foot hills surrounding the Kashmir valley and 107 species and six varieties spread over 51 genera and 22 families were studied and collected. Udar and Srivastava (1972) described a new species *Cyathodium denticulatum* Udar et Srivastava. from the Darjeeling region, which was characterized by thallus with distinct assimilatory and storage zone, air chambers in 2-3 layers and free margins of the involucre found distinct denticulate. Gangulee (1974) described with illustrations, 5 genera of Splachnaceae e.g. *Gymnostomiella*, *Splachnobryum*, *Voitia*, *Tayloria* and *Tetraphodon* from Eastern India. Bapna and Kachroo (1975) worked on *Riccia* species in India and concluded on the basis of their studies that *R. billardieri*, *R. melanospora* and *R. gangetica* are ecologically variable species. *R. billardieri* is a

complex in which *R. pimodii* Kachroo, *R. berrieri* Jones and *R. nigrosquamata* Jones are merged; *R. rautannenii* synonymised with *R. crystallina* and *R. palestina* with *R. frostii*; *R. melanospora* occur in ciliate and nonciliate both forms.

Chopra (1975) published his monumental work 'Taxonomy of Indian Mosses' which includes nearly 2000 species belonging to 329 genera under 56 families. Srivastava and Udar (1975) presented their comprehensive study on the genus *Fossombronia* Raddi. in India providing key and description of Indian species.

Mehra and Sokhi (1976) made a comparative morphological study of spermatozoids in the members of Hepaticae and Anthocerotae with a phylogenetic approach. Slack (1976) has summarized the earlier studies and suggested that bark factors such as water capacity, rate of drying and pH of host bark were important in host specificity and are dependent to some extent on geography and climate. Bapna (1977) studied *Anthoceros longii* from Mount Abu and concluded that the species is a distinct and valid one. He compared morpho-taxonomic characters of the species with *A. erectus*.

Udar and Singh (1979) instituted two new species of the genus *Notothylas* Sull., *N. pfliedereri* Udar et Singh and *N. anaporata* Udar et Singh and also provided an account of *N. dissecta* Steph. from region Agumbe in Western Ghats. It constituted the first record of its occurrence in Asiatic bryoflora. Bapna and Choudhary (1980) collected four species of *Fissidens* Hedw. viz. *F. involutus* sp. *curvato-involutus* (Dix.) Gangulee., *F. diversifolius* Mitt., *F. geminiflorus* Doz. et Molk. Var. *nagasakinus* (Besch.) Iwats. and *F. sylvaticus* Griff, from Rajasthan. This is the first record of this genus from Rajasthan and *F. gaminiflorus* var. *nagasakinus* is a new record for India.

Sharma *et al.*, (1981) carried out palynological studies on 20 species of liverworts of Garhwal Himalayas and noticed the diversity in sporomorphs. Shukla *et al.*, (1981) studied influence of moisture regime on the growth of *Plagiochasma appendiculatum*. Udar and Gupta (1981) made differentiation of the genus *Targionia* and described two morphological forms of it. They described *T. hypophylla* sensu stricto and *T. indica* Udar and Gupta sp. nov. from West Himalaya, Pachmarhi and South India. They also reported for the first time *T. lorbeeriana* Mull, from South India.

Udar and Singh (1981) described some more morphological details of *Notothylas pfliedereri* and investigated *N. khasiana* Udar et Singh, *N. himalayensis* Udar et Singh and gave their Latin diagnosis along with discussing the characters with other species. Concentration was given on some gametophytic and sporophytic characters in *Notothylas* Sull., for their significance as taxonomic parameters. Bharadwaj (1981) provided a comparative assessment of the Indian species of *Folioceros* primarily based on their sexuality, spore size, height of baculae on the sporoderm and stomatal frequency of the sporophyte. Udar and Shaheen (1982) described a new species of *Folioceros*, *F. pandei* sp. nov. from Kodai Kanal, Palani hills, Conoor and Nilgiris.

Udar and Asthana (1985) described a new sculptural variation of *Anthoceros* spores, which was discovered in *Anthoceros pandei* sp. nov. Udar and Asthana, described a species new to science. Srivastava and Asthana (1989) reported *Folioceros kashyapii* sp. nov. and *F. udarii* Asthana and Srivastava. from Nagaland and Kerala, respectively. Bapna and Chaudhary (1989) collected 16 species of the order Dicranales and Pottiales for the first time from Rajasthan and also gave caloric value of six species of liverworts.

Asthana and Nath (1994) discussed the distribution pattern of *Phaeoceros* Prosk. in Kumaon and Garhwal regions where *Phaeoceros laevis*, *P. laevis* subsp. *caroliniamis*, *P. himalayensis*, *P. kashyapii* and *P. udarii* occur in humid subtropical moist deciduous forests. Kumar *et al.*, (1994) studied the ecology of *Riccia frostii* growing on the banks of Ganga and concluded that silty clay soil texture and fairly high concentration of physico-chemical parameters were favourable to the numbers and diameters to the rosettes. Deora and Chaudhary (1995) have collected four species of the genus *Hyophila* from various parts of Rajasthan, these are *Hyophila comosa* Dix. et Varde., *H. involuta* (Hook). Jaeg., *H. rosea* Williams and *H. spathulata* (Harv.) Jaeg.

Deora and Chaudhary (1996) collected six species of *Bryum* from various regions of Rajasthan, these are *Bryum argenteum* Hedw., *B. capillare* L. ex Hedw., *B. cellulare* Hook., *B. paradoxum* Schwaegr., *B. plumosum* Doz., et. Molk. and *B. recurvulum* Mitt. This is the first record of the genus from Rajasthan and stated that Rajasthan can be divided into three major bryoecological zones, each zone having distinct climatic conditions resulting into different vegetation.

Negi and Gadgil (1997) studied the species diversity and community ecology of mosses of Garhwal Himalayas and concluded that the microhabitat and altitude seem to be the major ecological factors governing species diversity and composition. Esposito *et al.*, (1999) dealt with post-fire bryophyte dynamics in Mediterranean vegetation and found that after 2 years bryophytes were dominated on the plots which has experienced the highest fire and pioneer species were *Funaria hygrometrica*, *Barbula convohita* and *Bryum dunense*. Nakanishi (1999) studied species diversity of epilithic, epigeous and epiphytic bryophyte communities. Observations were based on dry weight of component species in relation to environmental gradients.

Chaphekar and Ghate (2000) discussed the possible use of *Plagiochasma* in the assessment of water quality, especially for heavy metals and experimented for water quality assessment. Deora and Chaudhary (2000) collected forty two moss species from Rajasthan state of which 25 species of mosses belongs to Mount Abu. They compared the distribution of the mosses with other parts of India and concluded that 36 species were common with Western Himalayas, 27 common with Eastern Himalayas and 24 common with South India.

Chaudhary and Sharma (2000) collected five epiphytic mosses from Udaipur, Rajasthan. These are *Erpodium mangiferae* C. Muell., *Levierella fabroniacea* C. Muell., *Stereophyllum ligidatum* Jaeg., *S. anceps* (Bosch. & Lac), *Fissidens gaminiflorus* var. *nagasakinus* Bosch. Dabhade and Patil (2001) studied palynology of some mosses from Western Ghats and described details of SEM study of 2 species of *Bryum* and 7 species of *Fissidens*. Joshi (2001) presented the floristic analysis of the 45 taxa of liverworts from Andaman Islands and compared them with that of Eastern Himalaya, Western Himalaya, South India and neighbouring countries of India.

Saxena *et al.*, (2001) highlighted the usefulness of bryophytes as subjects of biomonitoring studies and called for the establishment of bryophyte bank for environmental monitoring in India. Singh and Semwal (2001) reported *Notothylas udarii* Singh *et Semwal.*, new to science from Dehradun, India. Nair *et al.*, (2002) carried out a preliminary account on the diversity of bryophytes of Kerala, with brief history, its important implications of conservation. Sharma (2002) studied epiphytic mosses of South East Rajasthan and Gujarat. Singh (2002) worked on liverworts of

Khasi and Jaintia hills of Meghalaya and reported 247 species embracing 59 genera belonging to 23 families.

Kumar and Singh (2003) reported ten species of mosses and 12 species of liverworts from Great Himalayan National Park, Kullu, Himachal Pradesh. Singh and Nath (2004) collected epiphytic species *Frullania rotandistipula* for the first time from India from Eastern Himalayas (Mawaiben forest, Nongstoin) West Khasi hills, Meghalaya.

Sanadhya (2004) reported thirteen liverworts, ten hornworts and forty-four mosses from Gujarat. The distributional pattern and altitudinal range of each taxon was also provided in addition to the key to species. Srivastava and Verma (2004) recorded the epiphytic diversity of the hepaticae occurring in *Cinchona* plantation in Dodabetta, Nilgiri hills. They recorded a total of 13 species belonging to Jungermanniales and a single species of Metzgeriales. Tanwir (2005) studied the diversity of hepatic flora of district Poonch (North-West Himalaya). The survey has yielded a total 82 hepatic taxa belonging to 38 genera, 24 families and 4 orders. Chaudhary and Dulawat (2006) reported two species of *Vesicidaria*, *V. montagnei* (Bel.) Broth, and *V. reticulata* (Dozy and Molke) Broth. collected from Sitamata Wild Life Sanctuary, Rajasthan as the first record of this genus from Rajasthan. Tanvir and Langer (2006) carried out compilation study on the liverwort flora of Ladakh region of Jammu and Kashmir includes sixteen species of which seven (*Aneura pinguis* (L.) Dum, *Athalmia pinguis* Falc, *Cephalozia* sp., *Conocephalum conicum* (L.) Necker, *Marchantia nepalensis* L. et L., *Reboulia hemisphenica* (L.) Raddi. and *Riccia* sp. are being reported for the first time from the area.

Chaudhary *et al.*, (2006) collected eleven species of mosses from Elephanta Caves (Maharashtra). These are *Hydrogonium consanguineum* (Thwait. and Mitt.) Hilp., *Hyophila involuta* (Hook) Jaeg., *Funaria hygrometrica* Hedw., *Brachymerium turgidum* (Broth.), *Diaphanodon procumbens* (C.Muell) Ren. et Card., *Bryum capillare*, *Bryum coronatum*, *Anomobryum auratum* (Mitt.) Jaeg. *Entodon laetus* (Griff.) Jaeg., *Stereophyllum ligulatum* Jaeg., *Fissidens bryoides* sp. Schemdii. Nath and Gupta (2006) reported *Hyophila involuta* (Hook.) Jaeg. from Pachmarhi Biosphere Reserve. The species is distributed in various localities of Pachmarhi Biosphere Reserve. Pachmarhi is well known for its biodiversity and rich in bryophytic vegetation.

2.3 The dawn of symbiosis between plants and fungi

The symbiosis with Arbuscular mycorrhizal fungi (Glomeromycota) allowed the rootless early land plants to invade the poorly developed primaeval soils and go on terrestrialization to transform the biosphere was widely accepted. The occurrence of symbioses between hepatics and fungi has been known for over a century.

Schacht (1854) provided illustrations of fungal occupation in thalli of *Pellia*, *Preissia* and in rhizoids of species *Marchantia* and *Lunularia*. Janse (1897) found swollen rhizoid apices occupied by fungi in liverwort *Zoopsis*. Nemeec (1899) recorded the presence of fungal mycelium as a regular occurrence in leafy members of Jungermanniales. Peklo (1903) reported symbiotic fungi in various moss tissues like gametophyte and saprophyte. Bernard (1909) used the wide spread occurrence of fungus-hepatic associations as the basis of his theory that vascular cryptogams were descended from mycotrophic bryophytes. Ridler (1922) reaffirmed the occurrence of fungi in liverwort *Pellia* and *Lunularia*. Rayner (1927) in his landmark work reported fungal symbionts like mycorrhiza associated with bryophytes like *Baxbaumia*. Kashyap (1929) reported mycorrhizal presence in liverworts of Western Himalayas and Punjab Plain. Gavaudan (1930) found *Aneura* associated with mycorrhizal fungi as a normal feature of hepatic biology. Stahl (1949) reviewed all earlier work of mutualistic fungal association like Mycorrhiza with bryophytes. More recently, the use of combinations of histochemistry, light (LM) and electron microscopy (EM) has provided further refinement of our understanding. Pirozynski and Malloch (1975) believed in presence of fungal structures in fossil primitive plants like *Asteroxylon* and *Rhynia*; as its similarity with the present day VA mycorrhizae or earliest evidence of endomycorrhizae.

During cytological studies, Parke and Linderman (1980) observed hyphae and vesicles resembling structure of vesicular - arbuscular (VA) mycorrhizal fungi within the moss *Funaria hygrometrica* Hedw. growing on the soil surface of *Asparagus - Glomus epigaeus* pot cultures. Rabatin (1980) described the occurrence of the vesicular-arbuscular (VA) mycorrhizal fungus *Glomus tenuis* along leaves and stems of *Pogonatum* a moss. Grasso and Scheirer (1981) observed fungal hyphae in hydroid region (primitive) of moss *Polytrichum commune* Hedw. by scanning Electron Microscopy (SEM). Lingrone, (1988) found penetration of aseptate fungal hyphae between cells in gametophyte of hornwort *Phaeoceros* (Anthoceroophyta). Lingrone

and Lopes (1989) observed remarkable putative VA mycorrhizal endophytes in thalloid hepatic *Conocephalum conicum*.

Duckett *et al.*, (1991) by electron microscopic (EM) study of liverworts belonging to order Jungermanniales observed swollen rhizoids with fungal association but of ascomycetes.

The landmark footprint in the history of mycorrhizal fungus was recorded by Remy *et al.*, (1994) and discovered arbuscules in *Aglaophyton major*, an early Devonian land plant unequivocal evidence that mycorrhizae were established about 400 million years ago. These features found in both vascular plants and bryophytes. Such kind of mutualism was pivotal in the origin of the terrestrial flora.

Lingrone and Duckett (1994) confirmed the Zygomycetous glomalean infection of VA mycorrhiza in bryophytes like *Asterella*. Duckett and Read (1995) reported fungal infections among members of Metzgeriales and Anthocerotales showing Zygomycetous mycorrhizal association. Newsham *et al.*, (1995) reinforced the presence of VA mycorrhiza fungi in early land plants which broadly coincides with the evolution of terrestrial plants. Smith and Smith (1997) summarised the occurrence of *Paris-type* Vesicular Arbuscular (VA) mycorrhizal fungi (Glomalean) as endophytes in many bryophytes. Heijden *et al.*, (1998) suggested that the interaction between microbes and plant, particularly the mycorrhizal symbiosis increases nutrient uptake like phosphorus and also reflects plant diversity in ecosystem. Renzaglia *et al.*, (2000) considered bryophytes as the oldest extant lineages of land plants characterized by high rate of morphological and reproductive innovations and paraphyletic in origin. Read *et al.*, (2000) illustrated symbiotic association in bryophytes where three fungal phyla Zygomycota, Ascomycota and Basidiomycota are involved. Lingrone *et al.*, (2004) also emphasized that Zygomycetous fungi colonize wide range of lower land plants like liverworts, hornworts and less in mosses and reported that glomalean fungi forming VA mycorrhiza colonise liverwort *Pellia epiphylla* producing arbuscules and vesicles.

Wilkinson (2001) reviewed the mycorrhizal evolution and suggested that fungi predate the first vascular plant may be free living or probably forming mycorrhizal like relationship with the bryophytes as they are first terrestrial plants. Brundrett (2002) confirmed that the first bryophyte like land plants in early Devonian

(400 million years ago) had endophytic association resembling vesicular arbuscular mycorrhiza (VAM) even before roots evolved. He also hypothesized that roots gradually evolved from rhizomes (pteridophytes) and rhizoids (bryophytes) to provide suitable habitats for mycorrhizal fungi. Nebel *et al.*, (2004) indicated that the development of liverworts as earliest land plants was linked to the symbiotic association with Glomeromycota. Brundrett (2004) reviewed that mycorrhizal dependency has been established for many plants at realistic soil nutrient levels. Mycorrhizal formation is more efficient than direct nutrient uptake. These fungi were later replaced by ascomycetes and basidiomycetes in Jungermanniaceae and Aneuraceae. Russell and Bulman (2005) reaffirmed and recorded association between *Glomus* sp. with liverwort *Marchantia foliacea* as a specialized symbiosis..

Selosse (2005) proposed that liverworts may imitate mycorrhizas. He explored the liverworts and their fungal symbioses i.e. order Marchantiales, Monocleales, Haplomitriales, Treubiales, Fossombroniales and Metzgeriales association with *Glomus*. Jeffery *et al.*, (2006) reported highly differentiated glomeromycotean association with mucilage secreting primitive liverwort *Treubia* and *Haplomitrium* and consider its establishment even before evolution of rhizoids.

Zhang and Guo (2007) recorded AM fungal structures in mosses stem and leaf tissues but not in rhizoids of *Leucobryum glaucum*, *L. javense*, *Trichostomum crispulum* etc. The VA mycorrhizal spores like *Glomus*, *Acaulospora* and *Gigaspora* were observed among these mosses. Vyas *et al.*, (2007) reported AM fungi colonization in bryophytes like *Riccia*, *Asterella*, *Notothylas* and *Hypnum*. The *Glomus hoi*, *Glomus mosseae*, *Glomus* sp., *Acaulospora*, *Gigaspora* were found dominant among these bryophytes. Vyas *et al.*, (2008) observed vesicular arbuscular mycorrhizal association in bryophytes isolated from Eastern and Western Himalayas. The plants like *Riccia* sp., *Pellia* sp., *Notothylas* sp. *Plagiochasma* sp. *Asterella* sp. and *Marchantia* sp. were associated with AM fungi like *Glomus magnicaule*, *Gigaspora albida*, *Acaulospora scorbiculata* etc. Arora (2008) observed association of VA mycorrhizal fungi with liverworts like *Plagiochasma appendiculatum*, *Asterella angusta*, *Riccia discolor* and *Marchantia polymorpha*. The external and internal hyphae, appressoria, vesicles and *Glomus* sp. were recorded in these plants. Bidartondo and Duckett (2009) considered glomeromycetes as a basal trait of symbiosis with liverworts that long predates arbuscular mycorrhiza in other plants.

But in contrast, range of leafy liverworts like Aneuraceae may switch to basidiomycetes fungi. Pressel *et al.*, (2010) reaffirmed the fungal symbioses in *Treubia* and *Haplomitrium* showing hyphal entry in rhizoids and directly through epidermal cell wall.

Liepina (2012) examined 43 bryophyte species belonging to 28 families which are epigeic, epiphytic and epixylic where terricolous five species found associated with VA mycorrhizal association like *Conocephalum* sp., *Fossombronia* sp., *Pellia* sp., *Anthoceros* sp. and *Frullania* sp. etc. This is consistent with the idea that mycorrhizal associations depend on edaphic factors. Bidartondo *et al.*, (2013) in landmark statement suggested that the colonization of early land plants depends upon nutrient uptakes and its attributes and also begins the dawn of symbiosis between early land plants and fungi in symbiotic approach.

2.4 Antibiosis among bryophytes

The phenomenon of antibiosis has been reported to occur in the organisms which occur at lower level of evolution like bacteria, fungi etc. The bryophytes occupy comparatively a higher position in the evolutionary scale. The occurrence of antimicrobial substances in thalli of bryophytes has been reported since early 50's.

Madsen and Pates (1952) reported for the first time the antimicrobial activity of bryophyte *Sphagnum portoricense* and *Sphagnum strictum* against pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Smith (1955) presumed that *Sphagnum* bog occurrence in aquatic acidic habitat is due to decomposition. Ramaut (1959) described the antiseptic property of the *Sphagnum* bog to the acidity and anaerobic conditions of the ecological set up. Wolter (1964) screened antifungal activity of 18 species of bryophytes belonging to *Pellia epiphylla* and *Diplophyllum albicans* and 16 other mosses. Three species *Diplophyllum albicans*, *Plagiothecium denticulatum* and *Pogonatum aloides* showed antifungal activity. McCleary *et al.*, (1960) reported the occurrence of antibacterial activity against *Gaffkya tetragena* and *Staphylococcus aureus* in the extract of 3 species of *Sphagnum*. McCleary and Walkington (1966) examined 50 species of mosses of which 18 showed strong antibacterial activity while 7 exhibited less but positive activity and rest 25 were inactive. Gupta and Singh (1971) have reported antibacterial activity of petroleum

ether extracts of mosses *Barbula* and *Timella* against 33 species of bacterial pathogens.

The landmark work was reported by Banerjee and Sen (1979) by examining 52 species (40 genera) of the bryophyte where 29 species showed activity against at least one bacterium. The liverworts *Asterella sanguinea*, *Marchantia paleacea* and the moss *Brachythecium procumbens* showed the broadest spectrum of antibacterial activity. Cipla and Pascal (1986) found that aqueous and alcoholic extract of *Polytrichum juniperinum* were non-toxic to mice and possessed antimycotic effect against *Candida albicans*. Joshi and Desai (1988) detected antimicrobial activity of *Fossombronia himalayensis* in Petroleum ether extract. Castaldo-Cobianchi *et al.*, (1988-89) found that the acetone extracts of *Conocephalum conicum*, *Mnium undulatum* and *Leptodyctium riparium* were active against 8 pathogenic bacteria including *Pseudomonas aeruginosa*. Latiff *et al.*, (1989) screened 14 moss species belonging to 10 families of Malaysia for antibacterial activity. The ethanolic extract of the mosses were tested against *E.coli*, *S. aureus* and *Bacillus subtilis*. *S. aureus* was the most susceptible bacterium which was inhibited by all the moss extract. Lorimer *et al.*, (1993) isolated an antifungal and antibacterial bibenzyl (4-hydroxy-3' methoxy-bibenzyl) from New Zealand liverwort *Plagiochila stephensoniana*. The stilbenes present in the thallus possessed remarkable biological activity.

Grammes *et al.*, (1994) carried out *in-vitro* culture of *Fossombronia pusilla* and isolated or analyzed terpenoids, as same produced in natural condition of plants. The petroleum ether extract and terpenoids exerted antibacterial activity. Basile *et al.*, (1999) isolated pure flavonoids like flavones, apigenin etc. from mosses and tested against *Enterobacter cloacae* and *E. aerogenes*, *P. aeruginose* positively.

Subhisha and Subramaniam (2005) studied antifungal activities of steroid from bryophyte *Pallavicinia lyellii*, a liverwort. The fungi used were *Candida albicans*, *Aspergillus niger*, *A. fumigatus* and *Fusarium oxysporum*, showed broad spectrum of activity. Liang *et al.*, (2006) studied antibacterial activity in extracts of some bryophytes from China and Mangolia. About 60 bryophytes and their ethanolic extract were used for screening. Antibacterial activity was recorded in bryophytes like *Conocephalum*, *Frullania*, *Herbetus*, *Marchantia*, *Mastigophora* and *Porella* etc. Ilhan *et al.*, (2006) described the antimicrobial activity of 2 extracts of *Palustriella commutata* (Hdew.) Ochyra from Turkey. The acetone and methanol extracts of this

plant tested against 11 bacteria, 1 yeast and 8 moulds with positive effects. Singh *et al.*, (2007) reported antimicrobial activity of ethanolic extracts of 15 Indian mosses like *Sphagnum* sp., *Barbula* sp., *Brachythecium* sp., *Mnium* sp., *Entodon* sp. and found active against 12 micro-organisms.

Mewari and Kumar (2008) used crude methanol and flavonoid extracts of *Marchantia polymorpha* L. against three bacterial strain viz., *E. coli*, *P. mirabilis*, *S. aureus* and four fungal strains viz., *A. flavus*, *A. niger*, *C. albicans* and *T. mentagrophytes* for antimicrobial screening. Disc diffusion and micro broth dilution technique was used for experiment. Bodade *et al.*, (2008) described *in-vitro* screening of bryophytes like *Plagiochasma* sp., *Thuidium* sp., *Bryum* sp. and *Racomitrium* sp. for antimicrobial activity against 10 bacteria and 3 fungi. Agar diffusion method was used for screening of antibiotic activity. *E. coli*, *S. aureus* and *A. niger* were more sensitive to ethanol, acetone, chloroform and methanol extracts. Veljic *et al.*, (2008) reported antimicrobial activity of methanol extracts of mosses from Serbia by micro dilution and disc diffusion method against six bacterial and seven fungal species. Stalheim *et al.*, (2009) reported *sphagnum* named pectic like polymer can inhibit growth of food spoiling or food poisoning bacteria by lowering the pH.

Ücüncü *et al.*, (2010) recorded antibacterial activity of Turkish moss *Tortula muralis* (Hedw.), *Homalothecium lutescens* (Hedw.), *Hypnum cupressiformae* (Hedw.) and *Pohlia nutans* (Hedw.) against 6 bacteria and 3 fungi. Russell (2010) used 14 crude methanolic and ethanolic extracts of bryophytes from South Western British Columbia for screening of antibiotic activity against *E. coli*, *B. subtilis* and *K. pneumoniae* by disc diffusion method. Sabovljevic *et al.*, (2010) reported antibacterial activity of *in-situ* and *in-vitro* grown bryophytes like *Atrium* sp., *Marchantia polymorpha* and *Physcomitrella patens* against six bacteria positively.

Elibol *et al.*, (2011) reported six Turkish acrocarpic mosses like *Syntrichia* sp., *Grimmia* sp., *Bryum* sp., *Tortella* sp., *Orthotrichum* sp. and *Pleurochaete* sp. showing antibacterial activity. Savaroglu *et al.*, (2011) evaluated antimicrobial activity of some mosses viz., *Funaria* sp., *Hypnum* sp., *Polytrichum* sp. etc. The ethanolic, acetone and chloroform extracts of mosses found more effective than methanolic extract against 9 microorganisms. Pejin *et al.*, (2011) reported antimicrobial activity of dimethyl sulfoxide extract of moss *Rhodobryum ontariense* (Kindb.) by microdilution method against eight bacterial strains and five fungal

strains. It showed better inhibitory activity against *Trichoderma viride*. Khanam *et al.*, (2011) studied *in-vitro*, antibacterial activity of *Marchantia palmata*, with various extracts against *E. coli*, *P. aeruginosa*, *P. mirabilis* and *K. pneumoniae*.

Krishnan *et al.*, (2012) reported *in-vitro* microbicidal potentiality of *Targionia hypophylla* L. and *Bryum* species of bryophytes. Dey and De (2012) reviewed the antioxidative potential of bryophytes and considered it natural source of unique metabolites.

Dhondiyal *et al.*, (2013) reported antibiotic potential of *Lunularia cruciata* (L) Dum ex. Lindb (bryophyta) of Kumaon Himalaya using ethanol extract. Vats and Alam (2013) demonstrated and confirmed the antibacterial activity of *Atrium undulatum* (Hedw.), against three pathogens using well diffusion method. Junairiah *et al.*, (2013) reported antibacterial and antifungal activities of *Dumortiera hirsuta* active fractions against 3 pathogens.

Mukhopadhyay *et al.*, (2013) determined the antimicrobial and antioxidative potential of selected Eastern Himalayan mosses using agar well diffusion method. Devi and Kapila *et al.*, (2013) studied the antibacterial activity of aqueous extracts of six liverworts against six bacterial strains with significant positive results. Sharma *et al.*, (2013) reported the antimicrobial activity of the moss *Polytrichum commune* from the Solan region, Himachal Pradesh. against the microorganisms like *S. aureus* and *P. aeruginosa* with promising and significant results.

2.5 Phytochemical aspects of bryophytes

In recent years, bryophytes have been screened for their potential in pharmaceutical use all over the world. These plants found very rich in secondary metabolites and considered today as "remarkable reservoirs" of novel biologically active compounds. Brindha *et al.*, (1977) and Chopra and Kumar (1988) reported the presence of varied different chemical compound among bryophytes like carbohydrates, proteins, organic acids and lipids. Bryophytes, also possess the chemical constituents like flavonoids, phenolics, steroids, tannins, saponins and terpenoids as a potent source of medicinal properties.

Flowers (1957) reported the ethnobryology of many bryophytes from Gosnite Indians of Utah region. Basile *et al.*, (1999) isolated the pure flavonoids from the

mosses as a biologically active substance with potential antimicrobial activity. Asakawa (1981, 1984) reported the presence of terpenoids and lipophilic aromatic compounds in liverworts as potential source of antibiotics. Yoshihiro *et al.*, (2005) emphasized the biphasic effect of terpenol and phytol isolated from bryophytes against microorganisms like *S. aureus*. Xian *et al.*, (2006) reported the cytotoxicity of bryophyte *Marchantia convoluta* extracts to human cancer and liver cells.

The scenario of phytochemical analysis was changed due to the invention of advanced techniques like Gas Chromatography and Mass Spectroscopy (GC-MS), proton and carbon-13 Nuclear Magnetic Resonance (^1H and ^{13}C NMR), X-ray Crystallography and High Performance Liquid Chromatography (HPLC). Using these techniques, isolation and elucidation of many novel compounds from bryophytes were done by Marcham and Porter (1978), Asakawa (1981) and Huneck (1983).

Asakawa (2001) reported the chemical constituents from the bryophytes like acetogenins, monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and bis (bibenzyl)s from Japanese, Taiwanese, New Zealand, Argentinean and European regions. Ücüncü *et al.*, (2010) analyzed the chemical constituents of mosses like *Torula*, *Homalothecium*, *Hypnum* and *Pohlia* species using GC-MS techniques and recorded presence of many essential oils with antimicrobial activity. Russell (2010) reported that, the extracts of some bryophytes in South Western British Columbia, possess novel chemical compounds with antimicrobial activity. Fatoba *et al.*, (2010) done the phytochemical screening of many tropical African mosses and reported presence of antimicrobial potent compounds. Krishnan *et al.*, (2012) analyzed the phytochemical properties of bryophyte *Targionia hypophylla* with antimicrobial potential against selected gram positive and gram-negative bacteria.

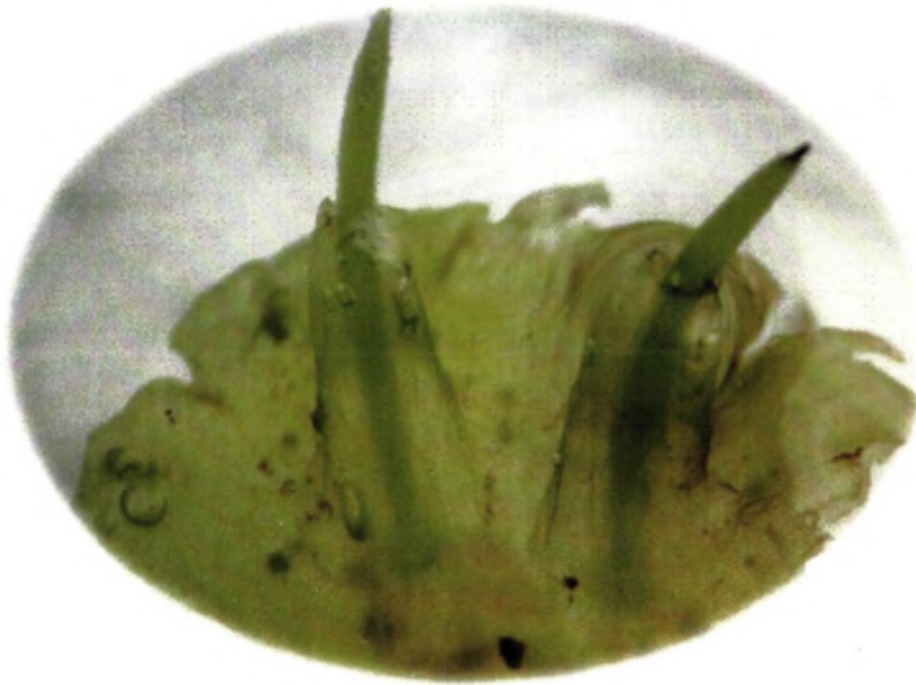
Naz *et al.*, (2013) isolated secondary metabolites from the moss *Funaria parviflora* and tested significantly against bacterial pathogens. Cansu *et al.*, (2013) isolated essential oil from the moss *Hylocomium* and *Leucodon* growing in Turkey and found that these oils possess a significant antibacterial activity.

Claude (2013) reported many chemical constituents like terpenoids from bryophytes like liverworts, hornworts and mosses with many lipids or sterol compounds.

Asakawa *et al.*, (2013) has done the most comprehensive work on chemical constituents of bryophytes like sesquiterpenoids, the fusicoccane type diterpenoids, and also the sesquiterpene hydrocarbon, isolepidozene comprising most elucidation of novel chemical compounds and their structure determination.

Recently, Asakawa *et al.*, (2014) in landmark findings reported the pungent, bitter, cytotoxic and antiviral terpenoid from bryophytes species like *Porella vermicosa*, , *Pallavicinea levieri*, *Plagiochila* sp. and *Chiloscyphus palyanthos* and also isolated the cytotoxic sesquiterpenoids lactones, plagiochilin like chemical compounds. He also found that the liverworts showed antiviral, insecticidal, anti-HIV, superoxide anion radical release, plant growth regulatory, neurotrophic, NO production inhibitory, muscle relaxant, antiobesity, piscicidal and nematocidal activity.

The reconnaissance of literature clearly revealed that the bryophyte though important in succession of vegetation during evolution of higher plants has not been undertaken for investigating its association with VAM fungi that played a significant role in co-evolution and conquest of land and forest ecosystem.



CHAPTER THREE
MATERIALS
&
METHODS

3. MATERIALS AND METHODS

3.1 Survey and site selection

For survey in Melghat Tiger Reserve (MTR), Permit No. 95 was granted and approved by the Chief Wildlife Warden of Maharashtra State and Principal Chief Conservator of Forest, Nagpur under wildlife protection rules, 1975. A log book was maintained while entering as well as exit in the forest area and certified by the forest office. The core area of Melghat forest like Chikhaldara plateau, Semadoh, Kolkhas, Ghatang, Gugamal, Tarubanda, Belkund and their allied sites like Churani, Vairat, Bhimkund, Amazari, Koha, Khongada, Parsapur, Raipur and Makhala were selected for the study.

3.1.1 Ecological studies

GPS (Global Positioning System) readings of plants locations were recorded for authenticity during survey by using handy GARMIN-72 made, GPS device (Table: 1.4). Using altimeter, the height of the various topographical regions was recorded from Mean Sea Level (MSL). Environmental assessment of climatic conditions like temperature and moisture were recorded throughout year using Eurolab made digital thermometer and hygrometer. Rainfall data was also acquired time to time during the course of work from tahsil office of Chikhaldara substation and Sipna Weather Observation and Information Centre, Chikhaldara.

3.1.2 Sampling of plants

Bryophytic thallus were collected from different sites preferably during rainy season of monsoon to winter i.e. from the month of July to February end from different terricolous, saxicolous or rupicolous and corticolous habitat of Melghat forest avoiding presence of ferns, grasses or small angiosperms in niche.

Thallus litter were cleaned carefully under tap water (distilled water for preservation) and cleaned thalloid gametophytes were preserved in 4 % formalin, 75% alcohol with seal tight plastic bottles. Plant thallus were pressed between blotting papers and dried in shade and finally preserved in acid free packets for Bryophytic Herbarium. Bryophytic plants were identified using flora like Kashyap (1929), Bapna and Kachroo (2000) and other monographs. Labelling and authentication of identified materials were done. All Bryophytic material collected during course of work has been deposited in Department of Botany, Sant Gadge Baba Amravati University, Amravati and accession was also sought out.

PLATE – 2
Instruments used and materialization



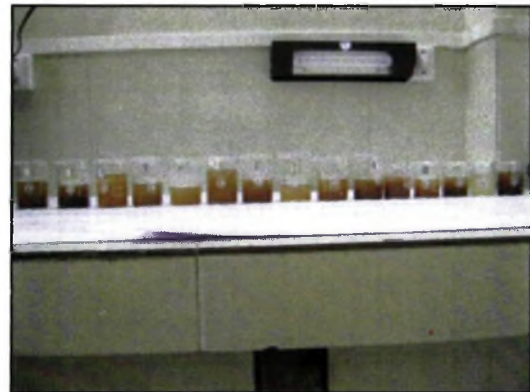
A) Eurolab hygro and thermometer



B) Garmin made handy GPS device



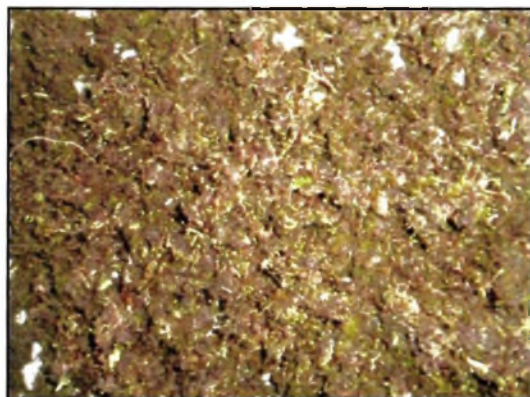
C) Soil sample from *Funaria* thallus



D) Different saturation of extracts



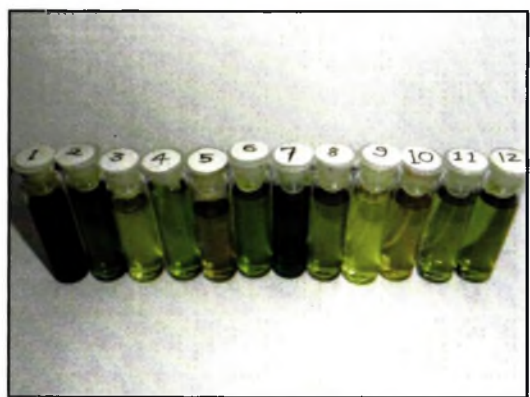
E) VSI soil and water analysis kit



F) *Funaria* plant in shade drying



G) *Plagiochasma* plant in shade drying



H) Plant extracts used for GC-MS

3.2 Soil analysis

Quantitative soil analysis was preferred than qualitative analysis for study of soil chemistry so that actual nutrient level can be assessed. Soil physico-chemical properties like Colour, Texture, pH, Temperature, TDS (Total Dissolved Solids), EC (Electric Conductivity) were evaluated using standard quality VSI Soil and Water Analysis Kit. The nutrient constitution like N (Nitrogen), P (Phosphorus), K (Potassium) and % C (Organic Carbon) were analysed in laboratory as per the procedure and protocols of Jackson (1973) and Mishra (1968).

3.2.1 Determination of soil pH

pH of soil was determined electrometrically using a pH meter (VSI -72 soil and water analysis kit); pH of soil samples were carried out by the procedure as described by Jackson (1973). 20g of air dried soil was weighed and taken into a beaker and 50ml distilled water was added. The suspension was stirred intermittently with glass rod for 30 minutes and kept aside for one hour. The pH electrode was inserted into the well stirred soil suspension and pH of soil was recorded.

3.2.2 Determination soil temperature

Temperature of the soil suspension was measured using VSI -72 soil and water analysis kit. The temperature electrode was inserted into the soil suspension and wet temperatures were recorded.

3.2.3 Electrical Conductivity (EC)

The saturated paste method has long been recommended method for assessing soil salinity in relation to plant growth (Jackson, 1973). 100g of soil was taken in a plastic cup and enough distilled water was mixed in the soil to make the paste. The paste tended to glistened when enough water was added to it. The saturated sample was allowed to stand for overnight. Again water was added to bring it back to saturation condition. The soil suspension was then vacuum filtered and extract was collected separately. Electrical Conductivity of this extract was measured with conductivity meter by placing electrode in it.

3.2.4 TDS (Total Dissolved Solids)

TDS measurement is based on the measurement of electrical conductivity of the sample which is closely proportional to dissolved solids. It offers a quick means of computing total dissolved solids. 20g of air dried soil was weighed and taken into a beaker and 50 ml distilled water was added. The suspension was stirred intermittently

with glass rod for 30 minutes and kept aside for one hour. The TDS probe electrode was inserted into the well stirred soil suspension and TDS value of soil was recorded using VSI -72 soil and water analysis kit.

3. 2.5 Estimation of Nitrogen

Available nitrogen was determined by potassium permanganate method (Subbaiah and Asija, 1956). In 800 ml Kjeldahl flask, 20g soil sample was taken. To this 20 ml of distilled water was added followed by 100 ml each of 0.32% KMnO_4 and 2.5% NaOH solutions. The frothing during boiling was prevented by adding liquid paraffin (1 ml) and bumping by adding a few glass beads. The contents were distilled in a Kjeldahl assembly at a steady rate and liberated ammonia was collected in a conical flask (250ml) containing 20ml of boric acid solution (with mixed indicator). With the absorption of ammonia, pinkish colour of boric acid solution turns green. Nearly 150ml of distillate was collected in about 30 minutes which was titrated with 0.02N H_2SO_4 to the original shade (Pinkish). Blank determination without soil was made for the final calculation.

$$\% \text{ Available N} = (A - B) \times (\text{N of Acid}) \times 0.014 \times \frac{100}{\text{Wt of Soil(g)}}$$

Where,

- Wt. - Weight of soil sample (Wt)
- A (ml) - Volume of std. acid required for soil
- B (ml) - Volume of std. acid required for blank
- N - Normality of Sulphuric acid

3. 2.6 Estimation of Phosphorus

Estimation of available phosphorus from soil described by Olsen, *et al.*, (1954) and modified by Watanbe (1965). The reagent used for the estimation was 0.5 M NaHCO_3 prepared by dissolving 42.0g of NaHCO_3 in distilled water to give one litre of the solution. The pH was adjusted to 8.5 with small quantities of 20% NaOH solution.

A little of Darco G 60 equivalent grade of activated carbon (free of phosphorus) was added to 2.5g air dried soil in 150ml conical flask, followed by 50ml of Olsen's reagent. A blank was run without soil. The flasks were shaken for 30 minutes on the reciprocating shaker and the contents were filtered immediately

through dry filter paper (Whatman no.1) into a clean and dry beaker. From the filtrate, phosphorus was estimated calorimetrically by Olsen's method, in the following way.

5ml aliquot of the Olsen's extract was pipetted into 25 ml volumetric flask and carefully acidified with 5N H₂SO₄ to pH 5. This was done by taking 5ml of the extracting reagent in a separate 25ml flask and determining the volume of acid required to bring the pH of solution to 5 using P-nitrophenol indicator, yellow colour of which disappears at this pH. On adjustment of the pH it was diluted to 20ml distilled water and 4ml of reagent (Dickman Bray's reagent having excess of acid for Olsen's method). The volume was made upto the mark and contents were shaken well. After 10 minutes the intensity of blue colour was read in a photoelectric colorimeter using 730-840 μm filter. A blank was run without soil.

Standard Curve for Phosphorous

For the preparation of the standard curve, different concentration 0, 2, 4, 6, 8 and 10 ml of 2 ppm P solution were taken in 25 ml volumetric flask separately, which correspond to 0.0, 16.0, 32, 0.48, 0.64 and 0.80 ppm P respectively. To this 5 ml of extracting solution (0.5 NaHCO₃) was added to each flask and pH was adjusted as above. Contents were diluted with 20 ml distilled water and 4 ml of Dickman and Brays reagent. Volume was made up and intensity of blue colour was read in photoelectric colorimeter using 730-840 nm filter or using red filter (660 nm). Graph was constructed by plotting readings on X-axis and concentrations of P on Y-axis.

$$\text{Factor (F)} = \frac{\text{Concentration of P}}{\text{Corresponding reading of above Concentration}} = \frac{0.32}{30} = 0.01$$

$$= 1 \text{ colorimeter reading} = 0.01 \text{ ppm (P) Phosphorus}$$

Calculations

$$\text{P (ppm in soil)} = \text{ppm P in aliquot} \times \frac{\text{Total volume of extract}}{\text{Aliquot taken}} \times \frac{1}{\text{Wt of soil (g)}}$$

$$\text{P (Kg/ha)} = \text{ppm P in soil} \times 2.24$$

$$\text{P}_2\text{O}_5 \text{ (Kg/ha)} = \text{P (kg/ha)} \times 2.29$$

$$\text{Conversion factors} = \text{P} \times 2.29 = \text{P}_2\text{O}_5$$

$$\text{P}_2\text{O}_5 \times 0.437 = \text{P}$$

3. 2.7 Estimation of Potassium

The available K includes both exchangeable and water soluble forms present in the soil sample. Potassium was estimated using 7N (NH₄OAc) ammonium acetate (pH- 7) on flame photometer (Jackson, 1973).

5g of soil sample was taken into 150 ml conical flask and 25 ml of 1 N ammonium acetate (extracting solution pH-7) was added. The contents were shaken for 5 minutes on a mechanical shaker and filtered immediately through a dry filter paper (Whatman no.1). First few ml of the filtrate was rejected. Potassium concentration in the extract was determined on flame photometer after necessary setting and calibration of the instrument.

Standard Curve for Potassium

From the stock solution, measured aliquots were diluted (in 50 or 100ml volumetric flasks) with the ammonium acetate solution to give 10 to 40 ppm of K. After fixing the appropriate filter and adjusting the gas and air pressure the flame photometer reading was set at zero for the blank, (ammonium acetate) and at 100 for 40 ppm K.

The curve was obtained by plotting the readings against the different concentrations (10, 15, 20, 25, 30, 40 ppm) of K.

Calculations

$$\text{Available K (kg/ha)} = R \times F \times \text{vol. of extract} \times \text{DF} \times \frac{2.24 \times 10^6}{\text{Soil wt (gm)} \times 10^6}$$

$$\text{Available K}_2\text{O (kg/ha)} = \text{Available K (kg/ha)} \times 1.20$$

Where,

R = reading from graph

F = Conc. of K / corresponding reading

DF = dilution factor, if any

G) Estimation of Organic Carbon

The organic carbon from soil was determined by wet oxidation method as described by Nelson and Sommers, (1996). 1g of finely ground soil sample passed through 0.5 mm sieved and taken into 500 ml conical flask and 10ml of 1N potassium

dichromate solution was added. Swirled the flask gently. 20ml of conc. H₂SO₄ was added and swirled the flask by hand for a minute and set aside on an asbestos pad for half an hour. After half an hour, 200ml distilled water was added, then 10ml of H₂PO₄ and 0.2g of NaOH and 3-4 drops of ferroin indicator was added. The content of the flask was titrated against 0.5 N ferrous sulphate solution, till the colour changed into brown-green blue to finally red. A blank titration was carried at the beginning without soil.

Calculations :

$$\text{Organic Carbon\%} = (B - T) \times N \times 0.003 \times \frac{100}{\text{Wt of Soil(g)}}$$

B = ml of standard FeSO₄ required for blank.

T = ml of standard FeSO₄ required for soil sample.

N = Normality of standard FeSO₄ solution

3.3 Isolation and estimation of soil borne VAM fungi

Soil attached to bryophyte thallus and present in rhizosphere or mycorrhizosphere (Bansal and Mukherji, 1994) was collected, dried and preserved in zip lock polythene bags to avoid contact with air nitrogen. VAM spores were isolated from the soils closely attached to gametophytic thalli of bryophytes collected from different habitats. Isolation of VAM fungal spores from soil was done following the wet sieving and decanting method of Gerdemann and Nicolson (1963).

Estimation of VAM fungal spore population was done in 100g of soil suspended in 1000 ml of tap water. The mixture was stirred for 1-2 minutes and coarse particles were allowed to settle for half an hour. After that the suspension of the beaker decanted through a series of sieves which were arranged in descending order as mesh size 250 µm, 150 µm, 106 µm, 75 µm, 45 µm and 37 µm. The process was repeated for 4 to 5 times. AM spores retained on each sieve carefully poured into separate beakers with labels (Rodrigues and Muthukumar, 2008).

A circular filter paper (Whatman no.1, size 11 cm diameter) was taken and folded into four equal quadrants. The paper was reopened; two lines were drawn along the folds to divide the filter paper in four equal quadrants. Vertical lines were drawn on one half of the filter so as to divide into 10 columns with each column about

1 cm apart. Each column was numbered and direction of counting was marked by an arrow. The filter paper was folded in such a manner that the marked portion becomes the receiving surface for the sample during filtration. This filter paper along with spores was spread in a large petri dish. If there were cluster of spores on filter paper they were spread apart by the pressure of water from a pointed wash bottle with very fine edge needle or syringe. The dish was observed for spore characteristics under the Olympus stereo zoom binocular microscope. Two lines were focused in the field and by moving the petri plate, the spores were counted in every space between the two lines since the line were numbered and the direction set, it was easy to keep track of each spore on the filter paper as per protocol of Gaur and Adholeya (1994).

B) Mounting of spores

Intact spores were picked up using wet needle or match box stick or pipette and mounted in polyvinyl alcohol lacto-glycerol (PVLG) on glass slides. Melzer's reagent was used while mounting intact or crushed spores in PVLG for morphology based diagnoses (Koske and Tessier, 1983). The reagents prepared as followings.

PVLG		Melzer's Reagent	
Polyvinyl alcohol	: 16.6g	Iodine	: 1.5g
Lactic acid	: 100 ml	*Potassium iodide	: 5g
Distilled water	: 100 ml	Distilled water	: 100 ml
Glycerine	: 10 ml	*Potassium iodide for consistency	

C) Staining for VAM colonization

Structures produced by Glomeromycotan fungi are usually hidden by natural pigments and cell contents. Hence clearing procedure for removing these plant tissues using chemical agents was done. Bryophytes thalli of liverworts, hornworts and mosses along with rhizoids were preserved in FAA (1 Formalin: 0.5 Acetic Acid: 5 Alcohol: 3.5 Distilled Water) solution. All the thalli with rhizoids were cleared using NaOH and KOH treatment. All the material was stained by Trypan Blue for determining possible VAM colonization (Phillips and Hayman, 1970). As bryophytes tissues are more delicate and may be harmed during acidification process, alternate Acid Fuchsin (Kormanik and McGraw, 1982) stain was used for staining. All these slides then carefully observed under Carl Zeiss, trinocular research microscope (Axioscope-A-1) for morpho-taxonomic identification with magnification of 5x, 10x, 40x and 100x. All permanent glass slides were deposited in Department of Botany,

Sant Gadge Baba Amravati University, Amravati for accession. The stain composition was done as followings.

Trypan Blue - Stain		Acid- Fuchsin - Stain	
Trypan Blue	: 0.05g	Acid- Fuchsin	: 1.5g
Lactic Acid	: 50 ml	Lactic Acid	: 87.5 ml
Glycerine	: 10 ml	Glycerine	: 6.2 ml
Distilled water	: 40 ml	Tap water	: 6.3 ml

D) Identification of spores

Identification of VAM spores were done by using manual of Schenck and Perez (1990) and manual by Rodrigues and Muthukumar (2009). Online web based solution and data facility was referred and used from website URL <http://www.invam.edu>.

E) Preparation of Murographs

Morphological characters of the spores were recorded on sheet called murographs including detailed information of various contrasting characters of the spores. The murographs sheets were used for the taxonomic identification

- a) Occurrence of spores:** Occurrences of spores either singly, in loose clusters or in tightly packed sporocarps with or without peridium or spore within the spore were recorded.
- b) Size and shape:** The size and shape of the spores were varied. The shape, globose, ovoid, ellipsoid or irregular of the intact spore was recorded and dimensions were measured using an ocular micrometer.
- c) Colour:** Colour of the spores was determined by observing them under dissecting microscope with an incident light because under transmitted light the dense contents of some spores make them dark. The various colour observed under microscope as hyaline, yellow, reddish-brown, brown and black etc.
- d) Subtending hypha:** The hyphal attachment of the spores, whether simple funnel shape, constricted, bulbous or re-curved were recorded. Generally the species of *Glomus* has a prominent hypha but the spores without any hyphal attachment were identified with their specific characters such as wall layers, content and attachment of spores.
- e) Wall layers:** The number of wall groups, wall layers, thickness, type and their colour was recorded.

3.4. Antimicrobial activity of bryophytes

Bryophytes as medicinal plants have been documented in literature worldwide. Antibiotic activity of bryophytes was successfully reported while studying hepatics, hornworts and mosses (Banerjee and Sen, 1979). Antimicrobial potentials were elicited out using Water, Acetone, Methanol, Ethanol or Ether extracts (Singh *et al.*, 2006).

A) Preparation of samples

The bryophytic thalli collected were cleaned carefully and washed under tap water followed by shade drying. The materials were kept in oven at 60⁰C to remove excessive moisture and completely dried for grinding to powder form. Evaluation of antibacterial effects among eight bryophytes species viz. *Plagiochasma appendiculatum* Lehm et Lindenb in Lehm., *Targionia hypophylla* L., *Cyathodium tuberosum* Kash., *Asterella angusta* Steph., *Reboulia hemisphaerica* (L) Radii., *Riccia gangetica* Ahmad., *Anthoceros erectus* Kash., *Funaria hygrometrica* Hedw. and *Hyophila involuta* (Hook) Jaeg. against some human pathogenic microorganisms was done.

B) Preparation of aqueous extract

For aqueous extract preparation 20g of each herbal preparation was soaked in 200 ml of sterile distilled water in sterile reagent bottle for 40. hrs at room temperature. Each extract was stirred every 10 to 12 hrs. using a sterile glass rod and filtered through muslin cloth. The obtained aqueous extract was evaporated at low temperature under reduced pressure to obtain crude extract. The extracts were kept in sterile wide mouthed screw capped bottle and stored at 4⁰C for further use.

C) Preparation of solvent extract

Using Soxhlet apparatus, the powdered samples of plants were extracted in ethanol, methanol, petroleum ether, chloroform and acetone and different solvent fractions were obtained. The extract collected after 6-7 siphons so that all the plant powder becomes colourless and extract become concentrated. The concentrated extracts were evaporated by rotary evaporator in controlled condition of temperature to avoid destruction of active constituents of the preparations. Dried extracts were stored in labelled sterile wide mouthed screw capped bottles at 4⁰C and used for further study (Parekh and Chanda, 2008).

D) Microbial pathogens

The standard pathogenic bacterial and fungal strain cultures were procured from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in Nutrient broth (Hi-media laboratories, Mumbai, India) at 37⁰C for 18 hrs and then stored at 4⁰C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Subcultures were prepared from the stock for bioassay.

Table 3.2: Bacterial pathogens used in study

Sr. No.	Abbreviations used	Bacterial Pathogens	MTCC Number
1	<i>E. coli</i>	<i>Escherichia coli</i>	729
2	<i>P. vulgaris</i>	<i>Proteus vulgaris</i>	744
3	<i>K. pneumoniae</i>	<i>Klebsiella pneumoniae</i>	661
4	<i>S. flexneri</i>	<i>Shigella flexneri</i>	1457
5	<i>S. aureus</i>	<i>Staphylococcus aureus</i>	96
6	<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	424
7	<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>	98
8	<i>A. niger</i>	<i>Aspergillus niger</i>	343
9	<i>C. albicans</i>	<i>Candida albicans</i>	183
10	<i>R. oryzae</i>	<i>Rhizopus oryzae</i>	284

E) Disc diffusion method

Disc diffusion method was used for the antibacterial sensitivity test by following the standard methods (NCCLS, 1990). Nutrient agar medium was used to test antibacterial activity. Turbidity of inoculum was matched with McFarland turbidity standard. Inoculum was spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were kept over the lawn and pressed slightly along with positive and negative control. Ampicillin 10 mg/disc (Hi-Media) were used as positive while disc soaked in sterile distilled water and various organic solvent and dried were placed on lawns as negative control. The plates were then kept in refrigerator at 4⁰C for 30 minutes for diffusions and the incubated at 37⁰C for 18 to 24hrs. After incubations, zone of inhibitions were measured as diameter in mm; the experiment were carried out in triplicate and the averages diameter of zone of inhibition was recorded (Lalitha *et al.*, 1997).

The results were compared with the standard antibiotics like (10 µg/ml) Penicillin, Ampicillin and Tetracyclin and Nystatin.

3.5 Phytochemical aspect with qualitative phytochemical screening

Bryophyte extracts have demonstrated antimicrobial, antifungal, cytotoxic and many other kinds of biological activities (Basile *et al.*, 1999; Asakawa 2007; Sevlam 2010). Bodade *et al.*, (2008) confirmed that the antimicrobial activity in bryophytes due to presence of flavonoids, steroids, terpenoids and other polyphenolic compounds as per Harborne (1998). It involves testing of different classes of compounds.

The methods used for detection of various phytochemical compounds and followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug (Brindha *et al.*, 1977; Sadashivam and Manickam, 2005). The extracts were analyzed for the presence of phytoconstituents like alkaloids, flavonoids, tannin, phenolics, steroids, saponin and terpenoids.

A) Tests for alkaloids

Dragendroff's Test: About 0.2 g of the extracts (or 2-3 ml of filtrate) was warned with 2% H₂S04 for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

(Dragendroff's reagent: It is used for the detection of alkaloids. Boil 14g of sodium iodide with 5.2g basic bismuth carbonate in 50 ml glacial acetic acid for a few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals. To 40 ml of the red-brown filtrate add 160 ml of ethyl acetate and 1 ml water. Preserve the stock solution in amber-coloured bottle. When needed, add 20ml of acetic acid to 10ml of this stock solution and make up to 100ml with water.)

B) Tests for flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. The yellow solution that turns colourless indicates the presence of flavonoids.

Lead Acetate Test: To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

C) Tests for Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green or blue black solution indicates the presence of tannins.

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

D) Test for Saponin

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins by forming 1cm layer of foam.

E) Test for steroids

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of acid i.e. H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

F) Test for Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

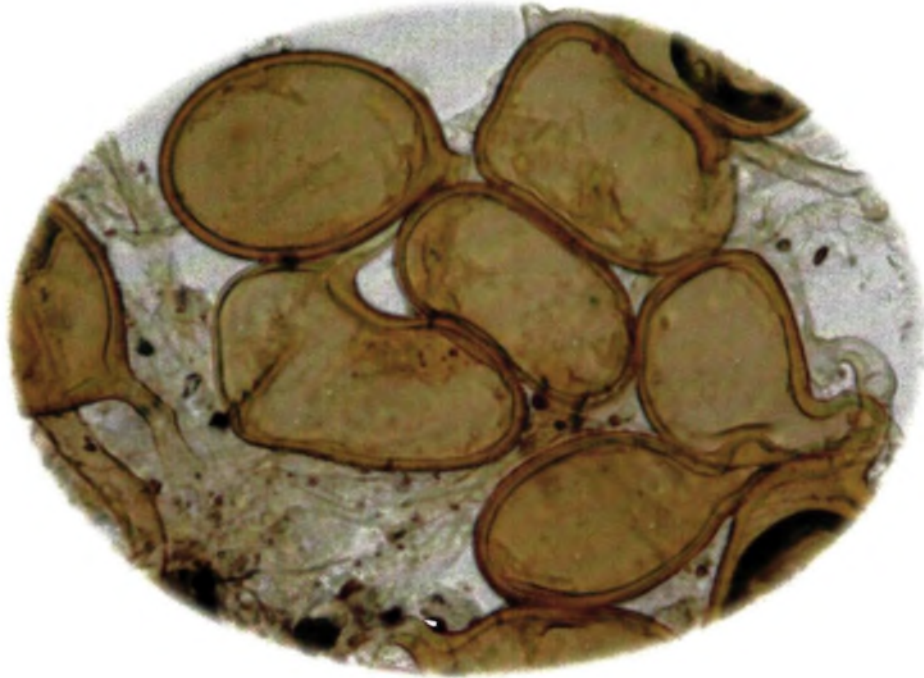
G) Test for Terpenoids

(Salkowski test): 0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration was formed to indicate positive results for the presence of Terpenoids.

3.6 GC-MS (Gas chromatography and Mass Spectroscopy) of bryophytes

About 12 Plant samples were extracted in Methanol and subjected to GC-MS analysis from Shivaji University, Kolhapur and results were obtained.

The methanolic extract obtained from these plants and subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40⁰C and held for 3 min and the final temperature of the oven was 480⁰C with rate at 10⁰C [min.sup.1]. A 2- μ L sample was injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The chemical components from the methanolic extract of plant was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.



CHAPTER FOUR
OBERVATIONS
&
RESULTS

4. OBSERVATIONS AND RESULTS

4.1. Physico-chemical analysis of soil

The soil is one of nature's most complex ecosystems, it contains thousands of different organisms, which interact and contribute to the global cycles that make all life possible. Melghat represents mostly tropical soil with considerably different conditions of weathering and rainfall within the tract. Patel (1968) divided the soils of Melghat forest into Bouldery soil, Clay, Alluvium, Lateritic loam and Gritty loam type.

The bouldery soil occurs mostly across the Melghat region and confined to the slopes. The soils found well drained and lacks moisture during dry season. The texture of soil varies as clay, loam or sandy loam and best vegetation of plants grow on this type of soil. The clay soil dominantly found in depressions and on flat areas. The clay soil generally found as black in colour and fertile. The soil occurs at low-lying areas and plains supporting good forest. The riversides and basins along the main rivers like Sipna, Gadga etc. represents a small area of alluvium type of soil. It varies from fine light brown silt to coarse masses favouring tree vegetation. The lateritic loam represents typical tropical forest soil occurring at hilltops and plateaus. It has characteristic red brown colour due to presence of iron oxides with small stones. Chikhaldara plateau generally resembles closely to lateritic showing fertility and trees growth. However, the Gritty loam occurs on the lower hills and derived from the weathering of the grey basalt rock. Besides its fair quality, it is not fertile in real sense to favour tree growth.

Soil serves as a medium for plant vegetation on the earth's surface. It consists of minerals as well as organic matter and air exhibits peculiar characteristics impressed by the physical and chemical action of tree roots, debris and forest humus. The soil dynamics are directly proportional to the growth of plants, ground cover vegetation, activity of organisms and climatic effects. Hence, several factors like climate, topology, organisms and parent material form a soil medium where root grows, anchor plants to absorb nutrients from soil and establishes a population or community (Brady and Weil, 2008). The soil analysis studied at various sites revealed diverse and varied observations during the course of work and the data obtained was analyzed in tabular form as well as in lucid graphical representation as below.

Table: 4.1.1 Physico-chemical analysis of the soil attached to the plant thallus.

Sr. No	Host Plant	Habitat	Colour	Texture (Type)	Location	pH (1-14)	Temp (°C)	TDS (mg/L)	*EC (mmhos/cm)	N (Kg/ ha) 281-420	P (Kg/ ha) 31-50	K (Kg/ ha) 181-240	C (%) 0.41-0.60
1	<i>Targionia hypophylla</i>	Saxicolous	Blackish	Clay	Semadoh	6.73	19.2	009	0.13	134.1	44.09	428.64	0.35
2	<i>Cyathodium tuberosum</i>	Terricolous	Blackish	Clay	Bhimkund	7.78	18.3	018	0.17	360.6	74.48	574.56	0.32
3	<i>Cyathodium cavernarum</i>	Rupicolous	Brownish	Lateritic	Semadoh	7.12	20.1	012	0.25	329.0	34.44	504.4	0.29
4	<i>Asterella angusta</i>	Saxicolous	Reddish	Bouldery	Belkund	8.06	21.0	031	0.26	428.3	45.0	683.7	0.33
5	<i>Reboulia hemisphaerica</i>	Terricolous	Reddish	Lateritic	Amazari	8.00	22	015	0.21	239.1	56.33	398.12	0.31
6	<i>Plagiochasma appendiculatum</i>	Terricolous	Reddish	Bouldery	Chikhaldara	6.89	18	010	0.14	403.2	89.97	478.2	0.47
7	<i>Plagiochasma intermedium</i>	Saxicolous	Whitish	Lime	Gawilgarh	8.00	16.2	009	0.21	211.1	14.60	398.1	0.31
8	<i>Plagiochasma rupestre</i>	Terricolous	Brownish	Lateritic	Koha	7.42	19	026	0.16	501.7	47.93	668.6	0.32
9	<i>Riccia gangetica</i>	Terricolous	Reddish	Lateritic	Khongada	7.56	20	012	0.29	239.1	41.52	396	0.41
10	<i>Riccia discolor</i>	Terricolous	Blackish	Clay	Churani	7.55	23	016	0.35	236	58.14	436	0.35
11	<i>Anthoceros erectus</i>	Terricolous	Blackish	Clay	Semadoh	7.18	24	009	0.18	266	102	346	0.34
12	<i>Folioceros udarii</i>	Saxicolous	Reddish	Lateritic	Semadoh	6.77	21	008	0.073	260	41.0	413	0.53
13	<i>Notothylas indica</i>	Terricolous	Brownish	Bouldery	Amazari	7.12	16	011	0.12	266	46.2	471	0.44
14	<i>Phaeoceros laevis</i>	Terricolous	Blackish	Alluvium	Semadoh	6.81	22	012	0.15	448	82.82	685.8	0.38
15	<i>Funaria hygrometrica</i>	Terricolous	Brown Red	Lateritic	Gugamal	7.6	19	011	0.21	250	27.14	200.8	0.41
16	<i>Brachythemium turgidum</i>	Epixylic	On tree	On tree	Gawilgarh	-	-	-	-	-	-	-	-
17	<i>Bryum coronatum</i>	Terricolous	Ash-black	Gritty-loam	Ghatang	6.89	26	012	0.09	241	32.87	680	0.47
18	<i>Stereophyllum decorum</i>	Epixylic	On tree	On tree	Bori	-	-	-	-	-	-	-	-
19	<i>Hyophila involuta</i>	Epilithic	Brownish	Lateritic	Kolkhas	7.85	24	013	0.25	321	16.50	463	0.37
20	<i>Hymenostylium recurvirostre</i>	Epilithic	Blackish	Clay	Madaki	6.90	29.1	017	0.14	230	14.50	390	0.43

* Mmhos/cm is equivalent to decisiemen/m (mmhos/cm multiply by 640 to estimate ppm/salt)

Fig: 4.1.1 pH analysis of the soil

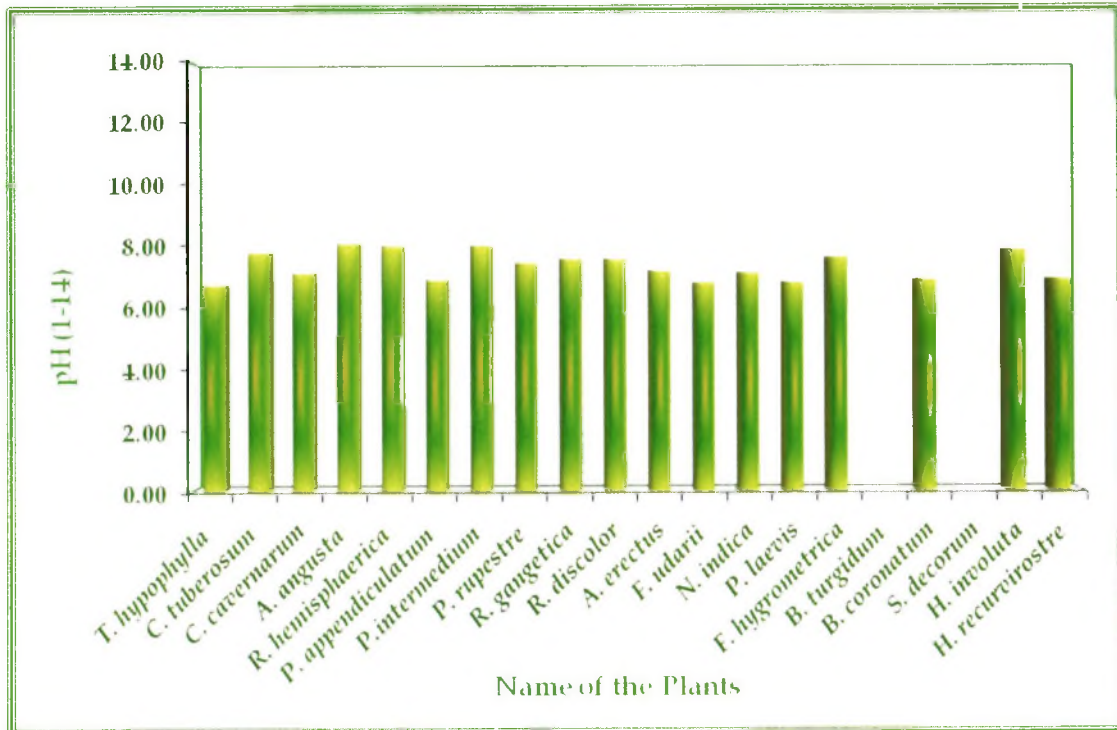


Fig: 4.1.2 Temperature (°C) analysis of the soil

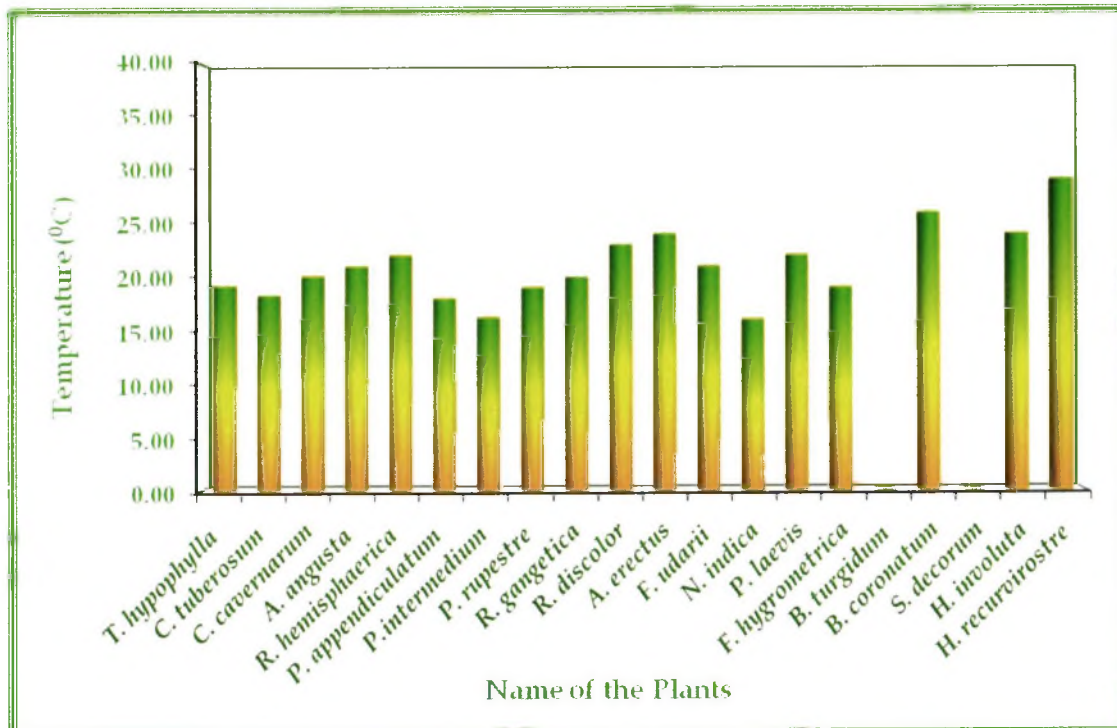


Fig: 4.1.3 TDS (mg/L) analysis of the soil

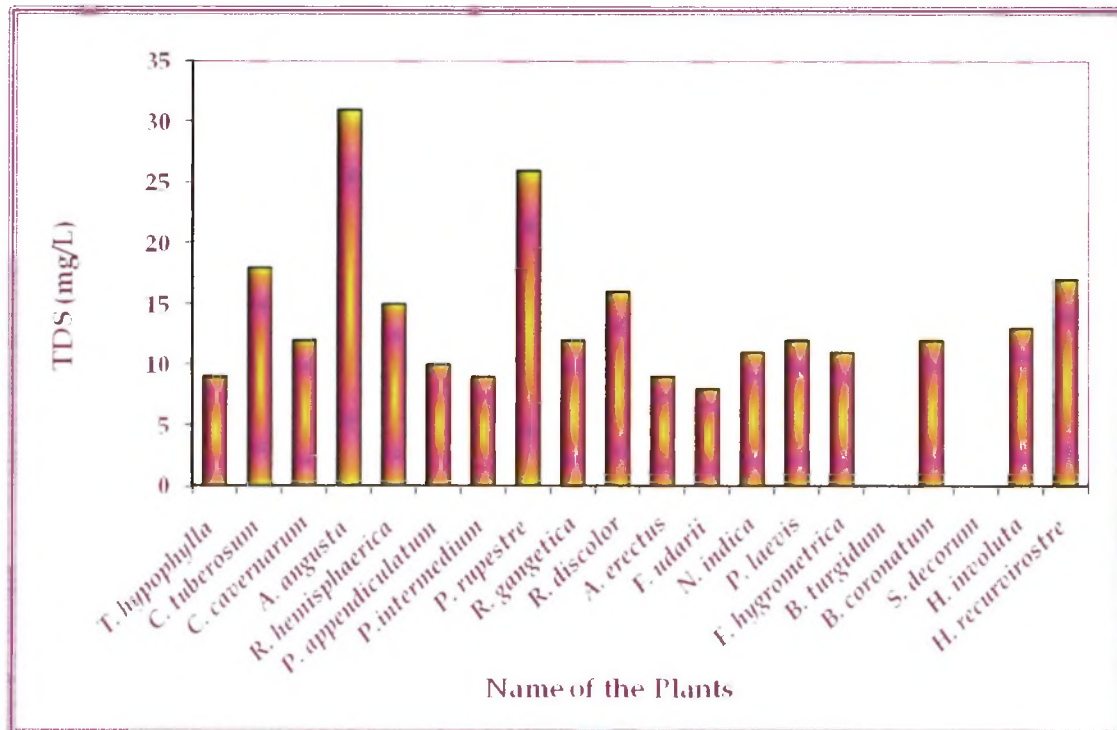


Fig: 4.1.4 EC (mmhos/cm) analysis of the soil

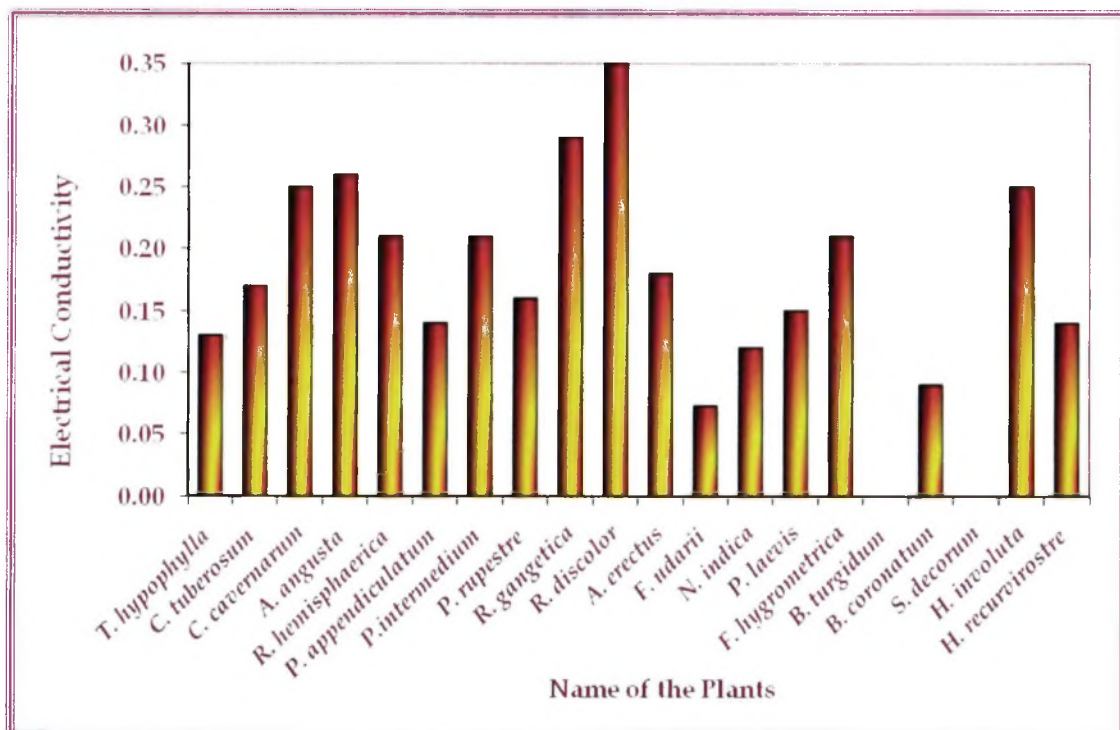


Fig: 4.1.5 N (kg/ha) Analysis of the soil

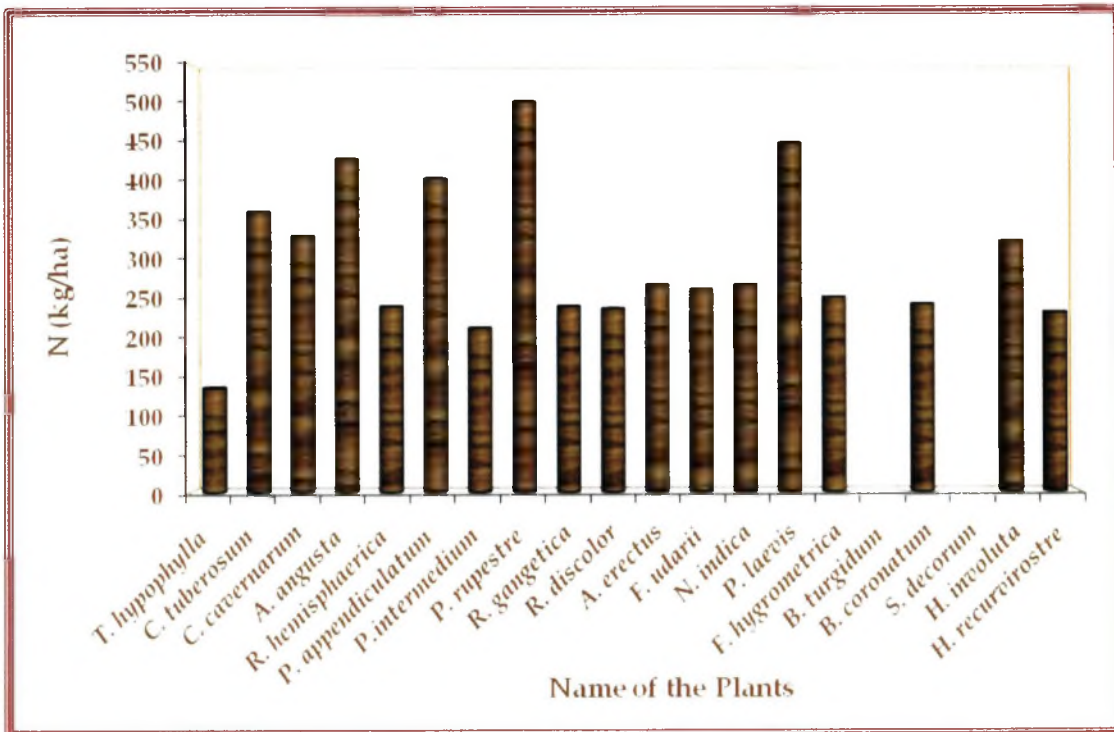


Fig: 4.1.6 P (kg/ha) Analysis of the soil

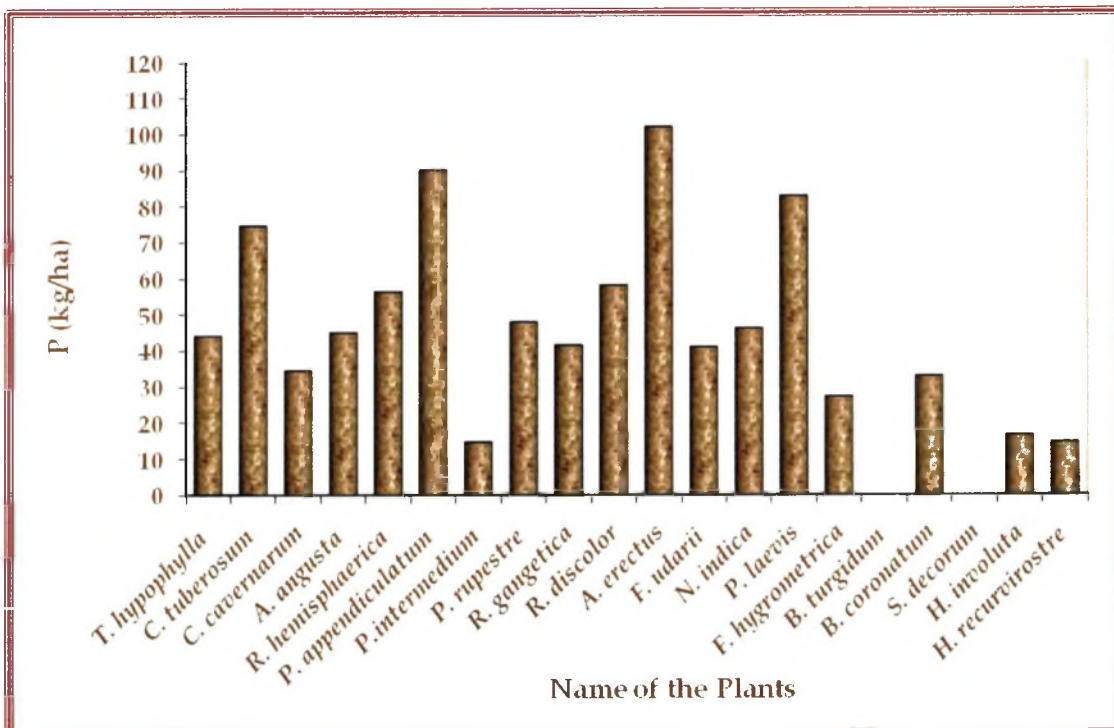


Fig: 4.1.7 K (kg/ha) Analysis of the soil

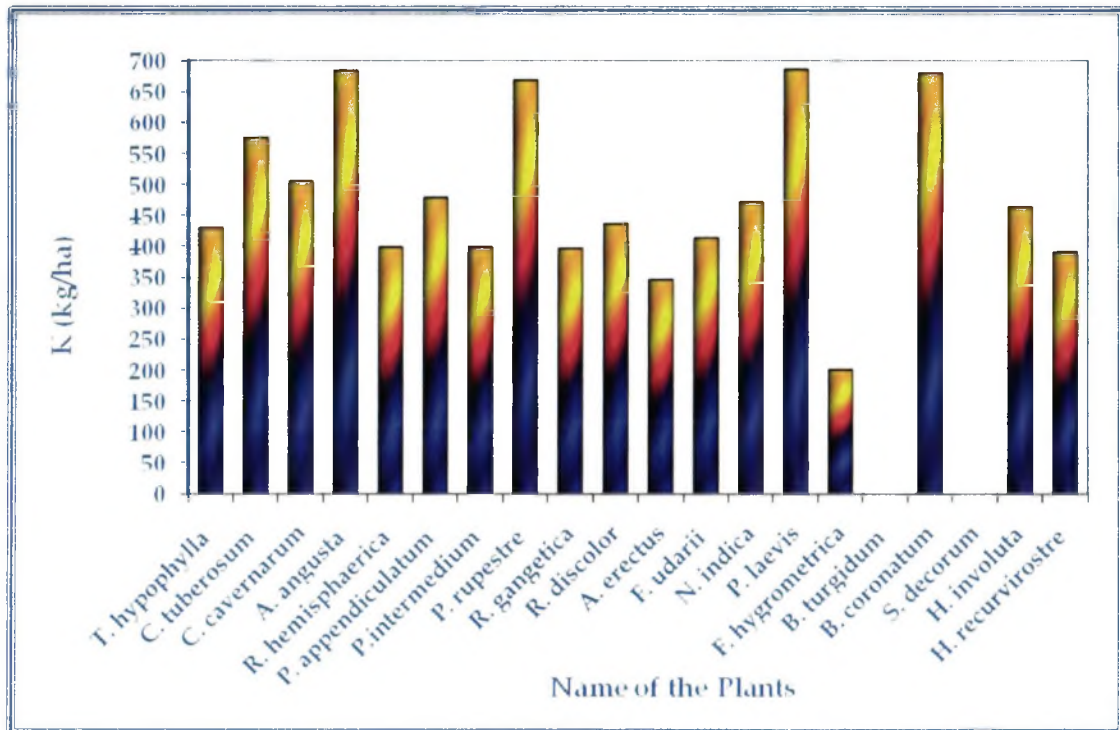
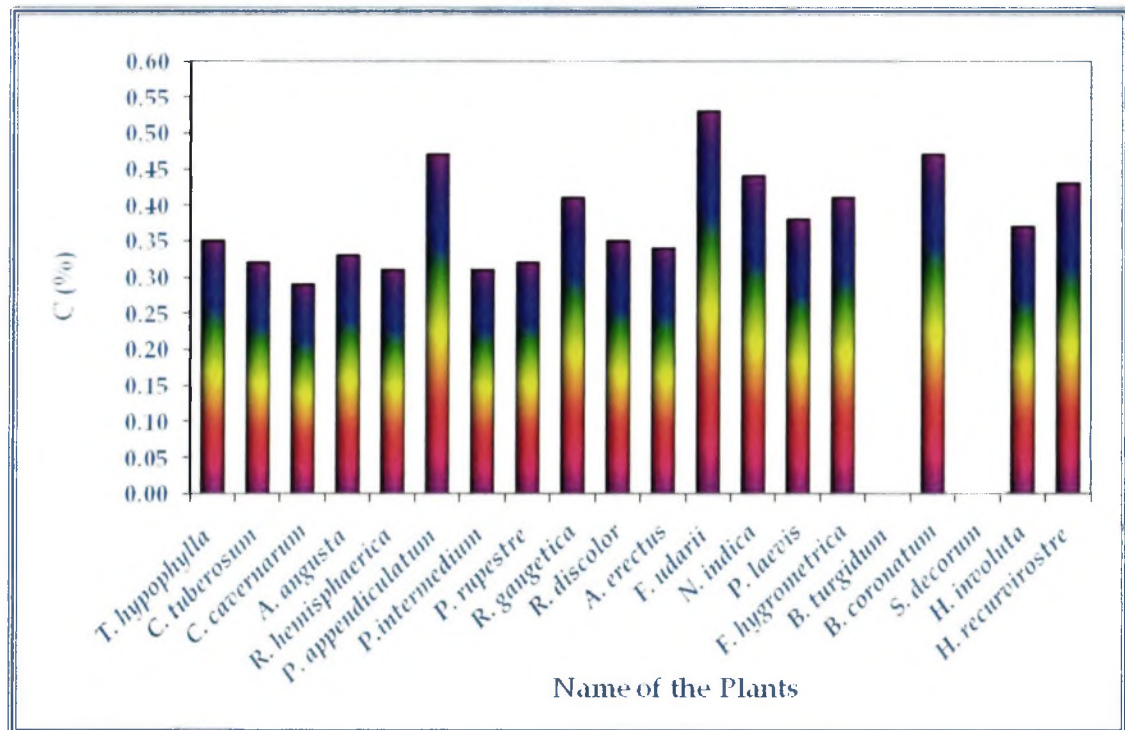


Fig: 4.1.8 C (%) Analysis of the soil



4.1.1 Soil texture

The soil texture exhibits different characteristics at different locations. The upper plateau of Chikhaldara showed bouldery type of brown coloured soil across the allied regions also. However, the lower parts at the slopes like Semadoh, Kolkhas, and Tarubanda represents red coloured lateritic type of soil. The region of Raipur, Makhala, and Ghatang exhibit mixed type of soil with some patches of gritty loam, brown coloured soil. The basins of rivers like Sipna, Gadga represents black alluvium type of soil and allied regions also showed black soil along with the plains also (Table: 4.1.1).

4.1.2 Soil pH

The pH value of the soil was observed in between 6.73 to 8.06 representing slight acidic, neutral and slight basic nature across the region (Table: 4.1.1). The upper plateau of Chikhaldara and its adjoining regions generally showed slight acidic to neutral pH value while lower plateau at Semadoh, Ghatang, and Kolkhas showed neutral to slight basic nature of soil. However, variations in pH values were observed across the different sites and among different bryophytic plants during the course of investigations. Plants like *Targionia* sp. found at pH value 6.73 while *Asterella* sp. found at 8.06 pH value near Belkund (Fig: 4.1.1).

4.1.3 Soil temperature ($^{\circ}\text{C}$)

The soil temperature observed in range of 16 $^{\circ}\text{C}$ to 29.1 $^{\circ}\text{C}$ among various samples, collected during the course of work (Table: 4.1.1). *Notothylas* sp. growing on brown coloured soil at Amazari showed lowest soil temperature of 16 $^{\circ}\text{C}$ while the plant *Hymenostylium* sp. near Madaki exhibited highest soil temperature of 29.1 $^{\circ}\text{C}$ collected during month of October (Fig: 4.1.2).

4.1.4 Total dissolved soils (TDS)

The total dissolved solids among the soil suspension observed in the range of 008 mg/l to 031 mg/l. The plants growing in different habitat at different locations showed variations in values of total dissolved solids (Table: 4.1.1). The plant *Folioceros* sp. of terrestrial habitat and found on red soil at Semadoh showed less TDS value of 008 mg/L while *Asterella* sp. at Belkund showed the highest TDS value of 031 mg/L collected during the rainy season (Fig: 4.1.3).

4.1.5 Electrical conductivity (EC)

The electrical conductivity of soils of different locations was found in range between 0.073 mmhos/cm to 0.35 mmhos/cm (Table: 4.1.1). The terricolous hornwort *Folioceros* sp. found at Semadoh in red soil showed very less EC value of 0.073 mmhos/cm while *Asterella* sp. at Belkund showed 0.49 mmhos/cm. The electrical conductivity observed to be varying at different locations and habitats (Fig: 4.1.4).

4.1.6 Nitrogen content (N)

The presence of Nitrogen in the soil contributes to the vegetation stability in any habitat. The Nitrogen contents vary in different locations of the Melghat forest. Highest Nitrogen content was observed in Koha region among *Plagiochasma* sp. with 501.7 kg/ha and among *Phaeoceros* sp. at Semadoh region with 498 kg/ha collected during rainy season (Table: 4.1.1). The lower Nitrogen content were observed in *Targionia* sp. in black soil with saxicolous habitat with 134.1 kg/ha. However, most of the plants at different locations showed deficient to moderate nitrogen content in the soil.

The terrestrial habitat of most of the plants showed moderate nitrogen content than the rupicolous or saxicolous habitat (Fig: 4.1.5).

4.1.7 Phosphorus (P)

The phosphorus is a major nutrient block in plant metabolism, growth and development. The occurrences of phosphorus observed were variable from negligible condition to rich condition.

Higher phosphorus content observed in Semadoh region among *Anthoceros* sp. with 102 kg/ha and among *Plagiochasma* sp. at Chikhaldara with 89.97 kg/ha collected during rainy season (Table: 4.1.1). However, lower phosphorus content was observed in nutrient deficient mosses growing on rocks along roadside or pools or pebbles like *Hymenostylium* sp. with 14.50 kg/ha and among *Plagiochasma intermedium* with 14.60 kg/ha growing on rocks of Gawilgarh in whitish soil.

The moss *Hyophila* sp. also showed lesser content of phosphorus with 16.50 kg/ha growing on rocks at Madaki on Paratwada - Chikhaldara road. These soil samples collected during the month of December where winter season was prevailed in the region (Fig: 4.1.6).

4.1.7 Potassium (K)

The potassium content among the soils of Melghat region was found quite higher and sufficient with respect to bryophytes (Table: 4.1.1) and parallel to other species like *Bryum* sp. or *Anthoceros* sp. etc. The lowest potassium content was observed in *Funaria* sp. at Gugamal forest with 200.48 kg/ha at terricolous habitat.

The potassium played an important role in nutrient uptake and physiology of plant metabolism (Fig: 4.1.7).

4.1.8 Organic Carbon (C %)

The percentage of organic carbon among the soils of bryophytes of Melghat forest ranges between minimum from 0.29 % to 0.53 % at maximum (Table: 4.1.1). The bryophyte species *Cyathodium* of rupicolous habitat showed less 0.29 % organic carbon at Semadoh region while the species *Folioceros*, interestingly showed 0.53 % of maximum value in reddish lateritic soil in saxicolous and terrestrial habitat.

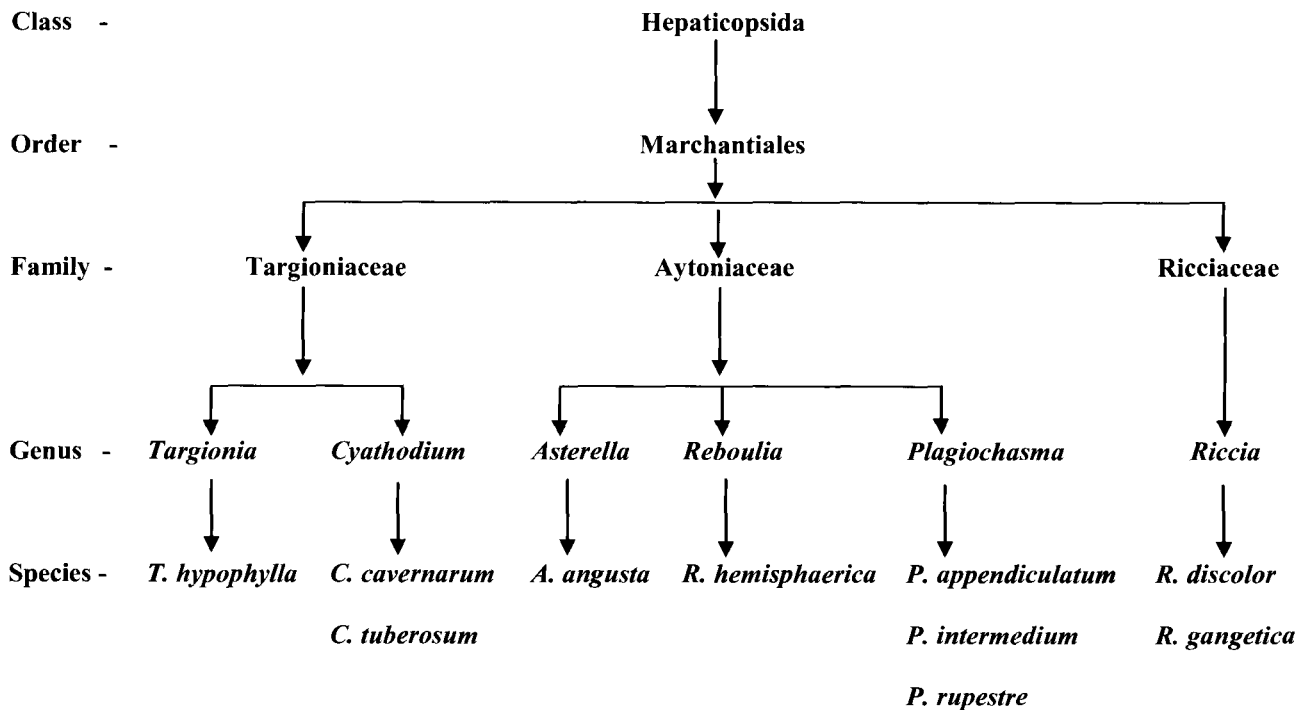
The variable value of organic carbon observed during present investigation but related to vegetation and community (Fig: 4.1.8).

4.2 Morpho-taxonomic studies of bryophytes

The division bryophyta includes comparatively small delicate group of plants and divided into three classes Hepaticopsida, Anthocerotopsida, and Bryopsida, generally known as liverworts, hornworts and mosses respectively. These group of plants have many distinctive features like gametophytic phase and sporophytic phase making them unique than vascular plants. Morpho-taxonomic studies of bryophytes was done by the author following description and arrangement of taxa of liverworts, hornworts and mosses from standard books of Kashyap (1927), Gangulee (1969-85), Chopra (1975), Dabhade (1998), Bapna and Kachroo (2000) and Choudhary *et al.*, (2008) with consultation.

In present investigations, the bryophytes of Melghat forest were first screened, identified, and arranged as per the latest classification in hierarchical manner so that their distribution can be measured substantially. The author presented the exhaustive information of all species under one umbrella of classification to provide the details of bryoflora at a glance. Identification keys of all the explored bryophytic species were also provided with full description of the plants as follows.

Fig: 4.2.1 A detailed outline classification of liverworts collected from Melghat region followed by Grolle's (1983)



4.2.1 Class: Hepaticopsida

Key to the class

Plant thalloid or leafy, numerous chloroplast per cells, rhizoids unicellular, capsule wall without stomata, opens by 2-4 valves, generally elaters present or absent.

..... **Hepaticopsida**

Key to the order

Gametophyte thalloid, thallus internally differentiated into air chambers and storage region; Rhizoids of two types, smooth walled and tuberculated; scales present; capsule wall one cell thick. **Marchantiales**

Key to the family

Air chambers in single layer with assimilatory filaments; absence of stalked female receptacle, archegonia apical; capsule wall with annular thickenings.

..... **Targioniaceae**

Key to the genera

Plants thick, green, air chambers with photosynthetic filaments; well developed, ventral scales in two rows; sporogonium enclosed within conspicuous, purplish two lipped involucre on ventral side. **Targionia**

Key to the species

Plant dioecious, spores found brown (55-65 μm) reticulate, two spiral elaters.

..... **hypophylla**

4.2.1.1 *Targionia hypophylla* Linn. Spec. Plant 1136 (1753); Steph., Sepc., Hep. 1 : 16. 1900; Macv., Std. Handb. Brit. Hep. 1 : 33 F. 1 - 3. 1926; Kash., Liverw. W. Himalaya. Punjab Pl. 1 : 57. Pl. 11 (1-3); Mahabale and Mahajan, J. Univ. Bombay, 23:24-43, f 1- 54, 1955.

Specimen Accession No: 510257

Photo Plate No: 3 (A-D)

Thalli light to dark green, in close clusters, overlapping, usually with their apical end projecting outward and downward. Thallus thin, somewhat brittle, abovate to linear - oblong, simple or once, rarely dichotomously branched, 10-15 mm. long and 3-6 mm. wide. Rhizoids numerous, arising from midrib, tuberculated or smooth walled. Air chamber in single layer separated by one row of cells, packed with

photosynthetic filaments. Storage region usually 6-10 cells high in middle containing some oil bodies.

Plants monoecious but often dioecious. Generally, male plants distinct from female i.e. smaller than female, 10 mm. long and 2-3 mm broad. Archegonia borne in groups of 4-6, on dorsal side of thallus, just back of apical notch; after fertilization the apical arches downwards, and the mature sporophyte appearing to be ventral. Sporophyte generally violet or bluish in colour at maturity but green in young condition. Spores dark brown, spherical, reticulate (Plate -3 ; D) 54-67 μm . rarely up to 75 μm , triradiate mark faint ; elaters with two spirals up to 250 μm long, simple or branched.

Field notes: Plants grow on moist soil, on rock cuttings, rock crevices, on exposed soil slopes. In exposed places, plants are light green and usually found creeping.

Locality: Paratwada - Chikhaldara road, Chikhaldara- Aamazari, Ghatang- Semadoh road, Makhala, Raipur.

Distribution: Western Himalaya, Mussoorie, Sikkim, Darjeeling, Khasi hills, Pachmarhi, Mt. Abu, Mahabaleshwar, Pachgani, Nilgiri Hills, Mysore, Chennai, Madagascar, Europe, America, Tasmania, New Zealand, Africa and Australia.

4.2.1.2 *Cyathodium tuberosum* Kash. New Phytol 13: 210. Liverw. W. Himalaya Punjab Pl.1:53 Pl.10 F.1-11.1929; Khanna. *J.Burma Rec.Soc.*17:270.1927.

Specimen Accession No: 510258

Photo Plate No:3 (E-H)

Thalli light green, fluorescent, sterile plants are smaller and narrower than female, once or twice dichotomously branched. Female plants large and usually unbranched. Plants generally dioecious. Rhizoids numerous, hyaline, dimorphic thick walled. Ventral scales irregularly distributed, uni-triseriate. Photosynthetic zone represented by unistratose empty air chambers.

Plants reproduce vegetatively by means of tubers present at both apices as well as at lateral margin of thallus. Tubers densely covered with rhizoids. Sporogonium differentiated into small foot, medium sized seta and capsule. Spores dark brown, spherical and spinate. Elaters reddish brown, pointed at one end and blunt at the other.

Field notes: Plants were growing in moist and shady places. Dominantly found near water percolating in rock crevices as less exposed to light. Mature sporangia found quite late in the month of September and October.

Locality: Chikhaldara, Semadoh, Semadoh-Chikhaldara Road, Paratwada-Chikhaldara Road, Makhala Forest.

Distribution: Sikkim, Gauhati, Kumaon, Nainital, Mahabaleshwar, Maharashtra, Chennai, Pune, Punjab, Rajasthan, Pachmarhi, Banaras, Lucknow, Manyamar and Java.

Key to the genera

Plants thin, pale green and translucent; air chambers empty ventral scales reduced; involucre bilobed to pouch like, open in front. *Cyathodium*

Key to the species

Plant dioecious, thalli light green, sporogonium differentiated, spores dark brown, spinate, elaters reddish brown. *tuberosum*

4.2.1.3 *Cyathodium cavernarum* Kunze. in. Lehm., Pugillus 6 : 17 1844; Schiffn; Ann Bryol. 11: 132-134. 1938; Khanna, J Burma Res. Soc. 16: 227 .1927.

Specimen Accession No: 510259

Photo Plate No: 4 (A-D)

Thalli 2-10 mm long, 2-5 mm broad delicate yellowish green, or green restricted to dark places, caves, or canopy of shade or open moist protected places. Thalli often dichotomously branched, fan shaped, dorsal epidermal cells thin walled, chlorophyllose, lower epidermal cell larger chlorophyllose. Air chambers usually in single row, partition between air chambers 1 -2 cells high with chloroplast. Pores on dorsal surface bounded by 2 -3 concentric rings. Rhizoids smooth walled, ventral scales simple, filamentous with mucilage papillae. Capsule ovoid, 0.68 mm in diameter, two walls single layered, generally blackish on maturity. Spores blackish brown, 28-50 µm in diameter, isopolar, baculate spinate, baculae 3-4 µm long and 1.25-2.5 µm broad at base. Elaters reddish brown, 4-9 in number, bispiral, 300-450 µm in length and 14-18 µm in width.

Field notes: Plant found growing in dense mats, overlapping on moist coarse soil, rock crevices and on walls of old building.

Locality: Gawilgarh fort, Kolkhas, Semadoh, Chikhaldara, Aamazari, Belkund, and Khongada.

Distribution: Eastern Himalayas, Darjeeling, Khasi and Jayantia hills, Shillong, Elephanta Caves, Khandala, Mahabaleshwar, Pachgani, Muktagiri, Western Ghats, Gujrat, U.P., Banaras, Lucknow, Burma, Cuba, Africa, Java and Mexico America.

Key to the Species

Plants yellowish green, fluorescent in colour, monoecious, capsule ovoid, spores blackish brown 28-50µm diameter, Elaters reddish brown and bispiral or trispiral.

..... *C. cavernarum*

4.2.1.4 *Asterella angusta* (Steph.) Kachroo. J. Hattori bot. lab. 19: 3. 1958

Specimen Accession No: 510260

Photo Plate No: 4 (E-H)

Thalli forming regular and irregular rosettes. Male and female plants in mixed clones and Thallus 12-25 mm long and 4 -6 mm broad, scales in two rows, appendage long lanceolate. Dioecious plants, ventral scales in two rows violet, pinkish with long appendages, prominent at apex. Rhizoids were tuberculated, smooth walled arising from ventral midrib. Surface cells polygonal, pores simple, large and air chambers of 2-3 layers. Antheridia in raised cushions on dorsal surface of the thallus or in patches; female receptacles hemispherical or disciform, stalked, terminal 4-8 lobed. Sporogonium with bulbous foot, short seta and spherical capsule. Spores dark brown rarely branched with single spiral 185-245 µm long.

Field notes: Plants grow on moist rocks crevices, on walls of old houses, or even on moist soil on ground.

Locality: Belkund, Amazari, Chikhaldara

Distribution: Shimla, Mussoorie, Darjeeling, Cherapunji, Pachmarhi, Sagar, Mahabaleshwar, Lonavala, Western Ghats, Amboli, Rajasthan, Gujarat, Nepal, Dalhousie and Ranikhet.

Key to the family

Air chamber into several layers without assimilatory filament; presence of stalked female receptacle; capsule wall without thickening..... *Aytoniaceae*

Key to the genera

Male receptacle naked, disc shaped or cushion like at an apex small female receptacle terminal on main shoot, stalked with rhizoidal furrow..... *Asterella*

Key to the species

Plant dioecious, male receptacles dorsal while female receptacles terminal on separate thalli. *angusta*

4.2.1.5 *Reboulia hemisphaerica* (Linn.) Raddi Opuse. Sci di. Bologna 2: 357. 1818; K Mull, Robenn. Krypt. Fl. 6: 256. 1940; Liverw. W. Himalayas Punjab Pl. 1:72. pl. 15(1-5) 1929.

Specimen Accession No: 510261

Photo Plate No: 5 (A-D)

Thallus 10-30 mm long, greenish or purple on ventral side lobes with emarginated or bilobed apex, pores little elevated with 3 -5 concentric rings of 6-8 cells each, radial walls thickened, scales purple with one row on each side of midrib, with two linear appendages. Male receptacle at apex of lobe, disciform surrounded by chafty scales. Female receptacle were hemispherical 4-9 lobed, Spores are brown, reticulate, lamellate with broad wing, 65-75 µm; elaters 2-3 spirals, coiled, 300-400 µm and rarely branched.

Field notes: Plants growing on moist rock crevices on wet soil often mixed with *Plagiochasma* or *Anthoceros* spp. Many times purple coloured pigmentation found on ventral as well as dorsal surface.

Locality: Amazari, Chikhaldara - Semadoh Road, Semadoh - Chikhaldara road.

Distribution: Mussoorie, Dalhousie, Shimla, Kulu, Khasi Hills, Kashmir, Darjeeling, Pachmarhi, Mahabaleshwar, Western Ghats, Nepal, Pakistan, Java, Korea, Japan, America, Europe and Africa.

Key to the species

Male receptacle disciform, female receptacle hemispherical 4 - 9 lobed. Spores reticulate 65-75 µm in diameter, spiral elaters. *R. hemisphaerica*

4.2.1.6 *Plagiochasma appendiculatum* Lehm *et.* Lindenb, Nov. minus cong. strip pug. 4: 14 1832; Kash., Liverw. W. Himalayas Punjab Pl. 1: 76 Pl. 16 (4-7). 1979; Kachroo, J. Hattori bot. Lab. 12: 45 F. 7 (A-R) 1954.

Specimen Accession No: 510262

Photo Plate No: 5 (E-H)

Plants generally monoecious; thalli lusty green, in large dense patches, usually 10-30 mm long and up to 10 mm broad, plant growing in moist places. Thalli dichotomously branched, lobes long, smooth on dorsal surface. Air chambers small in many rows without assimilatory filaments, partition walls one celled thick bearing chloroplast. Male receptacles horse-shoe shaped or lobed, protected by small purple scales, antheridia club shaped, develop acropetally in each lobe, pores on receptacle simple, position of receptacle variable. Male receptacle born in groups or after one another. Female receptacle sessile or short stalked usually without rhizoidal furrow, 2 to 5 lobed. Capsule with a definite lid, spores 74-85 μm in diameter, rounded, light brown, with a few large, thick in complete reticulations, tri-radiate mark distinct, elaters bispiralled, 100-200 μm long, simple or branched.

Field notes: The species found growing on rocks, exposed fissures and muddy drains, and on moist soil floor surface. When growing in moist, wet protected or undisturbed areas, the plants grow opulently, become larger and sterile. The species tolerates a wide range of habitat variations.

Locality: Chikhaldara, Semadoh, Belkund, Kolkhas, Aamazari, Ghatang.

Distribution: Jammu, Shimla, Nainital, Mussoorie, Saharanpur, Deharadun, Gharwal, Gauhati, Shillong, Kolkata, Pachmarhi, Mahabaleshwar, Khandala, Western Ghats, Nilgiri Hills, Rajasthan, Gujarat, Nepal, Pakistan, Manila, China, Taiwan, Philippines, Kenya and Yemen.

Key to the species

Scales appendiculate, large, broad, ovate, strongly constricted at base, elaters bi or trispiral. *P. appendiculatum*

4.2.1.7 *Plagiochasma intermedium* Lindenb. et. Gott. in Gott., Lindenb. and Nees, Syn. Hep. 513 (1846)

Specimen Accession No: 510263

Photo Plate No: 6 (A-D)

Monoecious, plants in dense patches, green 10-13 mm long and up to 5 mm broad; thalli plane, strap shaped, with a slightly wavy and crenate, purple margin. Thallus usually forked, growing point surrounded by incurved scales. Ventral scales in two rows, purple in colour, scales large, overlapping, lunate and appendiculate and margin of irregular cells. Rhizoids smooth and tuberculated and air chambers 2 - 4 layers in wings. Male receptacle sub-terminal or dorsal or on ventral lobe, horse-shoe shaped, encircled by scales, which are ovate to linear, apex acute and with marginal or terminal papillae. Female receptacle dorsal on thallus, sub-sessile, scales narrow, lanceolate with marginal or apical papillae. Spores often 70-90 μm brown, 1 - 3 alveoli at distal face, winged, elaters 200-271 μm long and 9 -14 μm wide.

Field notes: Plants generally found on old rocks, walls and on old forts, in creeping saxicolous habitat.

Locality: Gawilgarh fort at Chikhaldara and at Muktagiri temple walls.

Distribution: Nagpur, Mahabaleshwar, Khandala, Pachgani, Elephanta caves, Muktagiri, Pachmarhi, Bhopal, Sagar, Mt. Abu, Kota, Ranchi, Pathankot, Japan, Korea, Manchuria, China, Taiwan, Philippines, Mexico and Guatemala.

Key to the species

Scales appendages narrow, linear, slightly constricted at base, elaters lack spiral bands and found tubular. *P. intermedium*

4.2.1.8 *Plagiochasma rupestre* (Forst.) Steph. Spec. Hepat. 1 : 80 (1898)

Specimen Accession No: 510264

Photo Plate No: 6 (E-H)

Monoecious or dioecious, thallus creeping up to 2 mm. long and 2.5-5 mm broad, greenish white or bluish green, sometimes purple or more rarely red or brown. Pores simple minute, not elevated over surface, bounded by a ring of 4-5 cells. Scales red, cells of the margin not differentiated and devoid of papillae. Female receptacle in median part of thallus. Archegoniophore stalk of 0-4 mm long, epidermis tinged yellow, red or violet. Involucres 1-3, yellowish green or brownish, sometimes tinged red or violet. Elaters have many spirals, yellowish, orange or red spores 80-100 μm in diameter brown or dark brown in colour.

Field notes: Plants found on calcareous walls with *Hyophila involuta*, on old walls dominantly during rainy season and lustrous fruiting during September and October.

Locality: Gawilgarh fort, rock crevices at Chikhaldara and at Muktagiri temples.

Distribution: Ootacamund, Pondicherry, Gujarat, India Nepal, Srilanka, Taiwan, Iran, Greece, New Caledonia, Italy, Portugal, Tunisia, Turkey, Yugoslavia, Africa and South America.

Key to the species

Thallus velvety, bluish green or purple, creeping, cuticle covered by water repelling granular depositions, pores without hyaline rings. *P. rupestre*

4.2.1.9 *Riccia gangetica* Ahmad. Current Sci. 11.433. 1942. Pande and Udar, J Indian Bot. Soc. 36: 573. F. 33 - 44. 1957.

Specimen Accession No: 510265

Photo Plate No: 7 (A-D)

Plants generally monoecious, thallus bluish green, overlapping in patches or forming beautiful large well-defined rosettes. Thalli 1-3 times dichotomously branched, 2 to 14 mm long and 1-3 mm broad. Dorsal median groove prominent, lobes were ovate or linear. Rhizoids dense, simple and tuberculated while scales were hyaline or purple. Epidermal cells hyaline oval or spherical and thallus 2-3 times as broad as high. Antheridia and archegonia in 2-3 rows median line. Antheridial papillae, hyaline or pink, 150-180 µm projecting above the thallus, Archegonial neck slightly project above the surface. Capsule up to 500 µm in diameter. Spores tetrahedral, dark brown to black at maturity and 110-135 µm in diameter, with reticulation small 10-15 and margin dentate and crenate.

Field Notes: Plants grow on moist, wet, shady, or exposed soil on rocks, on coarse-grained soil.

Locality: Bhimkund, Churani, Semadoh-Paratwada Road, Makhala, and Ghatang-Semadoh Road.

Distribution: Western Himalayas, Gharwal, Assam, Shillong, Uttar Pradesh, Mahabaleshwar, Khandala, South India, Nilgiri Hills, Rajasthan and Gujarat.

Key to the Families

Presence of chlorophyll bearing assimilatory filaments; sporogonium without foot and seta. **Ricciaceae**

Key to the Species

Plant monoecious, spores unwinged thalli bluish green and broader. Dorsal groove present, spores 85-135 µm black, opaque, 10-16 reticulations across the outer face. ***R. gangetica***

4.2.1.10 *Riccia discolor* Lehm. et. Lindenb., Pugil. 4:1, 1832; Udar, Current Sci.

26:20. F. 1-4. 10-11, 1957; Pande and Udar J. Indian bot. soc. 36:567. F.1-16. 1957.

Specimen Accession No: 510266

Photo Plate No: 7 (E-H)

Plants generally dioecious, plants overlapping in dense patches, forming rosettes. Thalli green, one or twice forked and lobes oblong. Female plants larger than the male plants up to 15mm long and up to 7mm broad. Male thalli found smaller, 2-8 mm long and 2-5 mm broad with a groove that is narrow at apex and flat. Air spaces narrow and assimilatory filaments 6-8 cells high. Ventral surface bears simple and tuberculated rhizoids. Antheridia 1-3 in median rows, globular or slightly elliptical, up to 95 µm and turning violet in the middle after fertilization. Capsule in 1-2 rows, black at maturity, spores dark brown, 80 to 120 µm in diameter, reticulate, 6-10 areoles on the outer face and triradiate mark in conspicuous.

Field notes: Plant grow on moist soil or rocks in exposed situation dominantly during rainy seasons.

Locality: Churani, Chati-bilta, Muktagiri, Chikhaldara, Khongada fata.

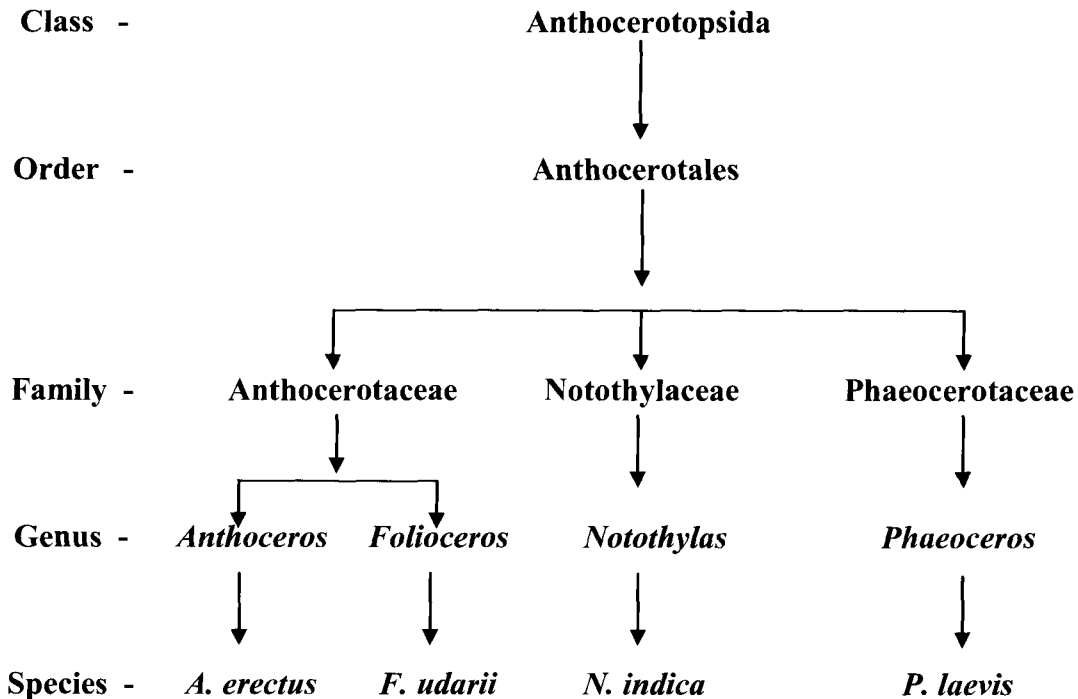
Distribution: Satpura, Maharashtra, Nainital, Mussoorie, Guwahati, Shillong, Sagar, Rajasthan, Coimbatore, Mysore, South India, Wayanand, Burma, Srilanka, Pakistan and Africa Continent.

Key to the Species

Plant without cilia, plants dioecious, spores tetrahedral, 35-120 µm, 6-10 reticulations across the outer face, triradiate mark inconspicuous.

..... ***R. discolor***

Fig: 4.2.2 A detailed outline classification of Hornworts collected from Melghat region followed by Asthana and Srivastava (1991)



4.2.2 Anthocerotopsida

Key to the class

Plant thalloid, single chloroplast per cell; capsule wall with stomata and chloroplast; capsule open by splitting into two valves. *Anthocerotopsida*

Key to the order

Class Anthocerotopsida includes a single order so the characters are same as above. *Anthocerotales*

Key to the family

Thallus spongy with schizogenous cavities; jacket cells of antheridium arranged in four tiers; spores dark coloured, brown or black.
Anthocerotaceae

Key to the genera

Proximal faces of spore with distinct and bold tri-radiate mark, pseudo elaters 1-4 celled short and thin walled. *Anthoceros*

Key to the species

Thallus harrowing at base and broader towards apex without spongy bodies at the apical margin; spores reticulate, blackish coloured..... *erectus*

4.2.2.1 *Anthoceros erectus*, Kash. New Phytol, 14 : 9-21, 1915; Liverw., 1:25, Pl. Im 14, 1929; Mehara and Handoo., Bot. Gaz., 114, (4) : 371-382, F.4-7, 9.13, Pl.15-16, 12, 19-21, 1960.

Specimen Accession No: 510267

Photo Plate No: 8 (A-D)

Plants generally dioecious, male thalli up to 4mm long, raised on cylindrical stalk and fanning at apex. Androecial chambers scattered, raised like humps on dorsal surface of thallus with 20-35 antheridia, while antheridial body up to 160 µm long. Female thalli up to 6 mm long, wide at apex with lobed and dissected margin. Epidermal cells reniform with their radial and end walls uniformly thickened. Spores dark, reticulate, 40-60 µm in diameter, thick usually 4-5 across the diameter on distal face. Elaters light brown, thin walled, 162-225 µm long.

Field notes: Often growing on moist soil, rocks, or slopes in hills. Some times in exposed situations and on alluvial deposits of creek banks or near river, ponds, or water streams.

Locality: Chikhaldara, Ghatang-Semadoh Road, Aamazari, Belkund, Khongada, Very common in Melghat forest.

Distribution: Maharashtra, Mahabaleshwar, Pachgani, Kaas plateau, Pachmarhi, Kulu, Manali, Shimla, Dalhousie, Darjeeling, Mt. Abu, Sikkim, Chennai, Travancore and Srilanka.

4.2.2.2 *Folioceros udarii* Asthana *et* Srivastava, Cryptogramie, Bryol, Lichenol 7(2): 149- 154, 1986.

Specimen Accession No: 510268

Photo Plate No: 8 (E-H)

The plants found were dioecious. Thallus broadly expanded and narrow at base, dorsal surface smooth, 4mm long, semi transparent during heavy rains, yellowish green in colour with greenish sporophytes. Antheridia scattered in the median portion, 13-20 rounded projecting on the surface. Female thalli 25-30 mm broad, lobed, cavernous, cells with single chloroplast and pyrenoids. Involucres up to 8 mm long, cavernous. Capsule wall found stomatiferous. Spores 27-35 µm, brown to

dark brown, spinose, triradiate, elaters vermiform 287-360 μm long, thick walled with narrow dark lumen and four celled.

Field notes: Plants found on moist and wet rocks, old house walls, and bridges exposed to light.

Locality: Belkund, Chikhaldara, Vairat, Semadoh.

Distribution: Pratapgarh, Mahabaleshwar, Khandala, Western Ghats, Amboli, Sawantwadi, Satara, Kaas Plateau, Kerala.

Key to the species

Plants dioecious, male thalli broad and female thalli expansive, spongy, spores, brown, spinose, Elaters vermiform. ***F. udarii***

4.2.2.3 *Notothylas indica* Kash. Proc. Lahore Philosoph. Soc. 4:54, 1925; Liverw. W. Himalayas.

Specimen Accession No: 510269

Photo Plate No: 9 (A-D)

Plants light green, prostrate repeatedly dichotomously branched forming orbicular or sub orbicular rosettes, 15-16 mm in diameter. Dorsal epidermal cells quadrate to sub-quadrate towards apex. Plate like lobed chloroplast with central pyrenoid complex. *Nostoc* colonies bluish green, spherical, ellipsoidal, irregularly distributed in thallus. Plant monoecious, protogynous, androecia present in groups 2 or 3 along the anterior margins of young thalli bearing juvenile sporophyte. Sporangia exerted 1-2 in each involucre of 2-5-4 mm long, differentiated into a large bulbous foot, a small basal meristem. Capsule wall 3-5 layers thick and cells of epidermal layer are deep brown. Columella well developed and spores found brown or yellow brown, tetrahedral or oval of 33-45 μm with granular substances. Sterile cells 45 x 33 μm found with brown bands of thickening.

Field notes: Plants grow on moist soil in patches or on rock crevices with shade loving habitat. The floor bed in garden also shows association with mosses.

Locality: Semadoh-dominant vegetation, Chikhaldara, Belkund, Tarubanda, Koha.

Distribution: Konkan, Satpura, Mahabaleshwar, Chennai, Allahabad, Lucknow, Mt. Abu, Pachmarhi, Deharadun, Mussoorie, Matheran.

Key to the family

Thallus compact, without schizogenous cavities, antheridium irregularly arranged; capsule horizontal, determinate, marginal in position, entirely enclosed within involucre until maturity; elaters short and stumpy, spores yellow or light coloured.

..... **Notothylaceae**

Key to the genera and species

Thalli with dorsal ridge, occasionally lamellate; cells of the epidermal layer of capsule with stratified sheet-like thickening; spores 48.6-66 µm with prominent denticulate flange and without a distal hump..... ***N. indica***

4.2.2.4 *Phaeoceros laevis* (Linn) Prosk. Subsp. *laevis* Prosk. et. Conm. VIII Cong. Intern. Bot. Paris. 14: 69. 1954. Bill. Torrey bot. club. 78:347. F.4, 21-27, 35, 1951; in K. Mull. Rabenh. Krypt. Fl. ed. 3.6:1313, 1958.

Specimen Accession No: 510270 Photo Plate No: 9 (E-H)

Plants found dioecious. Thalli lobed up to 15 mm long and 13 mm wide at apex. Gametophyte without large schizogenous cavities. Upper epidermis showed single chloroplast per cell, chloroplast ring shaped, 19-25 µm across pyrenoid bodies. Antheridium consists of many small cells, globose without tiered jacket cells. Epidermis with stomata having two guard cells. Sporogonium with persistent intercalary meristem and columella present. Spores found were yellowish 30-40 µm, in diameter, translucent yellow, outer papillose and inner face smooth. Elaters light yellow, 156-215 µm long, thin walled, may be 4 celled or 5 celled or rarely branched.

Field notes: Plants grow on moist soil with association of liverworts like *Plagiochasma appendiculatum*. Yellowish sporophytes at apical regions made a distinct character.

Locality: Semadoh - Ghatang Road, Makhala, Raipur at shady and moist places.

Distribution: Mahabaleshwar, Pachgani, Kaas plateau, Pachmarhi, Kodaikanal, Mussoorie, Darjeeling, Gangtok, Nepal, Pakistan, Japan, Taiwan, China, N. America and Europe.

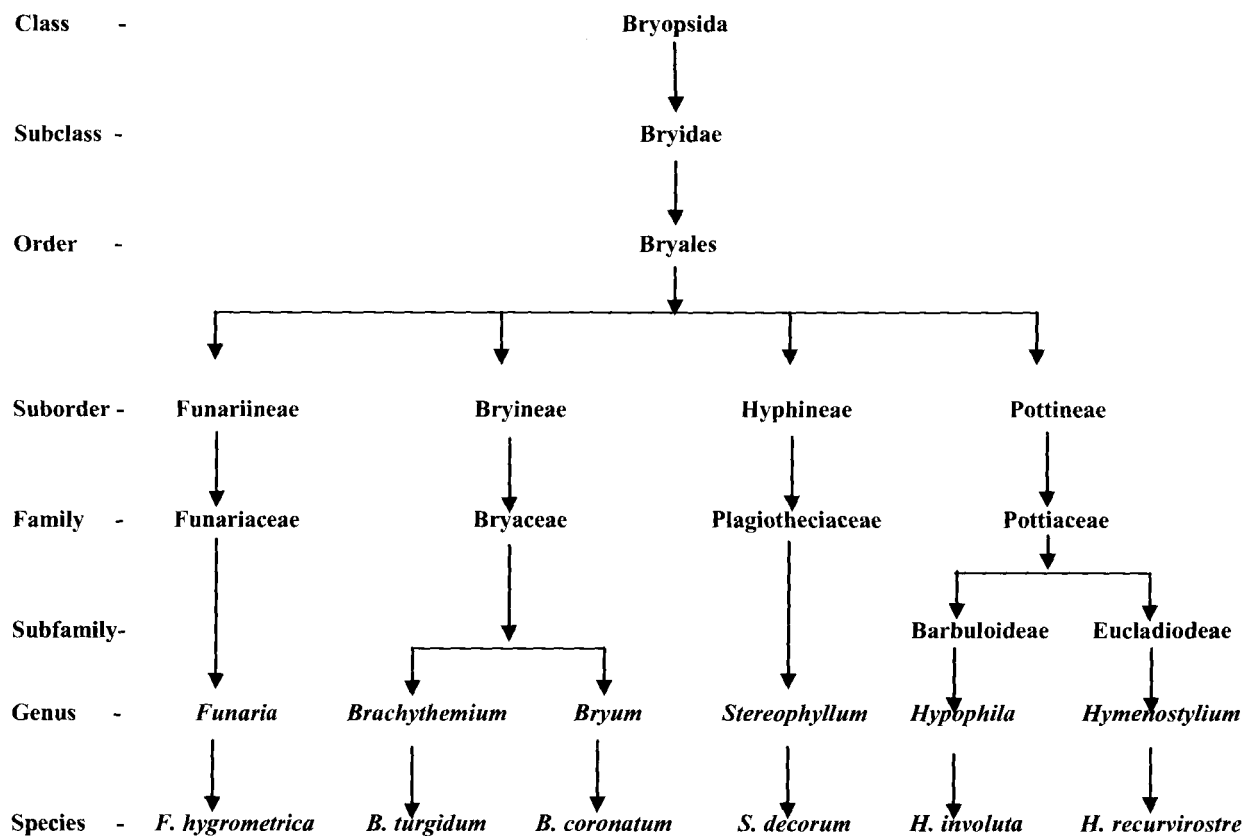
Key to the family Capsule erect, intermediate, dorsal in position on thallus, almost entirely projected out of the involucre at maturity; elaters long entirely distinctive in size and form not similar to spores. **Phaeocerotaceae**

Key to the genus and species

Sporoderm with hump like projections or lamellate markings, dioecious, proximal face of the spore with thick tri-radiate mark having short tubercles on it.

..... **Phaeoceros laevis**

Fig: 4.2.3 A detailed outline classification of Mosses collected from Melghat region followed by Goffinet *et al.*, (2008)



4.2.3 Bryopsida

Key to the class

Gametophyte leafy; leaves spirally arranged usually with costa; Rhizoids multicellular with oblique seta; capsule opens irregularly or by 4 longitudinal slits or by operculum; elaters absent. **Bryopsida**

Key to the subclass

Plant differentiated into stem, leaves and root like structure, rhizome with multicellular rhizoids; stem erect or creeping, seta present, capsule dehiscing by operculum mostly; peristome present. **Bryidae**

Key to the order

Protonema filamentous, capsule open by lid, peristome present and teeth articulated. **Bryales**

Key to the sub-order

Mostly annual or biennial mosses, leaves in rosettes terminally; sporogonia acrocarpous; capsule never cylindrical and peristome free. **Funariineae**

Key to the family

Stem with central strand, Upper leaves rosette forming; Mostly Autoecious; Male discoid with paraphyses and female sub terminal, seta elongated, capsule pyriform, pendulous. **Funariaceae**

4.2.3.1 *Funaria hygrometrica* Hedw., Spec. Musc. 172, 1801.

Specimen Accession No: 510271

Photo Plate No: 10 (A-D)

Plants found in loose to compact tufts, in large patches, green to yellowish green, simple and branched. Stem slender, erect and 5 to 10 mm high. Lower leaves found small, costa poorly developed and upper leaves large crowded at apex and upper cells sub-hexagonal, elongated, 9-20 μm long and 9-10 μm wide, middle cells 9-14 μm long, 10-13 μm wide and basal cells 14-19 μm wide and slightly narrower at margin. Seta found erect 2.2 cm long, terminal and reddish on maturity. Capsule horizontal to pendulous, curved, pyriform, oblique, and globose at back. Operculum large convex, mouth wide and bear two rows of teeth. Outer and inner teeth obliquely arranged in a spiral turn, and each row of 16 teeth transversely barred. Calyptra persistent, cucullate and spores rounded brown, with 25-26 μm in diameter.

Leaf: Lamina unistratose, cell thin walled and continuous over mid rib. Marginal cells narrow thick walled and midrib several layered thick.

Stem: Circular in outline with central strand of few thin walled cells. i.e. cortex.

Field notes: Plants grown on moist rocks, cemented old walls, bridges, and bricks and found along with *Hyophila involuta*. It is also growing epiphytically on bark of *Ficus bengalensis* L.

Locality: Gugamal forest gate, Semadoh, Chikhaldara-Ghatang Road, Aamazari, Raipur, Makhala.

Distribution: Cosmopolitan occurring worldwide.

Key to the genera

Autoecious, Calyptra obliquely placed, not lobed, operculum flat or convex; spores large..... *Funaria*

Key to the species

Plants in tufts, patches, green or yellowish, reddish on maturity, capsule pendulous, pyriform, operculum large, spores 14-15 μm in diameter.

..... *hygrometrica*

4.2.3.2 *Brachymenium turgidum* Broth. ex. Dix. Rev. Bryol. 35.94 : 1908.

Specimen Accession No: 510272

Photo Plate No: 10 (E-H)

Plants growing on trees, corticolous forming cushions with innovations at the apex, growing on branches. Plants erect 1-2 cm and high resolute in appearance. Leaves narrow, twisted when dry, leaf margin with extremely narrow border. Upper leaf cells 47-67 μm long, 14-20 μm wide, middle leaf cells 57-72 μm long, 19-23 μm wide. Leaf base cells 38-42 μm long, 23- 29 μm wide and elongated. Capsules erect with longer seta, turgid, oval and rounded. Spore sac becomes membranous in mature capsule. Spores globose, oval brown, 40 μm to 50 μm in diameter with minute papillae.

Leaf: Lamina cells more or less columnar, smooth thin walled continuous over midrib.

Stem: Irregularly sinuate in cross section, 9-10 cells thick in diameter and central strand and cortex present.

Field notes: Plant grow epiphytically on the bark of *Terminalia chebula* Retz, *Pterocarpus marsupium* Roxb. in addition, on certain palms or on garden trees.

Locality: Chikhaldara garden, Gawilgarh fort.

Distribution: Mahabaleshwar, Khandala, Pachgani, Kaas Plateau, Satara, Western Ghats, South India, Gujarat, Lingmala.

Key to the suborder:

Biennial or perennial mosses, stem circular, leaves orbicular or lanceolate; Sporangia terminal; capsules mostly inclined; Peristome well developed, teeth 16.

..... **Bryineae**

Key to the family

Leaf cells almost invariably smooth, isodimetric, more or less rhomboidal or linear or vermicular. Capsule (dry) smooth, Peristome teeth hygroscopic.

..... **Bryaceae**

Key to the genera

Leaves broader, capsule symmetrical, Peristome, processes mostly rudimentary; cilia rudimentary to absent. **Brachymerium**

Key to the species

Capsule large, oval, and turgid, spores oval, globose, brown, leaves narrow bordered. **B. turgidum**

4.2.3.3 *Bryum coronatum*. Schwaegr. Spec. Musc. Suppl. 1(2): 103 (1816)

Specimen Accession No: 510273

Photo Plate No: 11 (A-D)

Plants densely tufted, slender, bright to dull green, tomentose at base. Stem often found branched from base up to 1.5 cm high, with central erect, lightly contorted when dry, acuminate, and 2-3 mm long. Upper leaf cells narrow rhomboid to hexagonal 42-53 μm long and 14-20 μm wide. While the middle cells found 47- 58 μm long and 23-29 μm wide. Perichaetial leaves shorter, triangular, seta apical, erect, and acute at tip red to purple 2-3 cm long. Capsule pendulous, thick and 2 mm long in diameter. Operculum big and conical with thick wall. Spores 6 μm to 14 μm in diameter.

Leaf: Midrib 8-10 cells thick centrally thickened, with two layers.

Stem: Generally circular in cross section with irregular margin with 12-13 cells thick in diameter.

Field notes: Plants grow on walls, bridges, on rocks. Also found on ash of forest fire.

Locality: Dominantly found across the Semadoh – Paratwada and Semadoh-Chikhaldara road and adjoining areas or sites from monsoon to winter.

Distribution: Cosmopolitan, throughout India, Thailand, Taiwan, Japan, and Philippines.

Key to the genera

Plants small, compact tufts, gregarious, stem tomentose, leaves distant capsule pyriform, operculum distinct conical. **Bryum**

Key to the species

Costa ends in large (.5 mm) aristae; margin flat and entire. **B. coronatum**

4.2.3.4 *Stereophyllum decorum* (Mitt.) Wijk et. Marg., In Taxon 9:52 (1960)

Specimen Accession No: 510274

Photo Plate No: 11 (E-H)

Plants medium sized yellow green and glossy. Main stems creeping, 1.9 cm long, leaves dense; often bend to one side, erectopate. Costa strong, covering about leaf cells linear at apex. Apical cells 57-90 μm long and 5-9 μm wide, middle cells 104-115 μm long and 19-47 μm wide, and basal cells 19-34 μm long and 18-23 μm wide. Sporophyte present on main stem. Seta generally found elongated, red and smooth. Capsule erect or inclined and symmetrical with normal peristome.

Leaf: Lamina unistratose, cells squarish or rectangular, thin walled and midrib of 2-3 layered thick cells.

Stem: Ground tissue found in two zones i.e. inner 4-5 layered thin and outer 12-layered thick walled regions.

Field notes: Plants found growing epiphytically on the bark of *Ficus glomerata* Roxb., *Ficus virens*, *Carissa carandus* L, *Mangifera indica* L.

Locality: Ghatang - Chikhaldara Road, Bori, Makhala, Raipur, Koha

Distribution: Matheran, Trimbakeshwar, Bhimashankar, Malshej Ghats, Kaman, Rajasthan, Gujarat and East Nepal.

Key to the suborder

Plants terrestrial and corticolous, leaves symmetrical, pleurocarpic, radiculose.

Capsule erect to inclined, peristome well developed. **Hypninae**

Key to the family

Alar cells few and inconspicuous. Leaf cells firm walled, narrow, and more or less elongated. Leaves strongly complanate, leaves decurrent; cells oval-rhomboid or linear. **Plagiotheciaceae**

Key to the genera and species

Costa 2/3 of the leaf length; leaves ovate-lanceolate, leaves erectopatent, seta elongate red, capsule erect, peristome normal. ***Stereophyllum decorum***

4.2.3.5 *Hyophila involuta* (Hook) Jaeg. Ber. S. Gall. Naturw. Ges. 1871-72: 356, 1873.

Specimen Accession No: 510275

Photo Plate No: 12 (A-D)

Plants found in dense tufts, simple or branched, dark green up to 9 to 10 mm high, Radiculose below, rhizoids reddish and sex organs terminal. Stem covered with erect spreading leaves, oblong lingulate, the lower part found pale, sheathing and erect, toothed above. Apical cells 4 x 4.77 µm long and 3 x 4.77 µm wide, middle cells 3-4 x 4.77 µm long and 3-4 x 4.77 µm wide. Basal cells are 8-9 x 4.77 µm long and 3-4 x 4.77 µm wide. Seta apical, reddish brown, erect, 2.0 mm and long. Capsule erect, cylindrical, brown, 1.0 mm long without peristome. The spores are rounded, brownish, smooth and 10-15 µm in diameter.

Leaf: Lamina unistratose, quadrate to rectangular continuous over midrib.

Stem: Ovate in cross section, central strand with thin, narrow, and compactly arranged cells.

Field notes: Plant found growing on rocks, soil, on cement bridges, old bricks, walls.

Locality: All over Melghat, especially Chikhaldara, Semadoh, Ghatang, Tarubanda, Belkund, Koha.

Distribution: Cosmopolitan (Very common)

Key to the suborder

Mostly earth or rock mosses, leaves variable in shape, mostly non-bordered.

Peristome often with basal membrane..... **Pottineae**

Key to the family

Plants small, in tufts, stem with central strand, leaves heterogenous, capsule cleistocarpous, peristome with teeth twisted, operculum conical-rostrate calyptra cucullate..... **Pottiaceae**

Key to sub family

Caespitose, sometimes sturdy plants, leaves lanceolate, spatulate, lingulate from a broader base. Leaf margin rolled; lamina cells papillose. Peristome present.

..... **Barbuloideae**

Key to genera

Peristome absent; leaf cells mamilllose. Leaves lingulate or spatulate; perichaetial bracts slightly or not sheathing. **Hyophila**

Key to species

Upper leaf margin serrulate; leaf apex broadly pointed..... **H. involuta**

4.2.3.6 *Hymenostylium recurvirostre.* (Hedw) Dix. Rev. Bryol. Licn. 6.9631933.

Specimen Accession No: 510276

Photo Plate No: 12 (E-H)

Plants found slender, in compact tufts, stem elongate foliate, radiculose, branched and rust coloured with 1.5 to 2 cm high. Leaves incurved, flexuse when dry, widely spreading, not crisped but erectopotent, linear lanceolate and acuminate when moist with 1 to 2 mm long. Upper laminar cells chlorophyllose, quadrate to sub quadrate, smooth ranging 15 to 17 μm in diameter. Leaf base cells found rectangular to sub rectangular, 25 μm long and 15 μm broad. Seta found slender smooth erect and about 0.6 to 1 cm long. Sporogonia found terminal and capsule wide mounted, short, broadly ovoid, glossy and peristome absent. Operculum lid found with an oblique, subulaterostrate beak and spores round, slightly papillose with 14 to 16 μm in diameter.

Leaf: Leaf margin recurved on one side below, Nerve nearly procumbent ceasing just below apex.

Field notes: Plants grows luxuriantly on rocks in tufts.

Locality: Gawilgarh fort dominant vegetation on rocks, on roadsides and valleys.

Distribution: Common in South-East Asian countries, Mahabaleshwar, Khandala, Western Himalayas, Kangra, Ladakh, Kashmir, Darjeeling, Tibet, Nepal, Philippines and China.

Key to the sub-family

Leaves lanceolate or subulate, crisped when dry, and cells mostly papillose. Sporoangia acrogenous and lateral. Peristome absent, seldom fully developed operculum long and obliquely prostrate..... **Eucladioideae**

Key to the genera and species

Leaf margin plane or slightly bent. Operculum shorter, Sporangia terminal, leaves not crisped when dry and lanceolate when moist.

..... ***Hymenostylium recurvirostre***

Table: 4.2.1 Distribution of Bryophytes across the Melghat region.

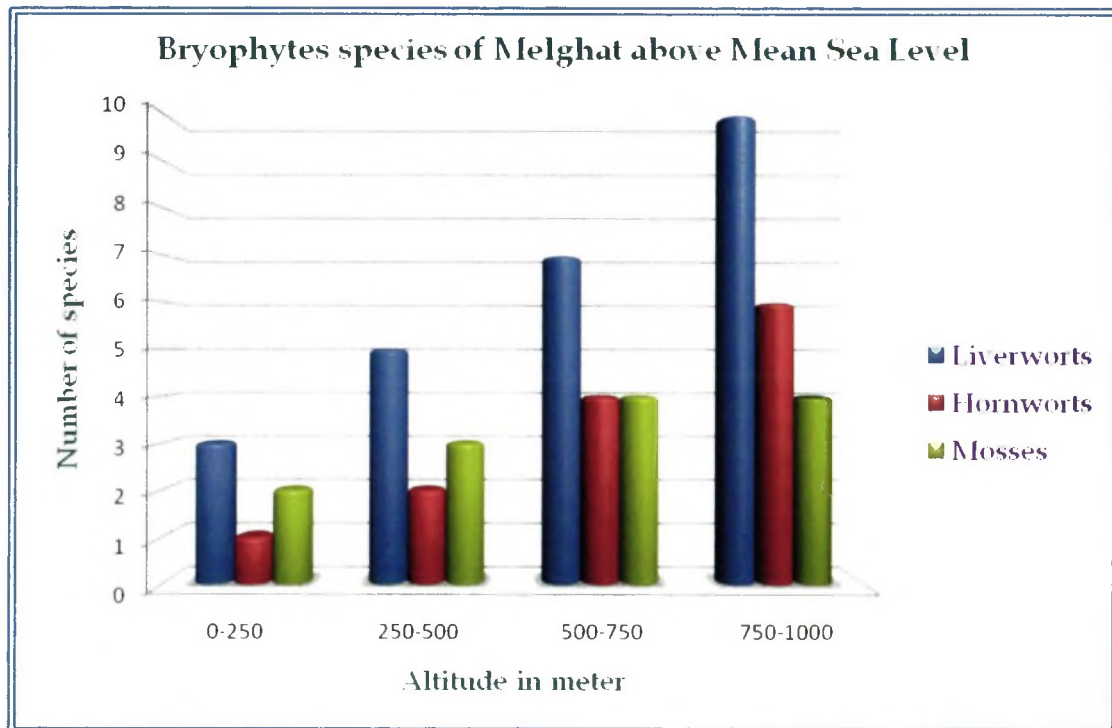
Bryophytes collected from the different localities are as follows.

Sr. No.	Bryoecological Zones	Distribution of species
1.	Ghatang	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Hyophila involuta</i> (Hook) Jaeg.,
2.	Semadoh	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i> Asthana. et. Srivastava., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,
3.	Makhala Forest	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. <i>Anthoceros erectus</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,
4.	Raipur	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,

5.	Kolkhas	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,
6.	Tarubanda-Koha	<i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Anthoceros erectus</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,
7.	Belkund	<i>Targionia hypophylla</i> Linn., <i>Cyathodium cavernarum</i> Kunze., <i>Asterella angusta</i> (Steph.) Kachroo., <i>Reboulia hemisphaerica</i> (Linn.) Raddi., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., 11. <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i> Asthana.et.Srivastava., <i>Notothylas indica</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
8.	Khongada-Parsapur	<i>Targionia hypophylla</i> Linn., <i>Cyathodium cavernarum</i> Kunze., <i>Asterella angusta</i> (Steph.) Kachroo., <i>Reboulia hemisphaerica</i> (Linn.) Raddi., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., 11. <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i> Asthana.et.Srivastava., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg.,
9.	Gugamal-Memna	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Brachymenium turgidum</i> Broth. ex. Dix., <i>Bryum coronatum</i> Schwaegr., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et.Marg., <i>Hyophila involuta</i> (Hook) Jaeg.,
10.	Chikhaldara Plateau	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., Kachroo., <i>Reboulia hemisphaerica</i> (Linn.) Raddi., <i>Plagiochasma</i>

		<i>appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma intermedium</i> Lindenb.et. Gott., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i> Asthana.et.Srivastava., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Bryum coronatum</i> Schwaegr., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et.Marg., <i>Hyophila involuta</i> (Hook) Jaeg., <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
a)	Chikhaldara Garden (Dominant Plants)	<i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg., <i>Brachymerium turgidum</i> Broth. ex. Dix. <i>Plagiochasma rupestre</i> (Forst.) Steph.
b)	Gawilgarh fort (Dominant Plants)	<i>Plagiochasma intermedium</i> Lindenb.et. Gott., <i>Cyathodium tuberosum</i> Kash., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
c)	Devipoint (Dominant Plants)	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Anthoceros erectus</i> Kash. <i>Plagiochasma rupestre</i> (Forst.) Steph
d)	Bhimkund (Dominant Plants)	<i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. <i>Riccia gangetica</i> Ahmad., <i>Anthoceros erectus</i> Kash. ., <i>Cyathodium tuberosum</i> Kash.,
11.	Vairat-Churani	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., Kachroo., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg., <i>Hyophila involuta</i> (Hook) Jaeg., <i>Hymenostylium recurvirostre</i> (Hedw.) Dix. <i>Hyophila involuta</i> (Hook) Jaeg.,
12.	Madaki	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,
13.	Bori	<i>Asterella angusta</i> (Steph.) Kachroo., <i>Reboulia hemisphaerica</i> (Linn.) Raddi., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma intermedium</i> Lindenb.et. Gott., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i>

		<i>Asthana.et.Srivastava.</i> , <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Bryum coronatum</i> Schwaegr., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg., <i>Hyophila involuta</i> (Hook) Jaeg.,
14.	Amazari	<i>Cyathodium tuberosum</i> Kash., <i>Reboulia hemisphaerica</i> (Linn.) Raddi., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg., <i>Hyophila involuta</i> (Hook) Jaeg.,
15.	Salona	<i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash. <i>Hyophila involuta</i> (Hook) Jaeg., <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
16.	Paratwada to Chikhaldara Road (3 Km)	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia gangetica</i> Ahmad., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Notothylas indica</i> Kash., <i>Funaria hygrometrica</i> Hedw., <i>Brachymerium turgidum</i> Broth. ex. Dix., <i>Bryum coronatum</i> Schwaegr., <i>Stereophyllum decorum</i> (Mitt.) Wijk. et. Marg., <i>Hyophila involuta</i> (Hook) Jaeg.,
17.	Semadoh to Chikhaldara Road (18Km)	<i>Targionia hypophylla</i> Linn., <i>Cyathodium tuberosum</i> Kash., <i>Cyathodium cavernarum</i> Kunze., <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb., <i>Plagiochasma rupestre</i> (Forst.) Steph., <i>Riccia discolor</i> Lehm.et. Lindenb., <i>Anthoceros erectus</i> Kash., <i>Folioceros udarii</i> Asthana. et. Srivastava., <i>Notothylas indica</i> Kash., <i>Phaeoceros laevis</i> (Linn.) Prosk., <i>Funaria hygrometrica</i> Hedw., <i>Bryum coronatum</i> Schwaegr., <i>Hyophila involuta</i> (Hook) Jaeg.,

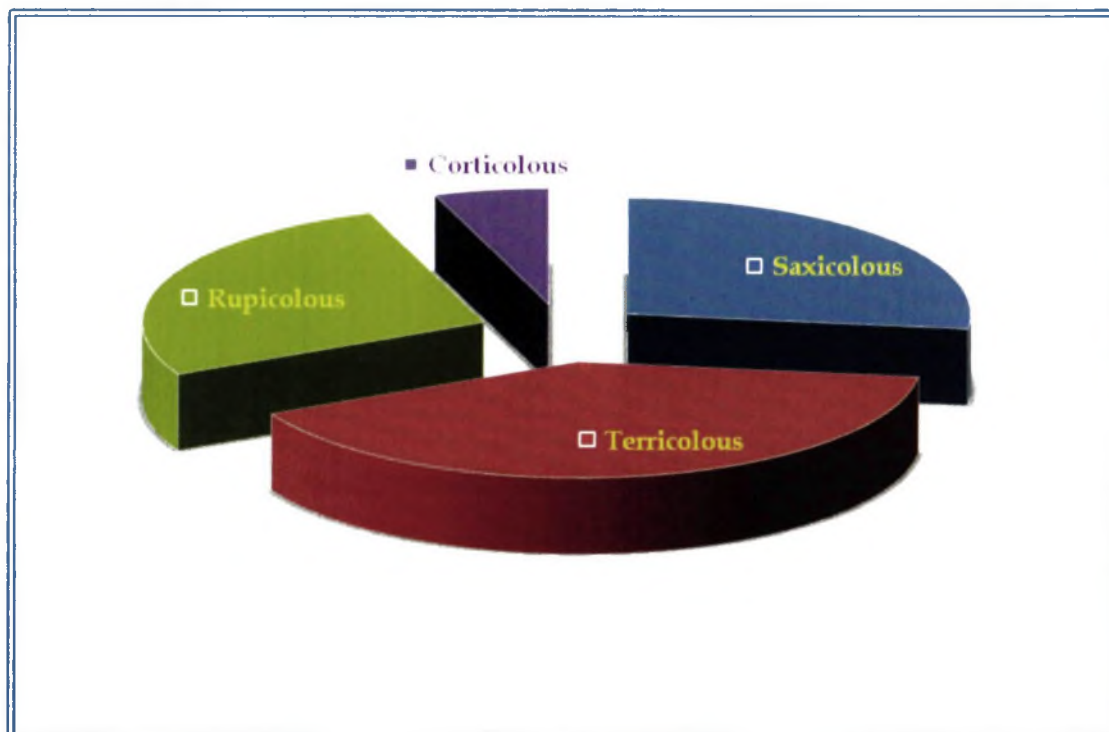
Fig: 4.2.4 Distribution of bryophytes species at various altitudes

4.2.4 Bryophytes distribution with reference to elevation

In Melghat region, the species richness and abundance varies from region to region and altitudes. The geographical features showed that the species richness and species diversity responds to different regions and localities. Maximum number of diversity of species found at above 750 m to 1000 m mean sea level where growth occurs luxuriantly due to ambience in favourable environmental conditions. In this region, the author recorded 10 liverworts, 5 hornworts and 6 mosses species. However, less density and diversity of species found at the elevation of below 250 m, which mostly comes under buffer regions. This region showed 3 liverworts, 1 hornwort and 2 species of mosses. It is noteworthy that, the moderate vegetation of bryophytes was found at elevation between 500 m to 750 m above mean sea level of Melghat forest. The region showed the presence of 7 liverworts, 4 hornworts and 4 members of mosses in abundance. Hence, variation in elevations responds differently to various geographical as well as environmental conditions that ultimately affect the bryoflora or vegetation of any region. Hence, the distribution of species or diversity of vegetation can be considered as directly proportional to the degree of higher elevation in the region of Melghat forest (Fig. 4.2.4).

Table: 4.2.2 Distribution of bryophytes in different habitats of Melghat region

Sr. No.	Name of the species	Saxicolous	Terricolous	Rupicolous	Corticolous
1	<i>Targionia hypophylla</i>	+	+	+	-
2	<i>Cyathodium tuberosum</i>	+	+	+	-
3	<i>Cyathodium cavernarum</i>	+	+	-	-
4	<i>Asterella angusta</i>	+	-	-	-
5	<i>Reboulia hemisphaerica</i>	+	+	-	-
6	<i>Plagiochasma appendiculatum</i>	+	+	-	-
7	<i>Plagiochasma intermedium</i>	+	-	+	-
8	<i>Plagiochasma rupestre</i>	-	-	+	-
9	<i>Riccia gangetica</i>	-	+	-	-
10	<i>Riccia discolor</i>	-	+	-	-
11	<i>Anthoceros erectus</i>	-	+	+	-
12	<i>Folioceros udarii</i>	-	+	-	-
13	<i>Notothylas indica</i>	-	+	+	-
14	<i>Phaeoceros laevis</i>	-	+	+	-
15	<i>Funaria hygrometrica</i>	+	+	-	-
16	<i>Brachythemium turgidum</i>	-	-	-	+
17	<i>Bryum coronatum</i>	-	+	+	-
18	<i>Stereophyllum decorum</i>	-	-	-	+
19	<i>Hyophila involuta</i>	+	+	+	-
20	<i>Hymenostylium recurvirostre</i>	+	+	+	-

Fig: 4.2.5 Distribution of bryophytes in different habitats

4.2.5 Distribution of bryophytes in different habitats

The Melghat bryophyte occurs at different habitats at different localities with response to edaphic, climatic and biotic factors. Liverworts mainly prefer terricolous and rupicolous habitats while hornworts dominantly found on soil substratum and few on saxicolous habitat. The mosses generally prefer and suitable for rocks, soil surface or even tree barks.

The liverworts *Targionia hypophylla* prefers terricolous, saxicolous and rupicolous habitat mainly at crevices of the rocks and on ground soil. The species *Cyathodium tuberosum* found on the rocks, on the soil and even on the small pebbles. However, *Cyathodium cavernarum* found on rocks near waterfalls or at the site of water percolation with saxicolous and terricolous habitat. The *Asterella angusta* and *Reboulia hemisphaerica* species are mostly saxicolous found on rocks at crevices, where shade and moisture is primary requisition and later needs light. The *Plagiochasma* species show dominantly saxicolous and rupicolous habitat in case of *P. intermedium* and *P. rupestre* while terricolous in *P. appendiculatum*. The *Riccia* species generally terricolous in habitat with few exceptions.

However, all the hornworts like *Anthoceros erectus*, *Notothylas indica*, *Phaeoceros laevis* and *Folioceros udarii* prefer rupicolous and terrestrial habitats.

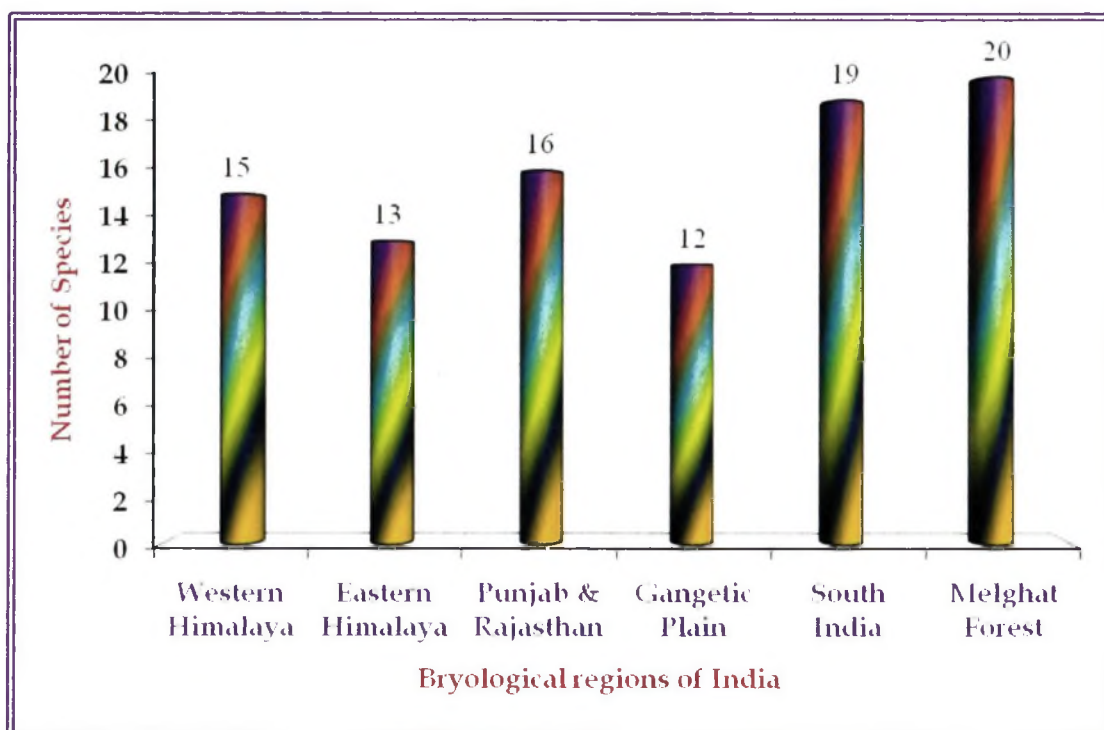
The mosses prefer a wide range of habitat due to their ability to survive in any habitat owing to specialized mode of nutrition. The species like *Bryum coronatum*, *Funaria hygrometrica*, *Hyophila involuta*, *Hymenostylium recurvirostre* are abundantly found on saxicolous, rupicolous and terricolous habitat while few corticolous mosses found on tress are *Brachythemium turgidum* and *Stereophyllum decorum* (Table 4.2.2).

Hence, out of 20 species of bryophytes, 10 species found at saxicolous habitat (50 %), maximum 15 species found on terricolous habitat (75%), near about 10 species found on rupicolous habitat (50%) and about 2 species of mosses are found on corticolous habitat (10%).

The bryophytes of Melghat region found mostly on terricolous, saxicolous and rupicolous habitat as compared to corticolous one (Fig. 4.2.5).

Table: 4.2.3 Distribution of bryophytes in Melghat region as compared with the Indian bryogeographical regions.

Sr. No.	Name of the species	Western Himalaya	Eastern Himalaya	Punjab & Rajasthan	Gangetic Plain	South India	Melghat Forest
1.	<i>Targionia hypophylla</i>	+	+	+	-	+	+
2.	<i>Cyathodium tuberosum</i>	+	+	+	+	+	+
3.	<i>Cyathodium cavernarum</i>	-	+	+	-	+	+
4.	<i>Asterella angusta</i>	+	+	+	+	+	+
5.	<i>Reboulia hemisphaerica</i>	+	+	-	-	+	+
6.	<i>Plagiochasma appendiculatum</i>	+	+	+	+	+	+
7.	<i>Plagiochasma intermedium</i>	-	+	+	+	+	+
8.	<i>Plagiochasma rupestre</i>	-	-	-	+	+	+
9.	<i>Riccia gangetica</i>	+	+	+	+	+	+
10.	<i>Riccia discolor</i>	+	-	+	+	+	+
11.	<i>Anthoceros erectus</i>	+	+	+	+	-	+
12.	<i>Folioceros udarii</i>	-	-	-	-	+	+
13.	<i>Notothylas indica</i>	+	-	+	+	+	+
14.	<i>Phaeoceros laevis</i>	+	+	+	-	-	+
15.	<i>Funaria hygrometrica</i>	+	+	+	+	+	+
16.	<i>Brachymenium turgidum</i>	-	-	+	-	+	+
17.	<i>Bryum coronatum</i>	+	+	+	+	+	+
18.	<i>Stereophyllum decorum</i>	+	-	-	-	+	+
19.	<i>Hyophila involuta</i>	+	+	+	+	+	+
20.	<i>Hymenostylium recurvirostre</i>	+	-	+	-	+	+
Total no. of species		15	13	16	12	19	20

Fig: 4.2.6 Comparison of bryophytes of Melghat with other Indian regions

4.2.6 Comparison of Melghat bryophytes with Indian bryogeographical regions

Pande (1958) divided India into six bryogeographical regions, namely the Western and Eastern Himalayas, Punjab and West Rajasthan, Gangetic plains, Central India, Deccan Plateau and the Western and Eastern Ghats. Here comparison with respect to species found in Melghat forest with the other parts of India is presented.

The Melghat forest comes under the region of Central India and shows affinity with South Indian part due to distribution and attachment of Maharashtra state. The occurrence of species *Cyathodium cavernarum*, *Plagiochasma intermedium*, *Folioceros udarii*, *Brachythemium turgidum* and *Hymenostylium recurvirostre* in Melghat makes difference between Western, Eastern Himalayas, and Melghat bryoflora. The presence of *Reboulia hemispherica* and *Plagiochasma rupestre* also makes the region unique as compared to Punjab and Rajasthan. The species like *Targionia hypophylla*, *Cyathodium cavernarum*, *Folioceros udarii*, *Stereophyllum decorum* etc. makes different the region as compared to Gangetic plains. However, most of Melghat bryophytes found to occur throughout the Indian regions (Table: 4.2.3).

The Melghat bryophytes resembles with the Central Indian and South Indian bryogeographical regions (Fig. 4.2.6).

PLATE - 3

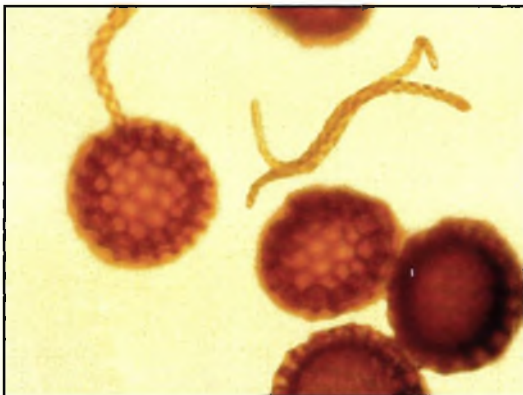
Morphological studies of *Targionia* and *Cyathodium* species



A) *Targionia hypophylla* Linn.



B) *T. hypophylla*, mature sporophyte



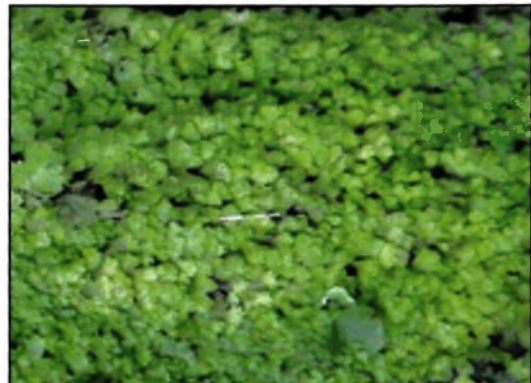
C) *T. hypophylla*, spores and elaters



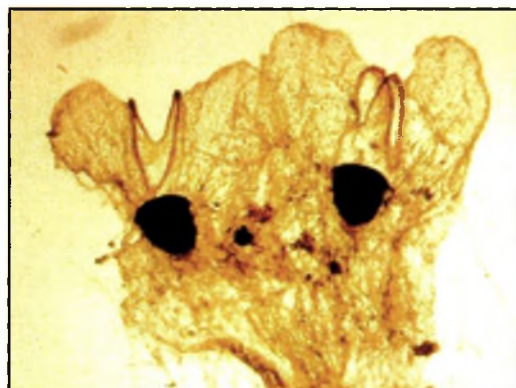
D) *T. hypophylla*, young sporophyte



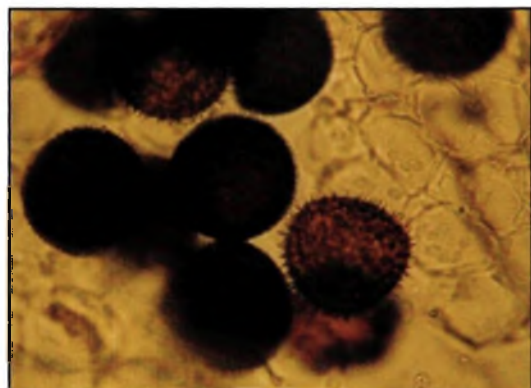
E) *Cyathodium tuberosum* Kash



F) *C. tuberosum*, thallus on rocks



G) *C. tuberosum* with capsule

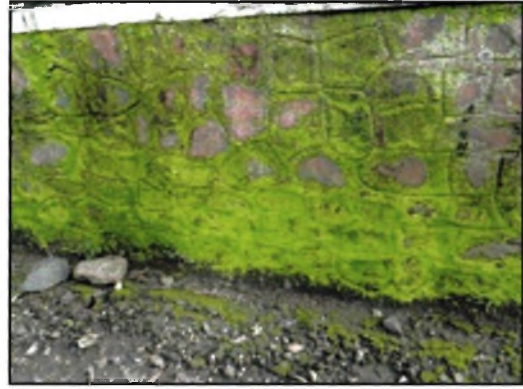


H) *C. tuberosum* with spiny spores

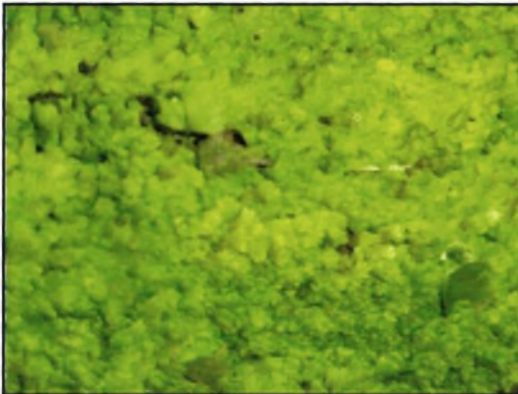
PLATE – 4
Morphological studies of *Cyathodium* and *Asterella* species



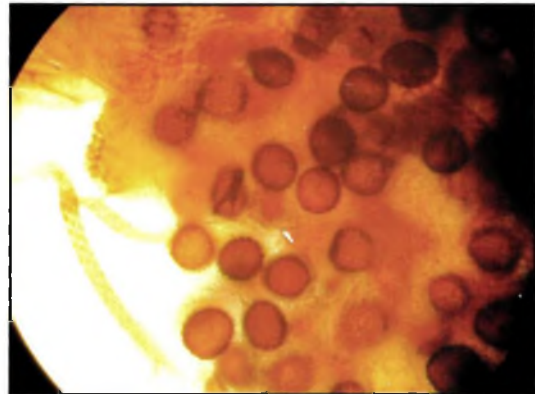
A) *Cyathodium cavernarum* Kunze.



B) *C. cavernarum* on wall



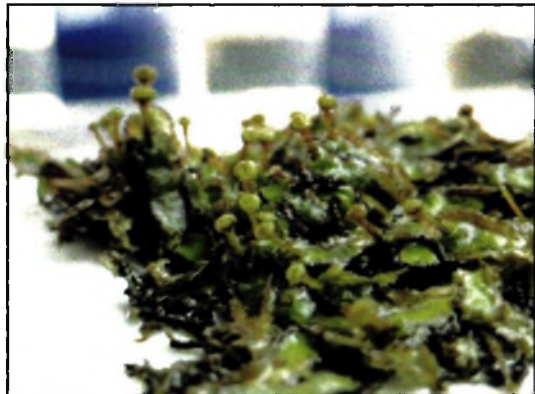
C) *C. cavernarum* fluorescent thallus



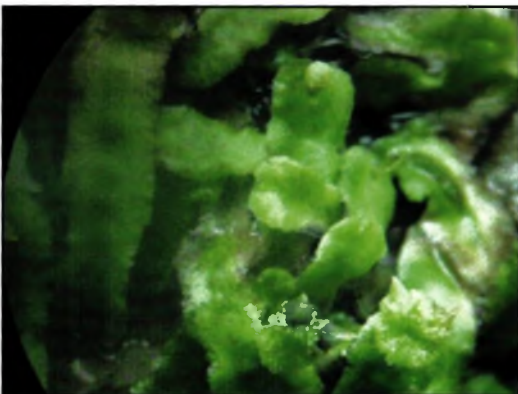
D) *C. cavernarum* spores & elaters



E) *Asterella angusta* (Steph.) Kachroo



F) *A. angusta* young sporophyte



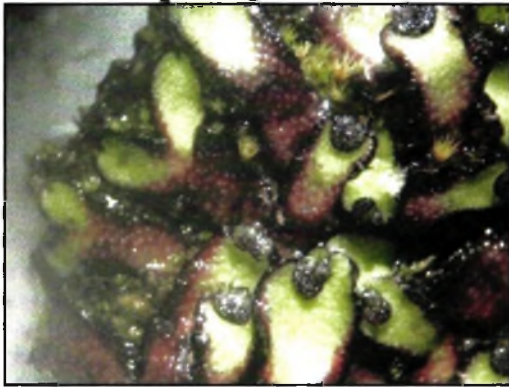
G) *A. angusta* mature black sporophyte



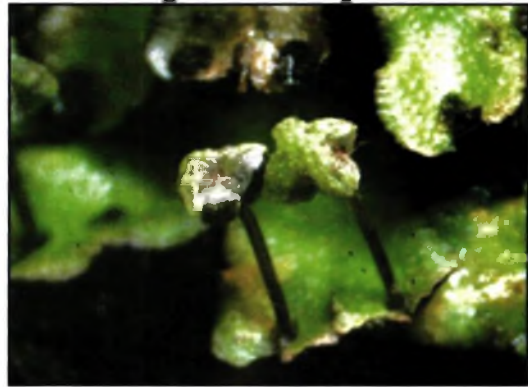
H) *A. angusta* with spores

PLATE – 5

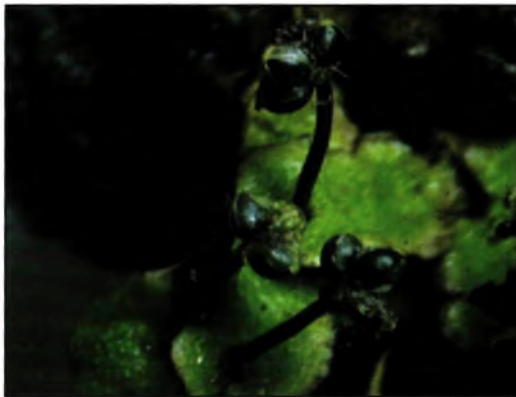
Morphological studies of *Reboulia* and *Plagiochasma* species



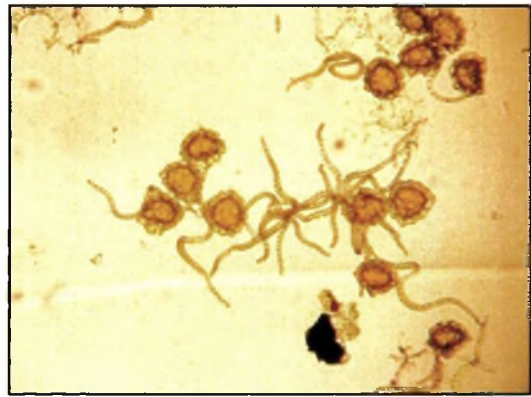
A) *Reboulia hemisphaerica* (L.) Raddi.



B) *R. hemisphaerica* female thallus



C) *R. hemisphaerica* sporophyte



D) *R. hemisphaerica* spores and elaters



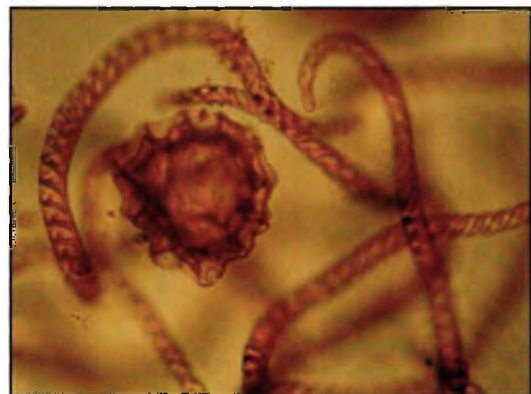
E) *Plagiochasma appendiculatum* L. et L.



F) *P. appendiculatum* male thallus



G) *P. appendiculatum* female thallus



H) *P. appendiculatum* spore-elaters

PLATE – 6
Morphological studies of different *Plagiochasma* species



A) *Plagiochasma intermedium* Linde. et. Gott.



B) *P. intermedium* purple margin



C) *P. intermedium* with sex organs



D) *P. intermedium* non-spiral elaters



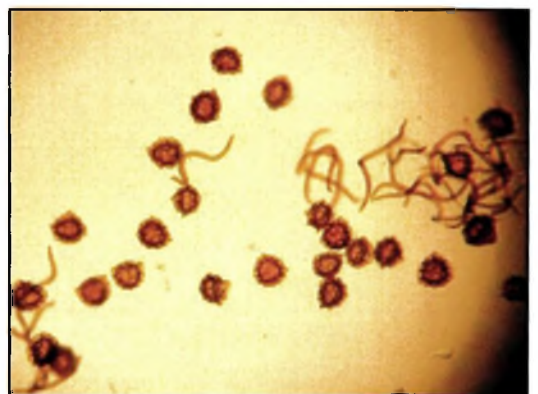
E) *Plagiochasma rupestre* (Forst.) Steph.



F) *P. rupestre* young thallus



G) *P. rupestre* with mature thallus.



H) *P. rupestre* spores and elaters

PLATE - 7
Morphological studies of different *Riccia* species



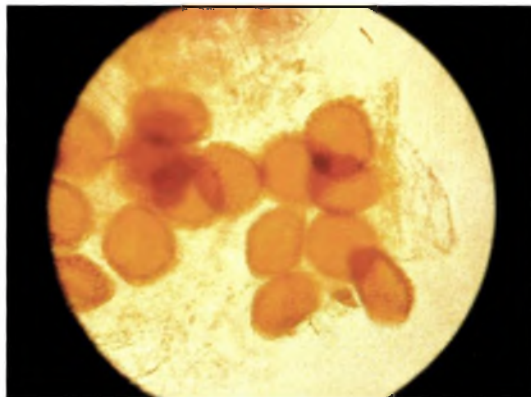
A) *Riccia discolor* Lehm. et. Lindenb.



B) *R. discolor* young rosette



C) *R. discolor* mature colony



D) *R. discolor* spores



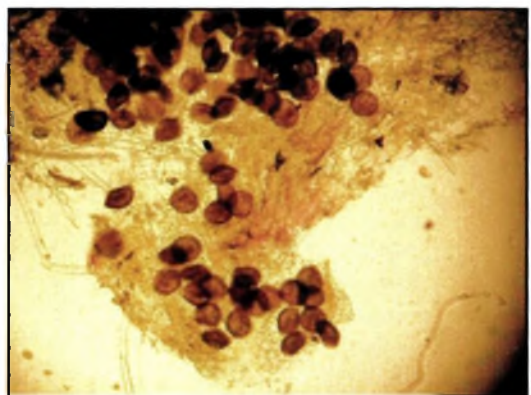
E) *Riccia gangetica* young



F) *R. gangetica* mature stage



G) *R. gangetica* with *Nostoc* colony



H) *R. gangetica* spores

PLATE – 8

Morphological studies of *Anthoceros* and *Folioceros* species



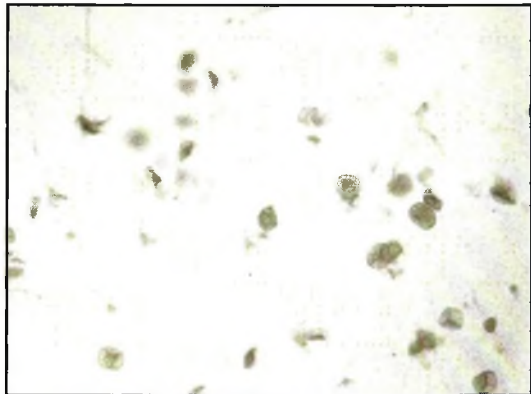
A) *Anthoceros erectus* Kash.



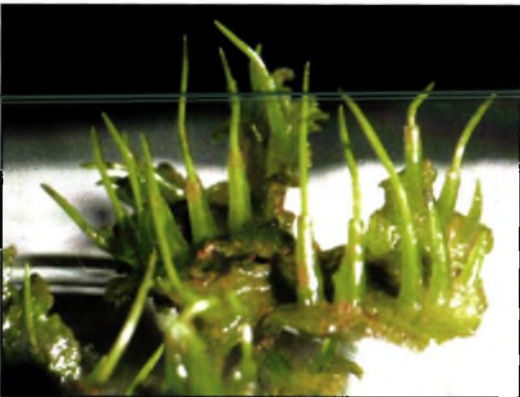
B) *A. erectus* young sporophyte



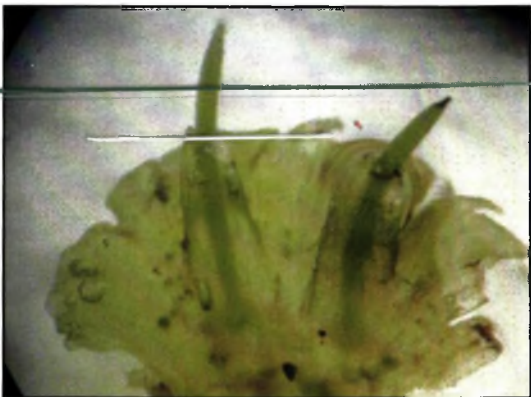
C) *A. erectus* with mature sporophyte



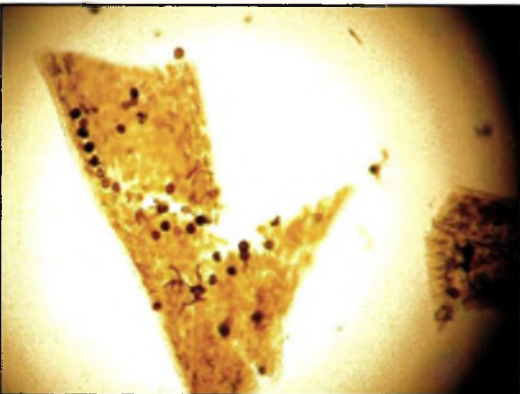
D) *A. erectus* spores



E) *Folioceros udarii* Asthana.et.Srivastava



F) *F. udarii* young sporophyte



G) *F. udarii* with dehiscent sporophyte



H) *F. udarii* with brown spores

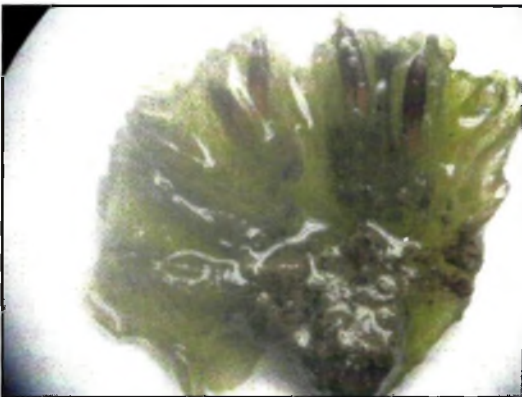
PLATE – 9
Morphological studies of *Notothylas* and *Phaeoceros* species



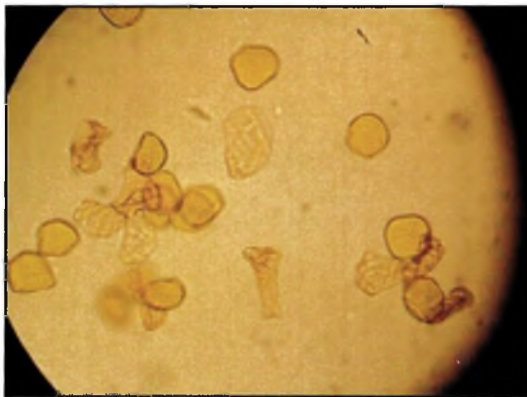
A) *Notothylas indica* Kash.



B) *N. indica* sporophyte



C) *N. indica* mature sporophyte



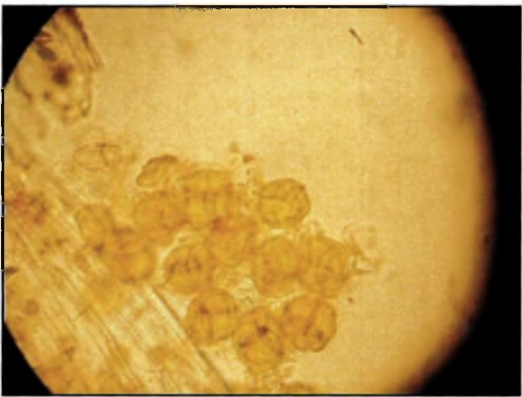
D) *N. indica* mature sporophyte



E) *Phaeoceros laevis* (Linn.) Prosk



F) *P. laevis* mature sporophyte



G) *P. laevis* dehiscent sporophyte



H) *P. laevis* with yellow spores

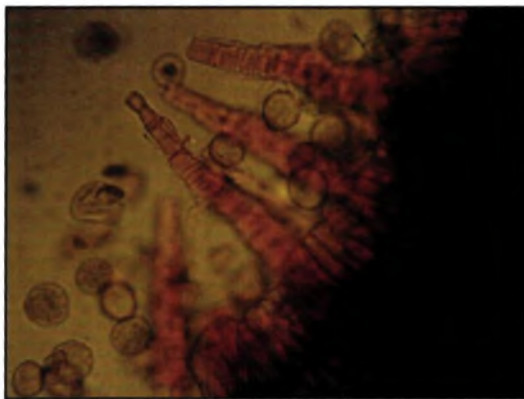
PLATE – 10
Morphological studies of *Funaria* and *Brachymerium* species



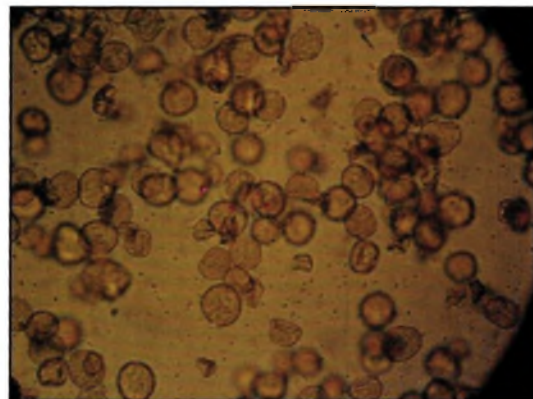
A) *Funaria hygrometrica* Hedw.



B) *F. hygrometrica* mature stage



C) *F. hygrometrica* capsule-teeth



D) *F. hygrometrica* with spores



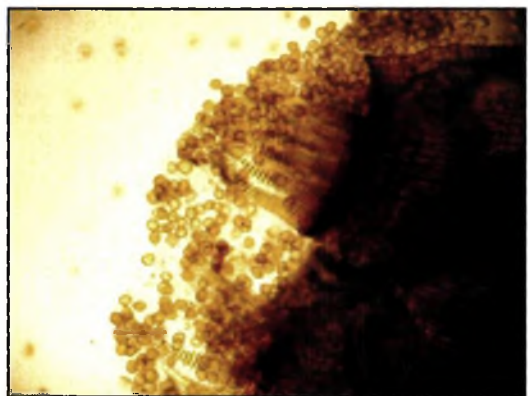
E) *Brachymerium turgidum* Broth. ex. Dix.



F) *B. turgidum* with sporophyte



G) *B. turgidum* with operculum lid



H) *B. turgidum* with many spores

PLATE – 11
Morphological studies of *Bryum* and *Stereophyllum* species



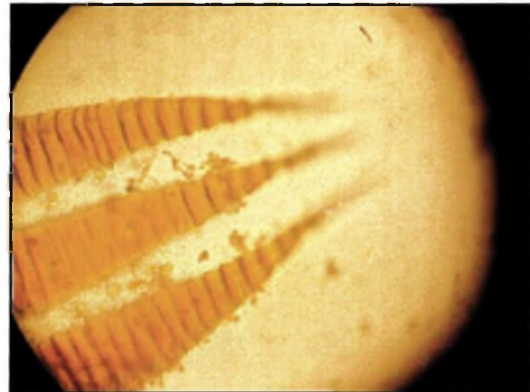
A) *Bryum coronatum* Schwaegr.



B) *B. coronatum* mature pink sporophyte



C) *B. coronatum* on forest fire ash



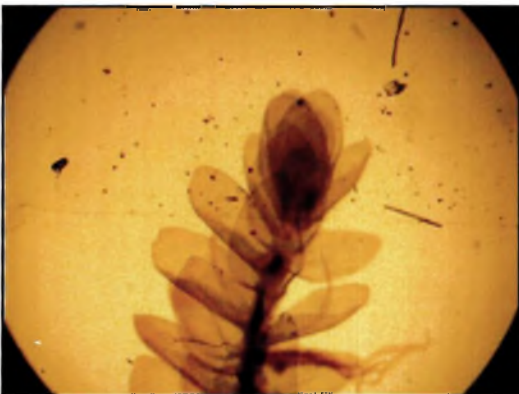
D) *B. coronatum* spores along teeth



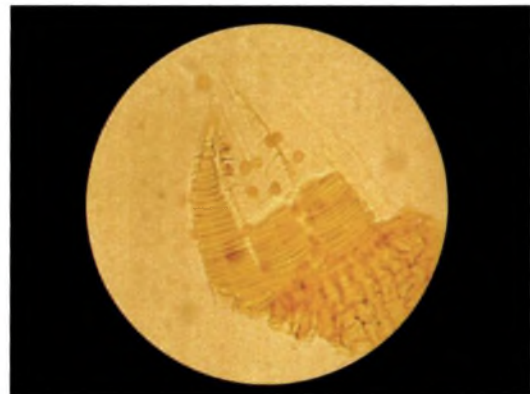
E) *Stereophyllum decorum* (Mitt.) Wijk. et Marg.



F) *S. decorum* capsule on cortex



G) *S. decorum* with leaf margin



H) *S. decorum* spores along teeth

PLATE – 12
Morphological studies in *Hyophila involuta* species



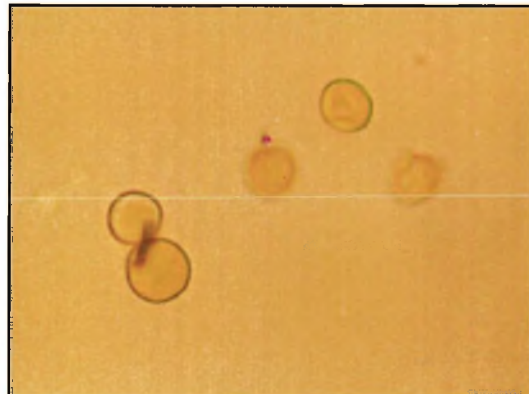
A) *Hyophila involuta* (Hook) Jaeg.



B) *H. involuta* with mature capsules



C) *H. involuta* single capsule



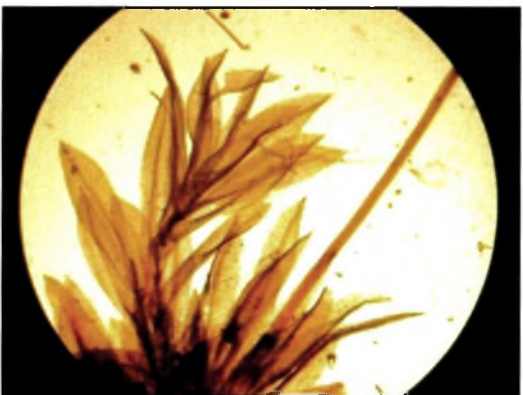
D) *H. involuta* rounded spores



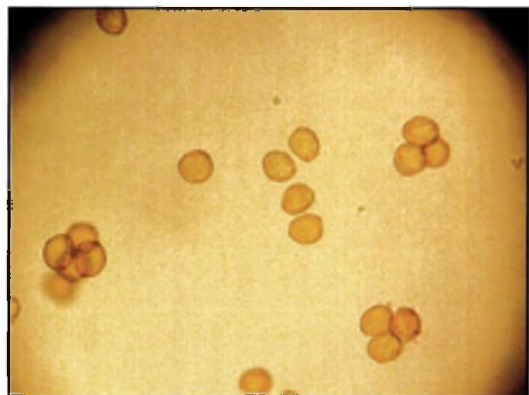
E) *Hymenostylium recurvirostre* (Hedw.) Dix



F) *H. recurvirostre* mature capsule



G) *H. recurvirostre* close leaf lamina



H) *H. recurvirostre* bunch of spores

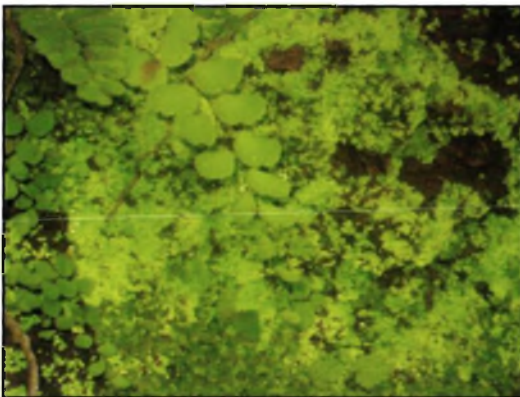
PLATE – 13
Association of Bryophytes with Algae and Ferns



A) *Targionia* with *Selaginella* fern



B) *Targionia* with silver fern



C) *Cyathodium* with *Adiantum* fern



D) *Cyathodium* with *Selaginella*



E) *Riccia* thallus with *Nostoc* algae



F) *Plagiochasma* with *Nostoc* algae



G) *Anthoceros* with *Nostoc* puffs



H) Fillicales in succession

PLATE – 14
Melghat Dry Scenario in Summer Season



A. Deciduous forest in summer



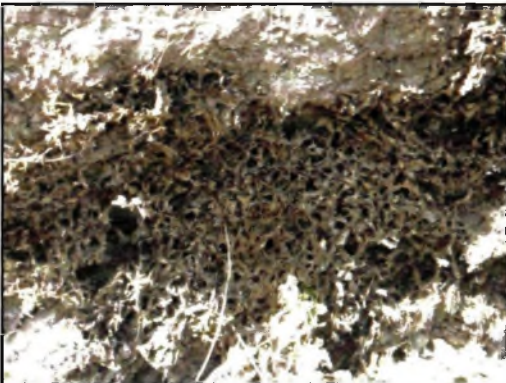
B. Dry patches on mineral rocks



C. Dried *Targionia* sp. thallus



D. Dried *Cyathodium* sp. on wall



E. Dehydrated *Reboulia* sp.



F. Barren view of *Asterella* sp.



G. Rolling back thallus of *Plagiochasma*



H. *Funaria* sp. on stone nugget

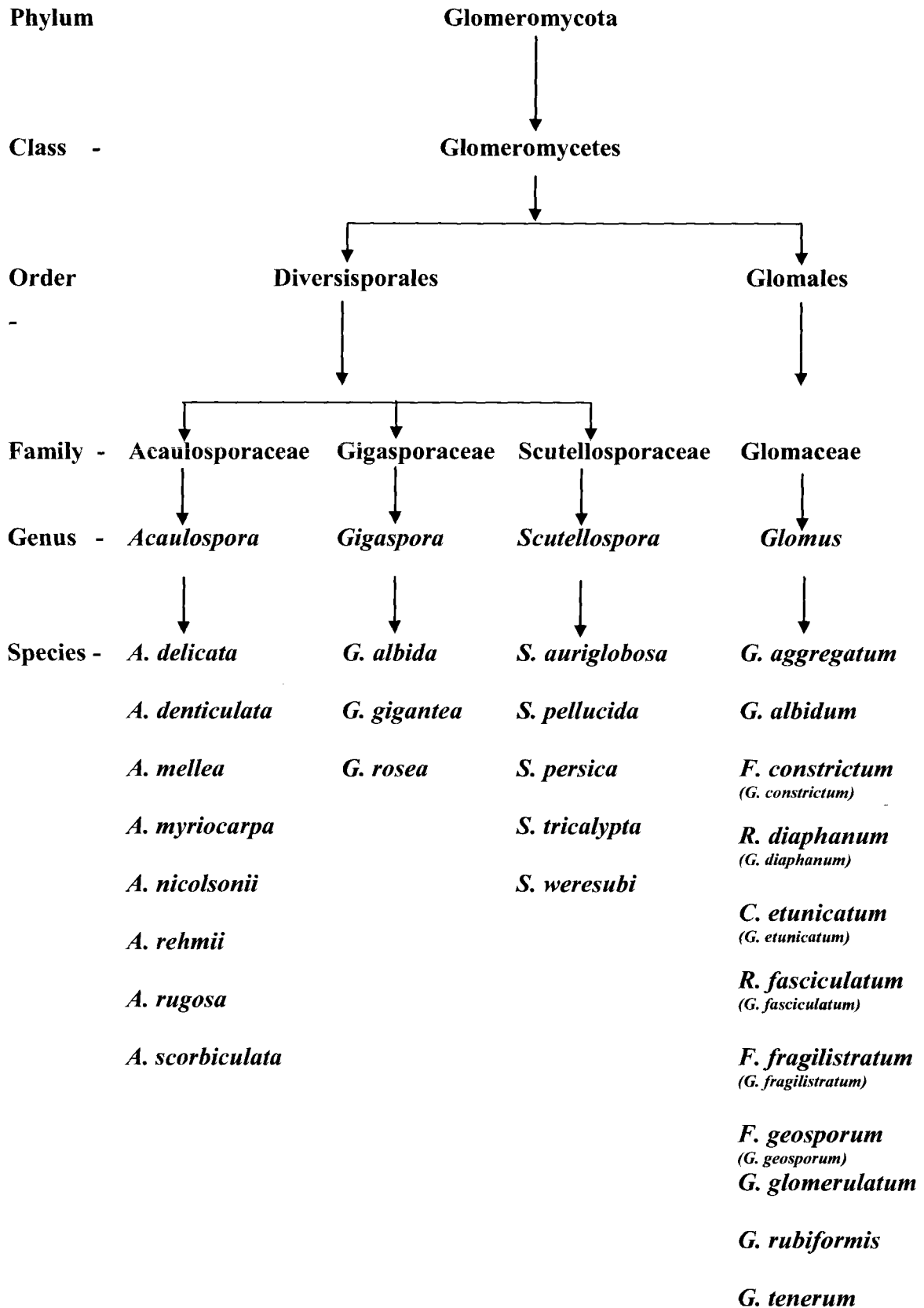
4.3 VAM fungal symbiosis in bryophytes

The state of art of fungal symbioses among early land plants as bryophytes have provided new insights to present era and using modern tools, their establishment was confirmed. The present study emphasized on the occurrence of AM fungi among bryophytes of Melghat forest. The soil borne fungus i.e. VAM or AM fungi (Vesicular Arbuscular Mycorrhiza) forms a symbiosis as a key attribute to the land plants. Various bryophytic plant forms were collected during the course of work. The litter matter was removed, soil cleaned, dried in shade and used for fungal spore isolation. The spores were isolated from soil of different bryophytic thalli of different species and slides were prepared and stored for taxonomic identifications. The plants belonging to the different habitat, habit or micro niche considered for the study to check VAM Fungal diversity among liverworts and hornworts dominantly found on the soil. Their soil characteristics were also analyzed during the work. However, the mosses are grown on wide range of habitats like soil, stones, walls, rocks and on tree bark but only terrestrial mosses were considered for the study. The corticolous mosses generally lack the VAM fungal association.

4.3.1 Morpho-taxonomic study of the AM Fungal spores

The spores isolated from the soil identified on the basis of morphological characters with taxonomic descriptions. Near about four groups of VAM, spores mainly *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were observed dominantly among all species of bryophytes of different terricolous, saxicolous, rupicolous and corticolous habitats. The maximum distribution and density of spores recorded in liverworts followed by hornworts and lesser in mosses species of the Melghat forest. The micrographs were made using the characters congruency with the observed spores. Based on morphological and cytological observations, the probable identifications of the spores recorded. Using manual of Schenck and Perez (1990) and Rodrigues and Muthukumar (2009) the spores were described as follows.

Fig: 4.3.1 A detailed outline classification of AM-Fungi collected from Melghat forest followed by Schüßler *et al.*, (2001)



4.3.1.1 *Acaulospora delicata* Walker, Pfeiffer and Bloss

(Plate No. 15 A-E)

Spores : Spores borne singly in the soil, hyaline to pale yellowish cream; sparkling nature of the spore content; shape globose to subglobose or rarely ovoid to abovoid; spore size ranges from 80 - 125 x 80 - 110 μm . Sporiferous saccule usually broader than long.

Spore wall : Generally four walled, but appears two under light microscope, outer two and inner two walls, the outer wall thin, hyaline, evanescent 1 μm thick, followed by adjacent pale yellow, laminated 3.5-5 thick wall. Soil particles often attached to the outer wall. The inner walls thin, hyaline, membranous and 0.5 to 1 μm thick, covered by minute granules.

Mycorrhizal association: *Plagiochasma* sp., *Riccia* sp.

Locality: Chikhaldara Plateau

GPS location: N = 21.40140, E = 77.29781; **Elevation:** 1029 m.

Congruency: Characters resembled with the observed spores.

4.3.1.2 *Acaulospora denticulata* Sieverding and Toro

(Plate No. 15 F)

Spores: Spores yellow brown to dark brown, globose to sub-globose, spores formed laterally on sporiferous saccule, 80-160 μm in diameter produced singly in soil.

Spore wall: Spore walls generally four walled, in two separable groups. The outermost layer found yellow brown of 0.5 μm thick with inseparable polygonal segments. The inner wall group composed of three hyaline layers, each one 0.5 to 1.5 μm thick, and walls may separate or attached to the wall four.

Mycorrhizal association: *Plagiochasma* sp., *Reboulia* sp., *Riccia* sp.

Locality: Amazari, Semadoh.

GPS location: N = 21.4439 : E = 77.41778 ; **Elevation:** 862 m.

Congruency: Characters resembled with the observed spores.

4.3.1.3 *Acaulospora mellea* Spain and Schenk

(Plate No. 15 G-H)

Spores: Azygospore formed singly in soil; borne laterally on hyphae tapering to a globose to sub-globose swollen hyphal terminus 90-100 μm diameter. Hyphal terminus remains attached to young spores but later collapse in preservation. Old

spores in soil also found devoid of a hyphal terminus. Azygospore generally honey coloured to yellow brown, globose to sub-globose 72-105 μm diameter, ellipsoidal or irregular 96-130 x 78-92 μm .

Spore wall: Spore wall composed of five layers of 4-11 μm thick. Layer one found yellow brown to dark brown, 2-6 μm thick, laminate, inseparable from layer two; layer two generally 0.5 μm thick; layer three distinctly hyaline to light yellow, membranous, 0.5-1 μm thick while layer four and five noticeably membranous.

Mycorrhizal association: *Plagiochasma* sp., *Asterella* sp., *Riccia* sp., *Reboulia* sp.

Locality: Chikhaldara Valley, Belkund

GPS location: N = 21.40504; E = 77.34579; **Elevation:** 1040 m

Congruency: Characters resembled with the observed spores.

4.3.1.4 *Acaulospora myriocarpa* Spain, Sieverding and Schenck

(Plate No. 16 A)

Spores: Spores formed singly in the soil, spores generally hyaline, globose to sub-globose 22 - 90 μm diameter or cylindrical, ovoid, pyriform to irregular, 23 - 95 (-114) x 28 - 80 (-96) μm in diameter.

Spore wall: Generally spore walls composed of four layers and total thickness found 1.5 - 3.5 μm layer one found rigid, 0.75 - 2 μm in thickness and layer two also rigid of 0.3-1.5 μm in thickness. However, layer three found above 0.3 μm thick and closely appressed to the layer two.

Spore contents: Hyaline and granular.

Mycorrhizal association: *Plagiochasma* sp., *Reboulia* sp., *Riccia* sp.

Locality: Belkund, Chikhaldara (Semadoh)

GPS location: N = 21.50342 : E = 77.33626 : **Elevation:** 506 m

Congruency: Characters resembled with the observed spores.

4.3.1.5 *Acaulospora nicolsonii* Walker, Reed and Sanders

(Plate No. 16 A)

Spores: Spores formed singly in the soil, laterally on the neck of a sporiferous saccule that collapse after the spore matures. Spores hyaline to pale yellow brown, globose to sub-globose to pyriform 93 - 218 μm in diameter.

Spore wall: Spore walls generally four layered, layer one found hyaline and 0.5-1 μm thick while layer two generally pale yellow brown, laminated and 3-10 μm in thickness. Layer three found as loosely adherent, pale yellow, brittle, unit wall and 0.5 to 1.5 μm thick. Layer four found membranous and 0.5 μm in thickness.

Spore contents: Appearing vacuolated, due to the presence of many oil droplets, but later becoming reticulate as the droplets apparently coalesce.

Mycorrhizal association: *Reboulia* sp., *Plagiochasma* sp., *Riccia* sp., *Targionia* sp., *Asterella* sp., *Anthoceros* sp., *Notothylas* sp., *Folioceros* sp., *Phaeoceros* sp.

Locality: Memna, Gugamal forest.

GPS location: N = 21.42339; E = 77.32551 : **Elevation:** 933 m

Congruency: Characters resembled with the observed spores.

4.3.1.6 *Acaulospora rehmii* Sieverding and Toro

(Plate No. 16 C)

Spores: Sporocarps unknown. Spores light yellow to brown, older spores often appearing dark red brown to black, globose to subglobose 82-175 μm in diameter. Spores sessile or formed on a short 2-4 μm long stalk on the neck of sporiferous saccule.

Spore wall: Composite spore wall consists of four wall layers. Layer one generally 3-13 μm thick including ornamentation of labyrinth form folds with depressions between ridges and 1-4.5 μm thick. Layer two found hyaline with 0.5-2.0 μm in thickness. However, layer three found 0.5 to 1.5 μm in thickness and layer four of 0.5 μm thick.

Mycorrhizal association: *Plagiochasma* sp., *Reboulia* sp., *Targionia* sp., *Riccia* sp.

Locality: Chikhaldara Valley

GPS location: N = 21.39417; E = 77.34684 ; **Elevation:** 1011 m

Congruency: Characters resembled with the observed spores.

4.3.1.7 *Acaulospora rugosa* Morton

(Plate No. 16 D-H, 17 A)

Spores: Spores formed singly in soil, borne laterally on hyphae, each ending in a globose to subglobose hyphal terminus 95-120 μm diameter. Spores generally sub hyaline to straw coloured or cream coloured.

Spore wall: Spore wall composed of five layers. Layer one generally hyaline, 1-1.5 μm thick, often forming folds 2-10 μm deep surrounding the intact spores and separating readily from wall layer 2 in crushed spores. The later two generally pale yellow, laminated, 1.2-3 μm thick while layer three was semi rigid, hyaline with 1-1.3 μm thickness. The fourth layer found hyaline, membranous, and fifth layer usually hyaline with 1-2.5 μm in thickness.

Mycorrhizal association: *Plagiochasma* sp., *Riccia* sp., *Reboulia* sp.

Locality: Ghatang, Chikhaldara

GPS location: N = 21.4439; E = 77.41778 ; **Elevation:** 862 m

Congruency: Characters resembled with the observed spores.

4.3.1.8 *Acaulospora scorbiculata* Trappe

(Plate No. 17 B)

Spores: Spores generally borne singly in soil, hyaline to light brown, globose to sub-globose, occasionally irregular, 100-240 μm in diameter. Spore surface evenly pitted with depressions 1-1.5 x 1.3 μm , separated by ridges 2-4 μm thick at the mouth of depressions, circular to elliptical or occasionally linear to Y-shaped.

Spore wall: Composite spore wall composed of four layers. First layer generally sub-hyaline to light greenish yellow, 3-6 μm thick, adhering it, the second layer with smooth, hyaline, 0.2-0.5 μm thickness. However, the third layer found hyaline with 0.5-1.0 μm thickness, while forth layer found roughen, hyaline with 0.2-1.0 μm thickened.

Mycorrhizal association: *Riccia* sp., *Asterella* sp., *Targionia* sp., *Plagiochasma* sp., *Reboulia* sp., *Anthoceros* sp., *Notothylas* sp., *Folioceros* sp., *Phaeoceros* sp.

Locality: Bhimkund, Bori, Salona.

GPS location: N = 21.39417; E = 77.3468; **Elevation:** 1011 m

Congruency: Characters resembled with the observed spores.

4.3.1.9 *Gigaspora albida* Schenck and Smith

(Plate No. 17 C)

Spores: Spores formed singly in the soil, colour dull white with a light greenish yellow, spherical 143-350 μm diameter.

Spore wall: Spore walls continuous, 4-12 μm thick with one to six layers. Layer one generally smooth and 1-2 μm thick. Layers two to six found laminated.

Germ tube produced directly through the spore wall near the bulbous suspensor separating it from the spore contents.

Sporogenous cell: Hyaline to yellow, 24-36 μm diameter attached to septate hypha with fine hyphal branches.

Mycorrhizal association: *Plagiochasma* sp., *Riccia* sp., *Reboulia* sp., *Asterella* sp., *Anthoceros* sp., *Phaeoceros* sp.

Locality: Bori, Salona, Chikhaldara Plateau

GPS location: N = 21.40558; E = 77.3499 ; **Elevation:** 1029 m

Congruency: Characters resembled with the observed spores.

4.3.1.10 *Gigaspora gigantea* (Nicolson and Gerdemann) Gerdemann and Trappe (Plate No. 17 D-E)

Spores: Zygosporangia formed singly in soil, colour when mature bright yellow with greenish tings, spherical, ellipsoidal and cylindrical or irregular 183-500 x 291 - 812 μm in diameter.

Spore wall: Spore wall with thin outer wall tightly covering a thick walled continuous endospore. Endospore wall found 2.5-7.5 μm thick. A bulbous suspensor 41-51 μm diameter with a slender hypha extending from the suspensor to the base of the spore and germ tube produced directly through the spore wall in the base region.

Mycorrhizal association: *Targionia* sp., *Asterella* sp., *Reboulia* sp., *Riccia* sp., *Funaria* sp., *Bryum* sp., *Plagiochasma* sp., *Anthoceros* sp., *Phaeoceros* sp.

Locality: Tarubanda, Koha, Belkund, Gugamal

GPS location: N = 21.346; E = 77.13605; **Elevation:** 640 m

Congruency: Characters resembled with the observed spores.

4.3.1.11 *Gigaspora rosea* Nicolson and Schenck (Plate No. 17 F-H, 18-A)

Spores : Azygospore produced singly in soil, predominantly globose, 230-294 (-305) μm in diameter, colour white to cream with a rose-pink tint on the spore wall near the hyphal attachment encompassing upto half the spore. Pink colour found variable from distinctly rose pink to barely detectable.

Spore wall: Generally 2.4 to 7.5 μm thick with 2-5 inseparable layers. Layer one found smooth.

Sporogenous cell: 28-40 μm diameter borne on a subtending hypha, the subtending hypha 7-14 μm wide, hyphal walls 1-2 μm thick and septate.

Mycorrhizal association: *Reboulia* sp., *Plagiochasma* sp., *Funaria* sp., *Targionia* sp., *Riccia* sp., *Anthoceros* sp., *Phaeoceros* sp.

Locality: Gawilgarh Fort, Chikhaldara

GPS Location: N = 21.38291; E = 77.33581; **Elevation:** 1044 m

Congruency: Characters resembled with the observed spores.

4.3.1.12 *Glomus aggregatum* Schenck and Smith emend. Koske

(Plate No. 18 B-C)

Sporocarps: Spores formed in sporocarps, 200-1400 μm in diameter, loosely aggregated spores lacking a peridium.

Spores: Spores produced in sporocarps with 20-210 μm in diameter. Colour pale yellow to yellow brown, subtending hyphae straight, constricted, swollen or irregular up to 12 μm wide at the spore base.

Spore wall: Spore wall composed of three layers, laminated 1-3 (-5) μm thick and often absent in mature spores. However, layer two found semi flexible, hyaline with 0.5-2 μm thickness. Layer three found laminated, smooth, yellowish brown with 2-4.5 μm in thickness. The subtending hyphae yellowish brown straight or curved, cylindrical to funnel shaped 6.4-14.3 (-21.6) μm wide at the spore base.

Mycorrhizal association: *Riccia* sp., *Asterella* sp., *Targionia* sp., *Plagiochasma* sp., *Reboulia* sp., *Anthoceros* sp., *Notothylas* sp., *Folioceros* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp. *Hymenostylium* sp.

Locality: Khongada-Parsapur-Belkund

GPS location: N = 21.37657; E = 77.1289; **Elevation:** 590 m

Congruency: Characters resembled with the observed spores.

4.3.1.13 *Glomus albidum* Walker and Rhodes

(Plate No. 18 D-G)

Spores: Spores borne singly in the soil on coenocytic hyphae. Mature spores 95-168 x 95-168 µm in diameter or may vary, shape found globose to subglobose, occasionally ovoid or irregular.

Spore wall: Continuous with hyphal wall with two layers. Layer one consists of an outer hyaline wall of 0.5-2 µm in thickness and the inner sub equal finely laminated wall with yellow colour having 0.5-2 µm thickness.

Spore contents: Spore contents showed crowded oil droplets, usually becoming angular from mutual pressure to give a reticulate appearance.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp.

Locality: Bhimkund Valley, Devi point Valley

GPS location: N = 21.39409; E = 77.32966; **Elevation:** 1074 m

Congruency: Characters resembled with the observed spores.

4.3.1.14 *Glomus citricola* Tang and Zang

(Plate No. 18 H)

Spores: The spores can be distinguished by its small clamydospores, size found 35-65 x 60-90 µm in diameter, and without the ephemeral hyaline outer wall of spores. Spores globose, yellow or red colour.

Spore wall: Spore wall composed of two layers. The outer wall found hyaline while the inner layer wall often minute perforated with thickened inward projections.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp. *Hyophila* sp., *Hymenostylium* sp.

Locality: Madaki (Paratwada - Chikhaldara Road)

GPS location: N = 21.38320; E = 77.40449; **Elevation:** 749 m

Congruency: Characters resembled with the observed spores.

4.3.1.15 *Funneliformis constrictum* (Trappe) Walker and Schüßler

(Plate No. 19 A) Old Name: *Glomus constrictum* (Trappe)

Spores : Spores formed singly or in loose clusters in the soil, shape globose to subglobose, colour dark brown to black, shiny smooth with 150-330 µm in diameter.

Spore wall: Spore walls 7-15 μm thick, composed of two layers, straight with a short funnel shaped projection. Attached hyphae found straight or recurved at the point of attachment with dark brown walls, 3-5 μm thick. Just beyond the point of attachment, the hypha constricted to 10-22 μm diameter. Just beyond the constriction, the hypha inflated to 15-30 μm diameter with yellow brown walls of 2-3 μm thickness. However, layer one found hyaline to pale yellow 0.8-2.5 (-8.5) μm in thickness. The layer two found laminate smooth brownish, orange to dark brown in colour with 7.5-12 μm in thickness. Most juvenile spores with one layer.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp. *Hyophila* sp., *Hymenostylium* sp.

Locality: Makhala forest, Semadoh, Gugamal forest

GPS Location: N = 21.531015; E = 77.378654; **Elevation:** 748 m

Congruency: Characters resembled with the observed spores.

4.3.1.16 *Rhizophagus diaphanum* (Morton and Walker) Walker and Schüßler

(Plate No. 19 B-C) **Old Name:** *Glomus diaphanum* Morton and Walker

Spores: Spores produced singly or in loose clusters in soil, hyaline, globose to subglobose 39-100 (-121) μm in diameter and sporocarps unknown.

Spore wall: Spore wall composed of two layers. The first layer found laminated with 2-4.4 (-6.5) μm in thickness. The second layer noticeably membranous 0.2- 0.8 (-1.3) μm in thickness, extended 5-12 μm into the subtending hypha and forms a septum enclosing the spore contents.

Spore contents: Hyaline and contain one to many oil globules. Subtending hypha 5.4-11 μm in diameter at the spore base and hyphal wall 1.4-3 (-3.7) μm in thickness.

Mycorrhizal association: *Plagiochasma* sp., *Riccia* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp. *Hyophila* sp., *Hymenostylium* sp.

Locality: Gugamal forest, Belkund forest etc.

GPS location: N = 21.4259; E = 77.3220; **Elevation:** 945 m

Congruency: Characters resembled with the observed spores.

4.3.1.17 *Claroideoglomus etunicatum* (Becker and Gerdemann) Walker and Schüßler

(Plate No. 19 D) Old Name: *Glomus etunicatum* (Becker and Gerdemann)

Spores: Spores formed singly in soil, colour light brown, globose to subglobose, size 68-162 μm in diameter, smooth or roughened from decomposition of the outer wall and adherent debris.

Spore wall: Spore wall composed of two layers with 4-13 μm thick. The ephemeral hyaline outer wall up to 5 μm in thickness and persistent yellow to brown laminate inner wall 2-8 μm in thickness.

Spore contents: Separated from attached hypha by a thin curved septum.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Amazari, Bori, Salona.

GPS location: N = 21.4439; E = 77.4178; **Elevation:** 862 m

Congruency: Characters resembled with the observed spores.

4.3.1.18 *Rhizophagus fasciculatum* (Thaxter) Walker and Schüßler

(Plate No. 19 E) Old Name: *Glomus fasciculatum* Walker and Koske

Spores: Spores borne singly in soil or in aggregates lacking a peridium, colour light brown to reddish brown with 75-149 μm in diameter.

Spore wall: Spore wall highly variable in thickness, 3-17 μm thick, perforated with thickened inward projections, and composed of three layers. The first layer generally smooth hyaline and unit wall 0.2-1.0 (-18) μm in thickness. The second layer generally pale yellow to pale brown, laminated with 0.8-14.3 μm in thickness. The third layer represents hyaline, membranous appearance with 0.1-0.9 μm in thickness.

Subtending hyphae often paler in colour than the spore, flattened, straight or slightly constricted proximally tapering to 1.5-2.0 μm in thickness distally.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Cosmopolitan

GPS location: N = 21.40504; E = 77.34579 ; **Elevation:** 1040 m

Congruency: Characters resembled with the observed spores.

4.3.1.19 *Funneliformis fragilistratum* (Thaxter) Walker and Schüßler

(Plate No. 19 F) **Old Name:** *Glomus fragilistratum* Skou and Jakobsen

Spores: Sporocarps unknown and spores formed singly in soil, colour yellow or bright yellow or pale orange, shape globose and 108-231 µm in diameter.

Spore wall: Generally, spore wall composed of six layers where layer one and layer two found hyaline with 1-1.5 µm and 1.4-3 µm in thickness respectively. However, the third layer found hyaline, unit wall of 1 µm in thickness along with fourth layer yellow, laminated with 5 µm thick. The layer five and six found hyaline with 1-2 µm in thickness.

Spore contents: Spore contents consist of oil globules of different sizes. Layers 1-4 of spore wall extend as subtending hyphae, 9-15 µm in diameter widening at the spore base with 1-2 hyphal septa positioned close to the spore.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp. *Hyophila* sp., *Hymenostylium* sp.

Locality: Ghatang-Semadoh Road

GPS location: N = 21.46943; E = 77.41451; **Elevation:** 683 m

Congruency: Characters resembled with the observed spores.

4.3.1.20 *Funneliformis geosporum* (Nicolson and Gerdemann) Walker and Schüßler

(Plate No. 19 G-H) **Old Name:** *Glomus geosporum* Nicolson and Gerdemann

Spores: Spores formed singly in soil, ellipsoidal, light to dark brown, 110-290 µm in diameter. Spores generally with straight to recurved funnel shaped subtending hyphae of 10-24 µm in diameter.

Spore wall: Spore wall composed 3 layers, 4-8 µm thick. The layer one found tightly adherent and found less than 1 µm in thickness. The second layer generally yellow brown to red brown, laminated with 3-16 µm in thickness. The third layer found yellow-brown, less than 1 µm thickness, membranous, forming a septum separating the spore contents from the subtending hypha.

Spore contents: Uniform sized droplets granular in appearance with maturity, separated by a septum that protrudes slightly into subtending hypha.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Semadoh-Chikhaldara Road

GPS location: N = 21.44079; E = 77.29781; **Elevation:** 743 m

Congruency: Characters resembled with the observed spores.

4.4.1.21 *Glomus glomerulatum* Sieverding

(Plate No. 20 A-D)

Sporocarps: Generally dark brown, globose to subglobose, rectangular or flattened or irregular shape with 290-680 μm in diameter.

Spores: Yellow to brown, globose to subglobose 40-70 μm in diameter.

Spore wall: Composed of 2 layers, composite. Layer one generally yellow to brown laminated and 4-9 μm in thickness. However, the layer numbers two generally hyaline, membranous, 0.5 μm in thickness.

Two to three hyphal attachments, straight to recurved, cylindrical to funnel shaped, yellow to brown, 5-7 μm in diameter. The pore of hyphal attachment is 1-2 μm in diameter, closed by the spore wall or a septum.

Spore content: Hyaline and oily

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Semadoh, Chikhaldara, Kolkhas, Ghatang, Belkund.

GPS location: N = 21.44392; E = 77.41778; **Elevation:** 732 m

Congruency: Characters resembled with the observed spores.

4.3.1.22 *Glomus rubiformis* (Gerdemann and Trappe) Almeida and Schenck

(Plate No. 20 E-H)

Sporocarps: Dark brown, 180-675 μm in diameter, surrounding a central plexus of hyphae. Peridium absent, individual spores partially enclosed in a thin network of tightly appressed hyphae.

Spores: Dark brown obovoid to ellipsoidal or sub-globose, 37-125 μm with a small pore opening into thick walled subtending hypha.

Spore wall: Generally laminate, 3-7.5 μm thick, up to 13.5 μm thick at the spore base. Perforated projections appear on the inner wall surface of subtending hyphae.

Mycorrhizal association: *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp., *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Chikhaldara valley and roadside vegetations.

GPS location: N = 21.40504; E = 77.34579; **Elevation:** 1040 m

Congruency: Characters resembled with the observed spores.

4.3.1.23 *Glomus tenerum* Tandy emend. McGee

(Plate No. 21 A)

Spores: Yellow brown or pale orange coloured, globose when distended, rarely pyriform found on soil surface as rounded structures. Hyphae thin walled whitish but on maturity becomes yellow with 5-7 μm in diameter.

Spore wall: Spore wall composed of two layers. The first layer found rough, hyaline and 1 μm thick. The second layer found smooth, hyaline, thickened up to 7 μm . There is no septum at the base of spore.

Spore content: Spore consists of very dense granular contents, sometimes a few oil globules. Subtending hyphae 8-12 μm wide.

Mycorrhizal association : *Riccia* sp., *Plagiochasma* sp., *Anthoceros* sp., *Notothylas* sp., *Asterella* sp., *Targionia* sp., *Reboulia* sp. *Phaeoceros* sp., *Funaria* sp., *Bryum* sp., *Hyophila* sp., *Hymenostylium* sp.

Locality: Semadoh - Belkund.

GPS Location: N = 21.34657; E = 77.13124; **Elevation:** 640 m

Congruency: Characters resembled with the observed spores.

4.3.1.24 *Scutellospora auriglobosa* (Hall) Walker and Sanders

(Plate No. 21 B)

Spores: Spores generally ectocarpic, globose or rarely polymorphic, 200-420 (-520) x 130-420 (-520) μm in diameter, colour pale yellow, brown transparent and shining. Moribund spore found brown in colour.

Spore wall: Generally, 2 to 4 layered, outer wall coloured with 6-16 μm in thickness. The inner walls found 0.1 μm thick and colourless to yellow.

Spores formed on a bulbous suspensor 40-70 μm diameter. Walls of the subtending hypha found 3-10 μm thick, yellow to light brown.

Mycorrhizal association: *Plagiochasma* sp., *Targionia* sp., *Riccia* sp., *Reboulia* sp. *Asterella* sp.

Locality: Amazari, Bori, Salona

GPS location: N = 21.4335; E = 77.37581; **Elevation:** 856 m

Congruency: Characters resembled with the observed spores.

4.3.1.25 *Scutellospora pellucida* (Nicolson and Schenck) Walker and Sanders

(Plate No. 21 C-D)

Spores: Spores formed singly in the soil, borne terminally on a bulbous suspensor like cells, glinting with oil droplets, hyaline to pale gray coloured, globose, ellipsoid, or irregular and size 58-183 x 58-241 μm in diameter.

Spore wall: Spore wall composed of six layers. The outer layer one found smooth, brittle, hyaline, unit wall 1-2 μm in thickness. While layer two found hyaline, laminated and 2-7 μm thick. The third layer generally hyaline membranous wall of 1 μm thick while wall four 1-2 μm thick followed by fifth wall of 5-8 μm thick and wall six found hyaline, single amorphous wall of 2-5 μm thickness.

Mycorrhizal association: *Plagiochasma* sp., *Targionia* sp., *Riccia* sp., *Reboulia* sp. *Asterella* sp.

Locality: Madaki Forest

GPS Location: N = 21.38320; E = 77.40449; **Elevation:** 749 m

Congruency: Characters resembled with the observed spores

4.3.1.26 *Scutellospora persica* (Koske and Walker) Walker and Sanders

(Plate No. 21 E)

Spores: Spores formed singly in the soil, terminally on a bulbous suspensor like cell, shape globose to subglobose to ellipsoid, size 270-354 x 281-384 μm in diameter, colour pale pinkish-orange or brownish orange or occasionally hyaline.

Spore wall: Generally composed of three layers. The first outer layer is an ornamented unit wall, brittle, hyaline, 0.5-0.8 μm thick with covering of low rounded

warts of 0.25-0.5 μm size. The second wall 5-12 μm thick, laminated, brittle, pinkish to orange to brown. The third layer found 0.5 to 1 μm thick, membranous and hyaline.

Suspensor like cell terminal on a septate subtending hypha, 5-10 μm diameter and subglobose, light bronze than the spore.

Mycorrhizal association: *Plagiochasma* sp., *Targionia* sp., *Riccia* sp., *Reboulia* sp. *Asterella* sp.

Locality: Memna Forest, Gugamal Forest.

GPS location: N = 21.42339; E = 77.32551; **Elevation:** 933 m

Congruency: Characters resembled with the observed spores

4.3.1.27 *Scutellospora tricalypta* (Herrera and Ferrer) Walker and Sanders

(Plate No. 21 F)

Spores: Generally spores formed free in the soil, colour very dark gray-brown to black, shape globose 303-397 μm or if ellipsoidal then 456 x 257 μm in diameter.

Spore wall: Spore wall consists of three easy recognizing layers. Dark gray-brown exospore to 9 μm thick, yellow to brownish-yellow mesospore, approximately 5 μm thick with yellowish spines up to 10 x 2 μm formed towards the outside and separated one from the other by as much as 10 μm hyaline membranous endospore to 3 μm thick surrounding a reticulate cytoplasm.

Subtending hypha flattening, attached laterally to the spore, 14-47 μm in diameter and up to 20 μm high with walls up to 4 μm thick, pores to 1.5 μm not continuous with endospore

Mycorrhizal association: *Plagiochasma* sp., *Targionia* sp., *Riccia* sp., *Reboulia* sp. *Asterella* sp.

Locality: Semadoh, Belkund, Chikhaldara

GPS location: N = 21.50342; E = 77.33626; **Elevation:** 506 m

Congruency: Characters resembled with the observed spores

4.3.1.28 *Scutellospora weresubi* Koske and Walker

(Plate No. 21 F)

Spores: Spores formed singly in the soil, shape globose to subglobose or irregular with size 125-265 x 135-414 μm in diameter, colour found translucent, glistening, pale pink to dark pink or reddish.

Spore wall: Generally, wall composed of six layers. The first layer i.e. outer found smooth, brittle, pink, laminated, with 0.5 µm thick and layer second found brittle, pink laminated with 3-8 µm in thickness. However, layer three and layer four generally 1 µm thick, wrinkled in crushed spore. the layer five found coriaceous with 2-8 µm in thickness and layer six found hyaline, membranous and 0.5 µm in thickness.

Mycorrhizal association: *Targionia* sp., *Plagiochasma* sp., *Reboulia* sp.

Locality: Chikhaldara Valley, Bhimkund Valley, Gawilgarh Valley.

GPS location: N = 21.38291; E = 77.33581; **Elevation:** 1044 m

Congruency: Characters resembled with the observed spores

Table: 4.3.1 Distribution of AM Fungal spores among the soil of bryophytes collected from Melghat forest.

Sr. No.	VA Mycorrhizal Spores	Occurrence in bryophytes species.
1)	<i>Acaulospora delicata</i> Walker, Pfeiffer and Bloss	<ul style="list-style-type: none"> ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
2)	<i>Acaulospora denticulata</i> Sieverding and Toro.	<ul style="list-style-type: none"> ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
3)	<i>Acaulospora mellea</i> Spain and Schenck	<ul style="list-style-type: none"> ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
4)	<i>Acaulospora myriocarpa</i> Spain, Sieverding and Schenck.	<ul style="list-style-type: none"> ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
5)	<i>Acaulospora nicolsonii</i> Walker, Reed and Sanders	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb.

		<ul style="list-style-type: none"> ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Notothylas indica</i> Kash. ▪ <i>Folioceros udarii</i>. Asthana et. Srivastava ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk.
6)	<i>Acaulospora rehmii</i> Sieverding and Toro	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
7)	<i>Acaulospora rugosa</i> Morton	<ul style="list-style-type: none"> ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph.
8)	<i>Acaulospora scorbiculata</i> Trappe	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Folioceros udarii</i>. Asthana et. Srivastava ▪ <i>Notothylas indica</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr.
9)	<i>Gigaspora albida</i> Schenck and Smith	<ul style="list-style-type: none"> ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk.
10)	<i>Gigaspora gigantea</i> (Nicolson and Gerdemann) Gerdemann and Trappe	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr.
11)	<i>Gigaspora rosea</i> Nicolson and Schenck	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi.

		<ul style="list-style-type: none"> ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw.
12)	<i>Glomus aggregatum</i> Schenck and Smith emend. Koske	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Notothylas indica</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
13)	<i>Glomus albidum</i> Walker and Rhodes	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr.
14)	<i>Glomus citricola</i> Tang and Zang	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw.

		<ul style="list-style-type: none"> ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
15)	<p>Current name: <i>Funneliformis constrictum</i> (Trappe) Walker and Schüßler</p> <p>Old name : <i>Glomus constrictum</i> Trappe</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
16)	<p>Current name: <i>Rhizophagus diaphanum</i> (Morton and Walker) Walker and Schüßler</p> <p>Old name : <i>Glomus diaphanum</i> Morton and Walker</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
17)	<p>Current name: <i>Claroideoglomus etunicatum</i> (Becker and Gerdemann) Walker and Schüßler</p> <p>Old name : <i>Glomus etunicatum</i> Becker and Gerdemann</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw.

		<ul style="list-style-type: none"> ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix. ▪
18)	<p>Current name: <i>Rhizophagus fasciculatum</i> (Thaxter) Walker and Schüßler</p> <p>Old name : <i>Glomus fasciculatum</i> (Thaxter) Walker and Koske</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Notothylas indica</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
19)	<p>Current name: <i>Funneliformis fragilistratum</i> (Skou and Jakobsen) Walker and Schüßler</p> <p>Old name : <i>Glomus fragilistratum</i> Skou and Jakobsen</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
20)	<p>Current name: <i>Funneliformis geosporum</i> (Nicolson and Gerdemann) Walker and Schüßler</p> <p>Old name : <i>Glomus geosporum</i> Nicolson and Gerdemann</p>	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk.

		<ul style="list-style-type: none"> ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
21)	<i>Glomus glomerulatum</i> Sieverding	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
22)	<i>Glomus rubiformis</i> (Gerdemann and Trappe) Almeida and Schenck	<ul style="list-style-type: none"> ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph.
23)	<i>Glomus tenerum</i> Tandy emend. McGee	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma intermedium</i> Lindenb.et. Gott. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb. ▪ <i>Anthoceros erectus</i> Kash. ▪ <i>Phaeoceros laevis</i> (Linn.) Prosk. ▪ <i>Funaria hygrometrica</i> Hedw. ▪ <i>Bryum coronatum</i> Schwaegr. ▪ <i>Hyophila involuta</i> (Hook) Jaeg. ▪ <i>Hymenostylium recurvirostre</i> (Hedw.) Dix.
24)	<i>Scutellospora auriglobosa</i> (Hall) Walker and Sanders	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
25)	<i>Scutellospora pellucida</i> (Nicolson and Schenck)	<ul style="list-style-type: none"> ▪ <i>Asterella angusta</i> (Steph.) Kachroo.

	Walker and Sanders	<ul style="list-style-type: none"> ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
26)	<i>Scutellospora persica</i> (Koske and Walker) Walker and Sanders	<ul style="list-style-type: none"> ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
27)	<i>Scutellospora tricalypta</i> (Herrera and Ferrer) Walker and Sanders	<ul style="list-style-type: none"> ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.
28)	<i>Scutellospora weresubi</i> Koske and Walker	<ul style="list-style-type: none"> ▪ <i>Plagiochasma rupestre</i> (Forst.) Steph. ▪ <i>Targionia hypophylla</i> Linn. ▪ <i>Asterella angusta</i> (Steph.) Kachroo. ▪ <i>Reboulia hemisphaerica</i> (Linn.) Raddi. ▪ <i>Plagiochasma appendiculatum</i> Lehm.et. Lindenb. ▪ <i>Riccia gangetica</i> Ahmad. ▪ <i>Riccia discolor</i> Lehm.et. Lindenb.

Table: 4.3.2 AM fungal spores among bryophytes species of Melghat forest

Sr. No.	Host Plant	<i>Acaulospora</i>	<i>Gigaspora</i>	<i>Glomus</i>	<i>Scutellospora</i>
1	<i>Targionia hypophylla</i>	+	+	+	+
2	<i>Cyathodium tuberosum</i>	-	-	-	+
3	<i>Cyathodium cavernarum</i>	-	-	-	+
4	<i>Asterella angusta</i>	+	+	+	+
5	<i>Reboulia hemisphaerica</i>	+	+	+	+
6	<i>Plagiochasma appendiculatum</i>	-	+	+	+
7	<i>Plagiochasma intermedium</i>	+	-	+	+
8	<i>Plagiochasma rupestre</i>	+	-	+	+
9	<i>Riccia gangetica</i>	+	+	+	+
10	<i>Riccia discolor</i>	+	+	+	+
11	<i>Anthoceros erectus</i>	+	+	+	-
12	<i>Folioceros udarii</i>	+	-	+	-
13	<i>Notothylas indica</i>	+	-	+	-
14	<i>Phaeoceros laevis</i>	+	+	+	-
15	<i>Funaria hygrometrica</i>	+	-	+	-
16	<i>Brachythemium turgidum</i>	-	-	+	-
17	<i>Bryum coronatum</i>	-	-	-	-
18	<i>Stereophyllum decorum</i>	-	-	-	-
19	<i>Hyophila involuta</i>	-	-	+	-
20	<i>Hymenostylium recurvirostre</i>	-	-	+	-

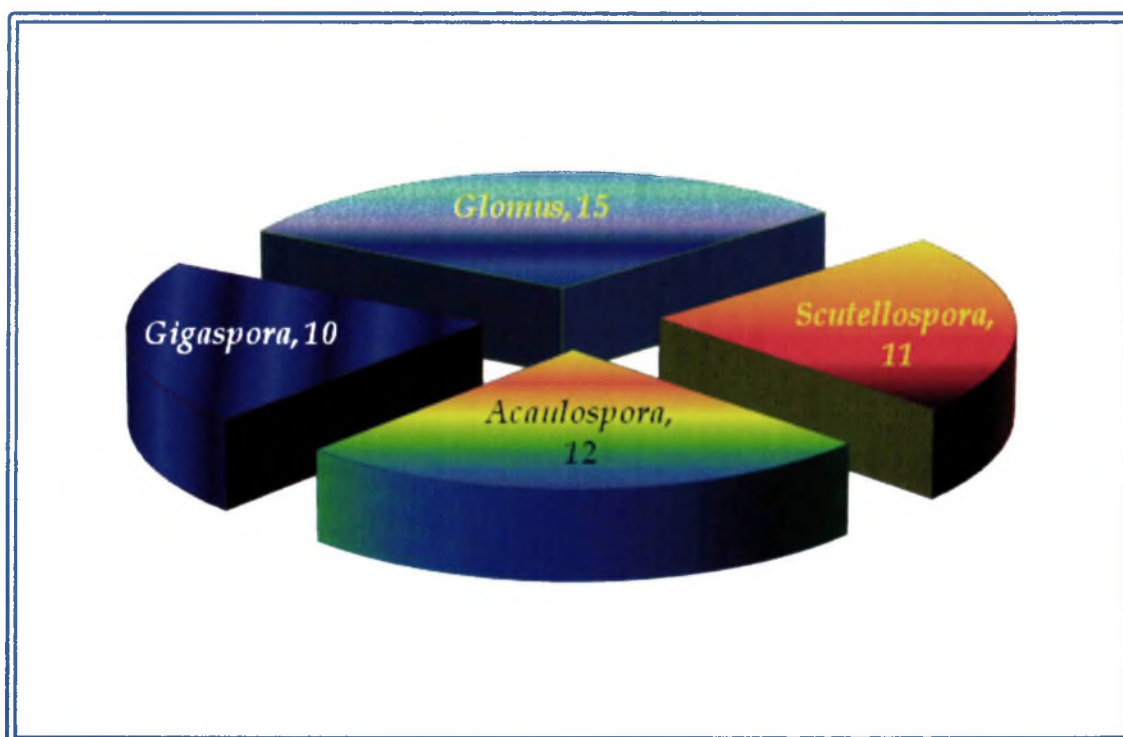
Fig: 4.3.2 AM fungal spores distribution among bryophytes

Table: 4.3.3 AM Fungal spores and root colonization among bryophytes species of Melghat forest

Sr. No.	Host Plant	Habitat	Location	% Rhizoidal Colonization	Spores Per 100g of soil
1	<i>Targionia hypophylla</i>	Saxicolous/clay	Semadoh	48.23	359
2	<i>Cyathodium tuberosum</i>	Terricolous/clay	Bhinkund	-	-
3	<i>Cyathodium cavernarum</i>	Rupicolous/marsh	Semadoh	-	-
4	<i>Asterella angusta</i>	Saxicolous/clay	Belkund	57.12	259
5	<i>Reboulia hemisphaerica</i>	Terricolous /marsh	Amazari	63.90	342
6	<i>Plagiochasma appendiculatum</i>	Terricolous /marsh	Chikhaldara	78.02	478
7	<i>Plagiochasma intermedium</i>	Saxicolous /clay	Gawilgarh walls	34.12	132
8	<i>Plagiochasma rupestre</i>	Terricolous /clay	Koha	72.06	421
9	<i>Riccia gangetica</i>	Terricolous/forest soil	Khongada	23.85	213
10	<i>Riccia discolor</i>	Terricolous/forest soil	Churani	25.12	190
11	<i>Anthoceros erectus</i>	Terricolous/forest soil	Semadoh	33.47	210
12	<i>Folioceros udarii</i>	Terricolous/forest soil	Semadoh	13.21	132
13	<i>Notothylas indica</i>	Terricolous/clay	Amazari	29.12	198
14	<i>Phaeoceros laevis</i>	Terricolous/clay	Semadoh	26.12	157
15	<i>Funaria hygrometrica</i>	Terricolous/clay	Gugamal	22.43	214
16	<i>Brachythidium turgidum</i>	Epiphytic/tree	Gawilgarh	-	-
17	<i>Bryum coronatum</i>	Terricolous /clay	Ghatang	21.01	137
18	<i>Stereophyllum decorum</i>	Epixylic/tree	Bori	-	-
19	<i>Hyophila involuta</i>	Epilithic/clay	Kolkhas	-	78
20	<i>Hymenostylium recurvirostre</i>	Epilithic/clay	Madaki	-	69

4.3.3 Density and occurrence of AM fungal spores

The AM Fungal density, diversity and dominant taxon were found from different experimental sites and recorded from different bryophytic thalli. Plants like *Plagiochasma appendiculatum* (478), *Plagiochasma rupestre*, *Asterella angusta*, *Targionia hypophylla*, and *Reboulia hemisphaerica* showed maximum number of spore density while plants like *Riccia discolor*, *Riccia gangetica*, *Anthoceros erectus*, *Notothylas indica*, *Phaeoceros laevis*, *Folioceros udarii*, *Funaria hygrometrica* and *Bryum coronatum*, *Hyophila involuta* have shown intermediate density whereas *Hymenostylium recurvirostre* (69) depicted minimum value of spore density. Maximum rhizoidal colonization of 78 % found in *Plagiochasma appendiculatum*

while less found in 13.21 % in *Folioceros udarii* species and no colonization recorded in *Cyathodium* and few mosses (Table: 4.3.3).

Table: 4.3.4 Structural details of VA mycorrhizae of liverworts among bryophytes species of Melghat forest

Sr. No.	Host Plant	Hyphae diameter μm	Appressoria formed	Looped hyphae	Vesicle shape	Vesicle size μm	Associated mycorrhizal fungi
1	<i>Targionia hypophylla</i>	2.5	+	+	Round	20.02	Glomus sp.
2	<i>Cyathodium tuberosum</i>	-	-	-	-	-	-
3	<i>Cyathodium cavernarum</i>	-	-	-	-	-	-
4	<i>Asterella angusta</i>	3.1	+	+	Round	27.02	Glomus sp.
5	<i>Reboulia hemisphaerica</i>	4.2	+	+	Round	32.90	Glomus sp.
6	<i>Plagiochasma appendiculatum</i>	5.3	+	+	Elliptical	40.53	Glomus sp.
7	<i>Plagiochasma intermedium</i>	4.9	+	+	Elliptical	24.51	-
8	<i>Plagiochasma rupestre</i>	3.7	+	+	Round	27.02	-
9	<i>Riccia gangetica</i>	2.9	-	-	-	-	-
10	<i>Riccia discolor</i>	2.1	-	-	-	-	-
11	<i>Anthoceros erectus</i>	1.3	-	-	Globular	13.51	Glomus sp.
12	<i>Folioceros udarii</i>	1.4	-	-	-	-	-
13	<i>Notothylas indica</i>	1.1	-	-	Globular	17.21	Glomus sp.
14	<i>Phaeoceros laevis</i>	1.2	-	-	Globular	13.51	Glomus sp.
15	<i>Funaria hygrometrica</i>	+	-	-	Circular	40.53	Glomus sp.
16	<i>Brachythidium turgidum</i>	-	-	-	-	-	-
17	<i>Bryum coronatum</i>	+	-	-	Circular	38.12	Glomus sp.
18	<i>Stereophyllum decorum</i>	-	-	-	-	-	-
19	<i>Hyophila involuta</i>	+	-	-	-	-	-
20	<i>Hymenostylium recurvirostre</i>	+	-	-	-	-	-

4.3.2 Rhizoidal colonization among bryophytes

Although the occurrence of VAM fungal spores were a normal phenomenon from soil based isolation but their rhizoidal infections or root infections among the host plant determines a key attribute for host and fungus association. It has also been noted that, the collection sites were full of other plant vegetation. Bryophytes often allow the other vegetation of small grasses, small fronds, ferns, smaller angiosperms to grow in association rather combinations with the vegetation in particular microhabitat or niche. Hence, rhizoidal infections of VAM fungi needed to be explored for the authentic association between host plant and the fungus. Fresh thalli were isolated from selected sites and preserved for cytological study. The plant materials i.e. thallus and rhizoids were stained by Trypan blue and acid Fuchsin stain to study rhizoidal colonization of AM fungi (Plate: 25-28)

It was observed that the thallus of *Plagiochasma appendiculatum* possess larger scales and rhizoids while thallus near about 2-3 cm long or broad. Due to such giant thallus structures, heavy and vigorous vesicles were observed in this plant (Plate; 25 A-B). Vesicles and inter or intra cellular hyphae were clearly observed among the thallus structure. Vesicles were well connected by hyphae, hyphae ramifying across the cells with appresoria formation. Vesicles were rounded, globose or subglobose and 13-40 μm in diameter with blue coloured in Tryphan blue (Plate; 26 A-D) or pink coloured in acid Fuchsin (Plate; 28 A,F,G). No arbuscular structures were observed in all the bryophytic materials. Swollen infected rhizoids were also observed dominantly in *Plagiochasma* sp., and *Reboulia* sp. (Plate; 25 E-F) as a key feature of rhizoidal infections of VAM fungi. However, the ramifying hyphal network was reported very significantly in many rhizoids of *Plagiochasma* sp. (Plate; 25 A-B), *Reboulia* sp. (Plate; 26-A), *Riccia* sp. (Plate; 26-H), *Targionia* sp. (Plate; 26-F) and *Asterella* sp (Plate; 25 A-B).

The *Targionia* sp. showed presence of vesicles, single or in groups among the thallus and clearly observed as rounded, oval with 8-13 μm in diameter. Likewise, the thallus of *Riccia* sp. and *Asterella* sp. also showed infected fungal hyphae and vesicles. The most fascinating fungal infections were observed with hornworts *Anthoceros* sp., *Notothylas* sp. and *Phaeoceros* sp. (Plate; 27 D-F) with intra-radial vesicles on ramifying hyphae among all the thallus parts.

These vesicles were present in groups with globose, ovoid, circular in shape with distinct spore walls of 7-13 μm in diameter. Interestingly, the most delicate, sensitive plants i.e. mosses generally lack the true VAM fungal association. Nevertheless, the terrestrial mosses like *Funaria* sp., *Bryum* sp. (Plate; 25 G-F), *Hymenostylium* and *Hyophila* sp. (Plate; 27 G-H), showed presence of fungal infection in stem and leaves of the plant parts. Swollen or thick rhizoids were observed as a sign of AM fungal infections among many of the plant species like *Plagiochasma*, *Reboulia*, *Riccia*, etc. Dark pink coloured 13-16 μm *Glomus* spores were observed in *Funaria* plant at basal region (Plate; 28 B-C). The cortical mosses generally found to be without VAM fungal association.

4.3.4 Spores in spore syndrome

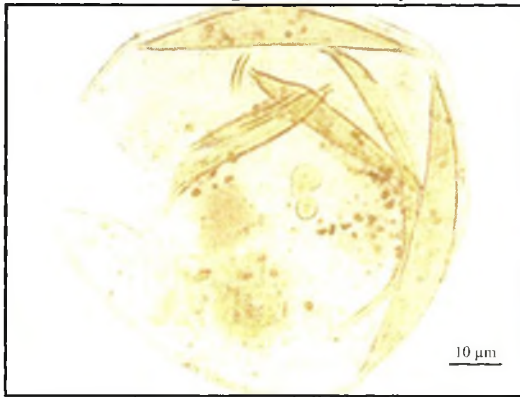
The presences of AM fungal spores inside the dead spores of other VAM fungal species were reported among few spores (Plate: 22 A-E).

Various studies have reported the presence of AM fungal spores inside the dead spores of other VAM fungal species (Koske *et al.*, 1986). This suggests that spores of AM fungi acts as a microhabitat when they are dead, apart from their normal role as propagules. It also suggests the ability of different AM fungal species to sporulate in close proximity to each other.

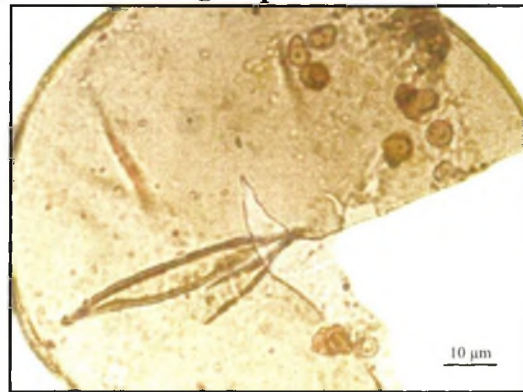
4.3.5 Spore germination shield

The unique occurrence of spore germination shield was observed in few spores inside the spore content (Plate: 22 F-H).

PLATE – 15
Morpho-diversity of *Acaulospora* VAM fungal spores



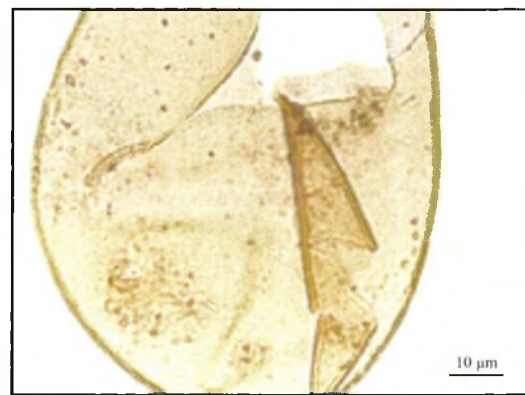
A. *Acaulospora delicata* (40x10)



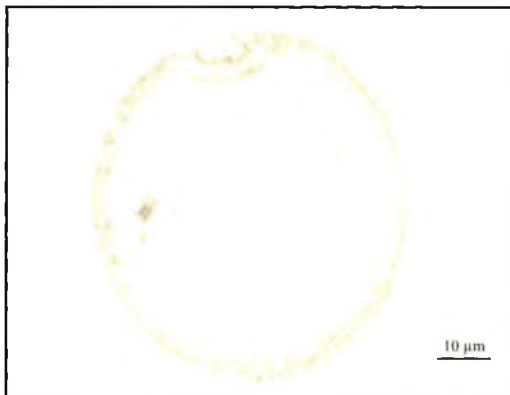
B. *Acaulospora delicata* (40x10)



C. *Acaulospora delicata* (40x10)



D. *Acaulospora delicata* (40x10)



E. *Acaulospora denticulata* (40x10)



F. *Acaulospora denticulata* (40x10)

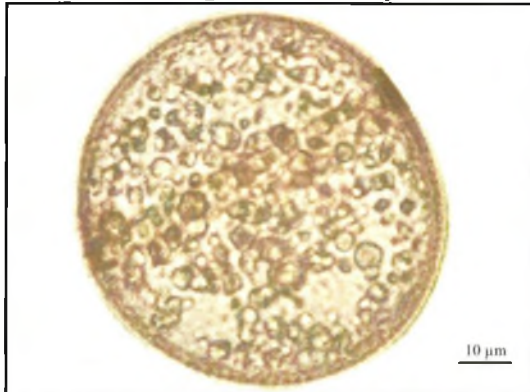


G. *Acaulospora mellea* (40x10)



H. *Acaulospora mellea* (40x10)

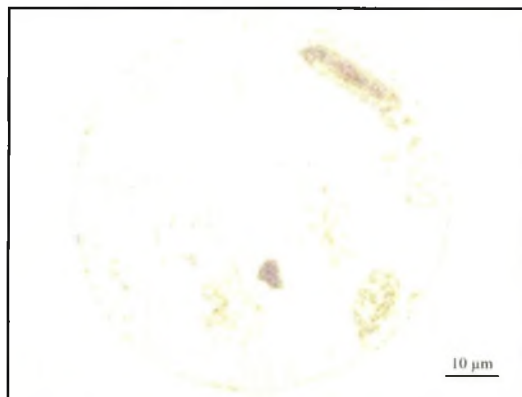
PLATE – 16
Morpho-diversity of *Acaulospora* VAM fungal spores



A. *Acaulospora myriocarpa* (40x10)



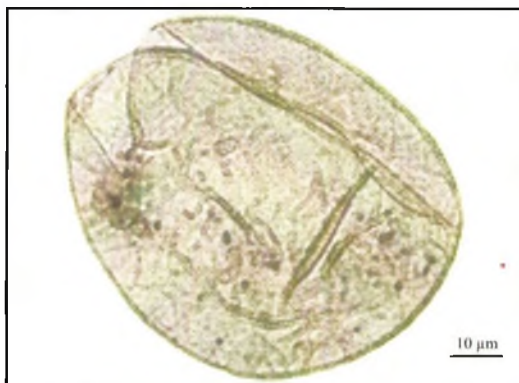
B. *Acaulospora nicolsonii* (40x10)



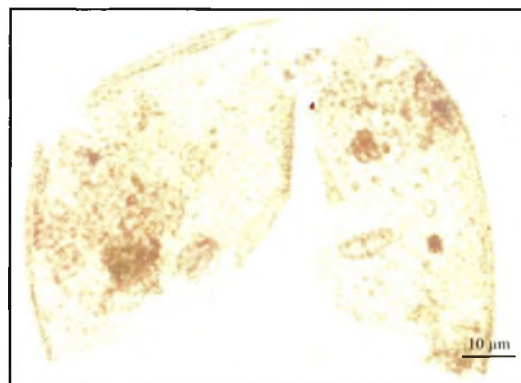
C. *Acaulospora rehmi* (40x10)



D. *Acaulospora rugosa* (40x10)



E. *Acaulospora rugosa* (40x10)



F. *Acaulospora rugosa* (40x10)



G. *Acaulospora rugosa* (40x10)

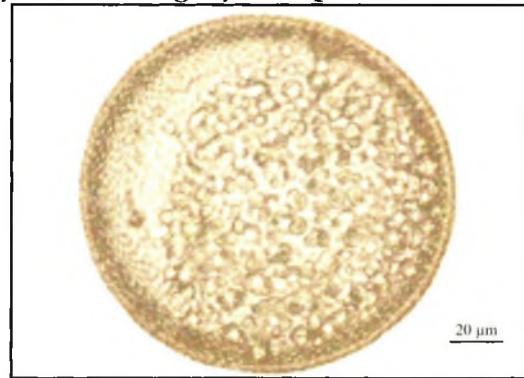


H. *Acaulospora rugosa* (40x10)

PLATE – 17
Morpho-diversity of *Acaulospora* and *Gigaspora* spores



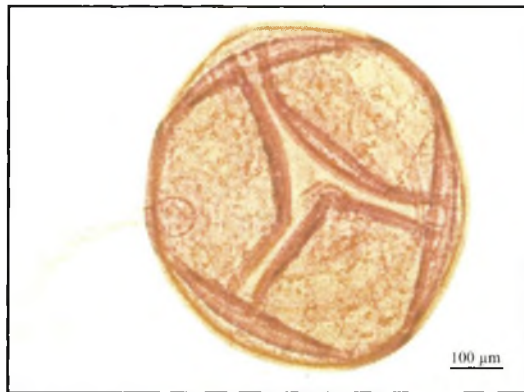
A. *Acaulospora rugosa* (40x10)



B. *Acaulospora scorbiculata* (40x10)



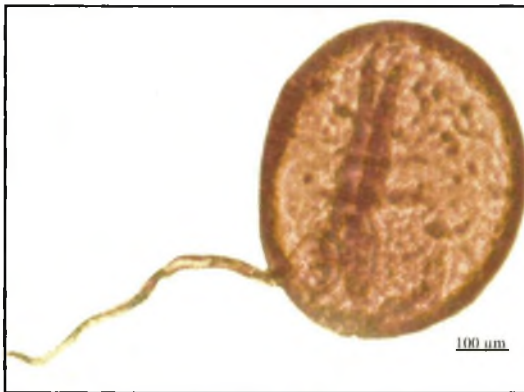
C. *Gigaspora albida* (40x10)



D. *Gigaspora gigantea* (40x10)



E. Bulbous suspensor in *G. gigantea* (40x10)



F. *Gigaspora rosea* (10x10)

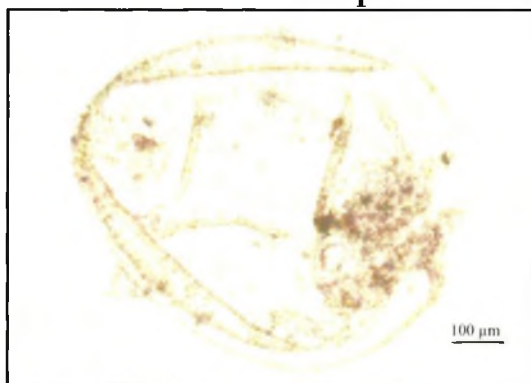


G. Bulbous suspensor in *G. rosea* (40x10)

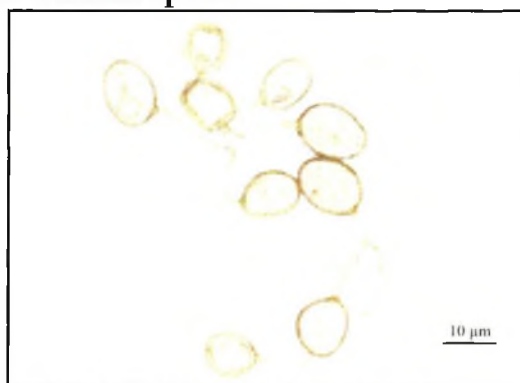


H. *Gigaspora rosea* (10x10)

PLATE – 18
Morpho-diversity of *Glomus* spores



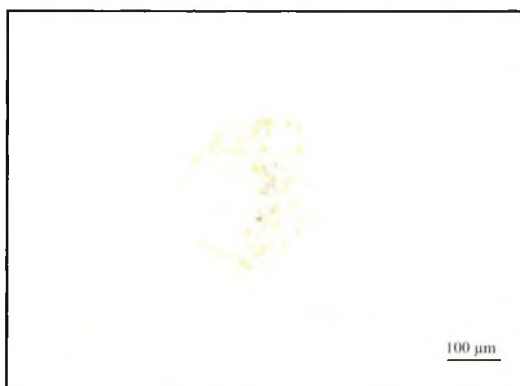
A. *Gigaspora rosea* (40x10)



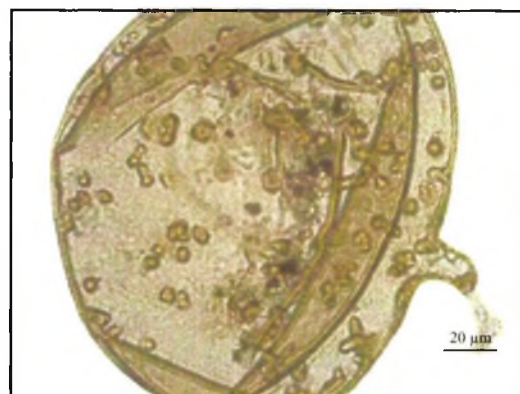
B. *Glomus aggregatum* (40x10)



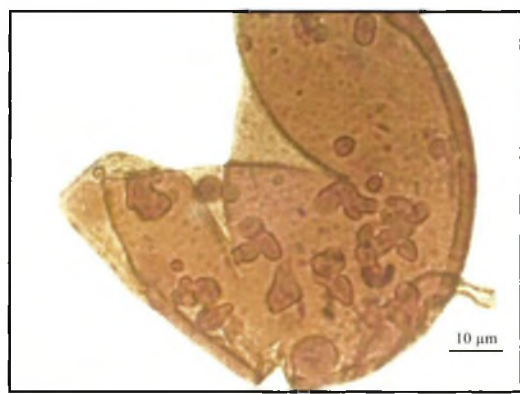
C. *Glomus aggregatum* (100x10)



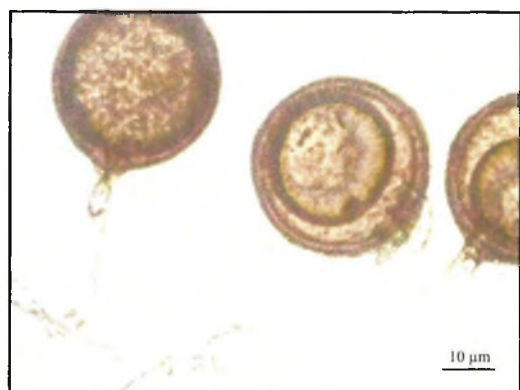
D. *Glomus albida* (10x10)



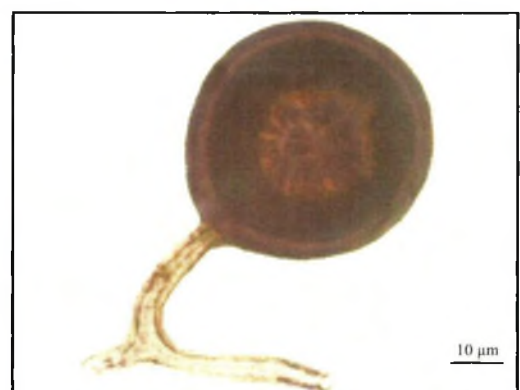
E. *Glomus albida* (40x10)



F. *Glomus albida* lipid bodies (40x10)

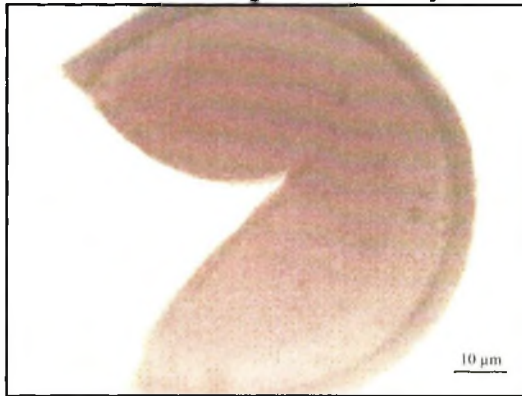


G. *Glomus citricola* (40x10)

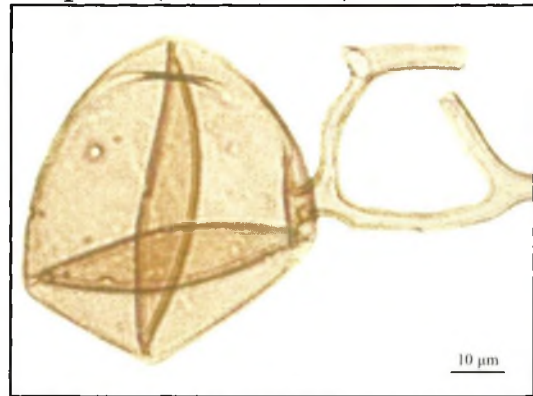


H. *Glomus citricola* (40x10)

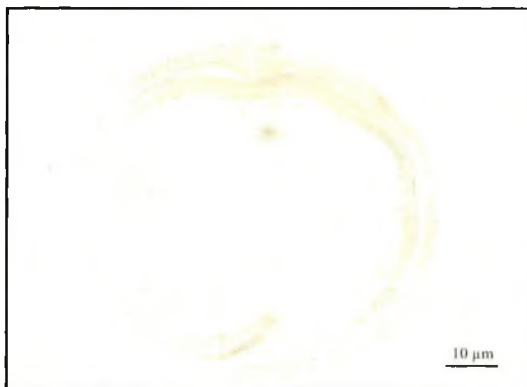
PLATE – 19
Morpho-diversity of *Glomus* spores (New Names)



A. *Funneliformis constrictum* (40x10)



B. *Rhizophagus diaphanum* (40x10)



C. *Rhizophagus diaphanum* (100x10)



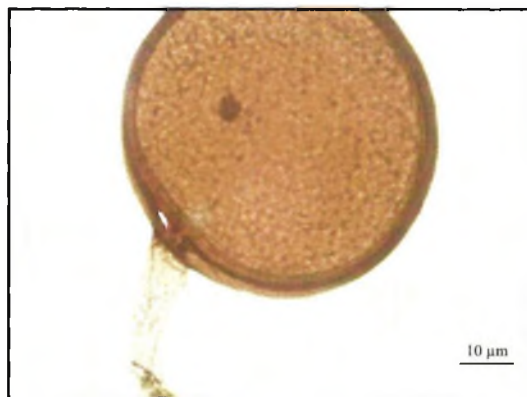
D. *Claroideoglomus etunicatum* (40x10)



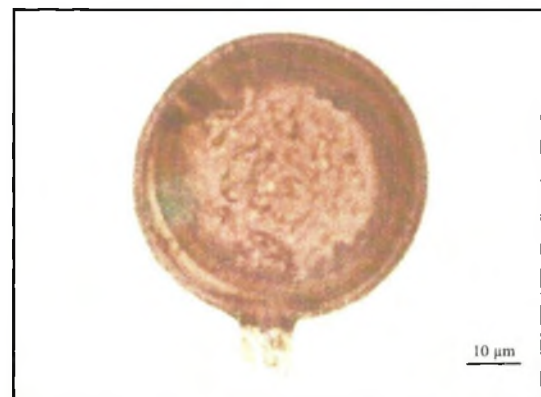
E. *Rhizophagus fasciculatum* (40x10)



F. *Funneliformis fragilistratum* (40x10)

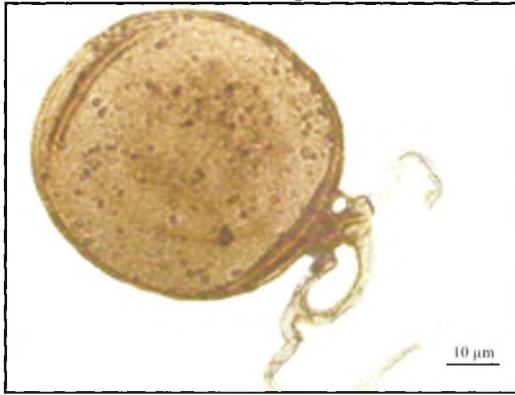


G. *Funneliformis geosporum* (40x10)



H. *Funneliformis geosporum* (40x10)

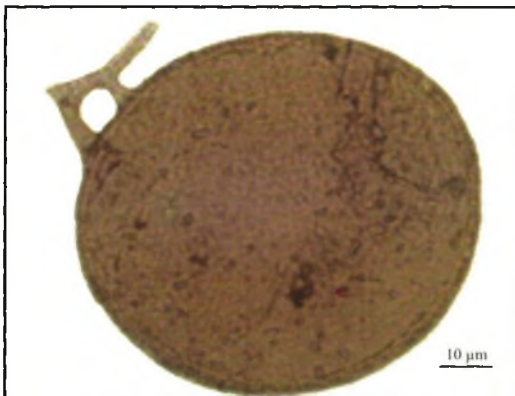
PLATE – 20
Morpho-diversity of *Glomus* species spores



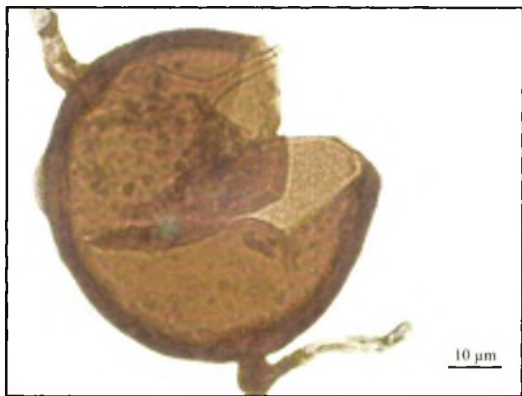
A. *Glomus glomerulatum* (40x10)



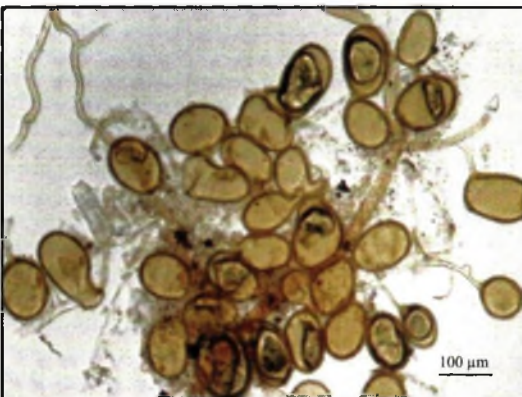
B. *Glomus glomerulatum* (40x10)



C. *Glomus glomerulatum* (40x10)



D. *Glomus glomerulatum* (40x10)



E. *Glomus rubiformis* (10x10)



F. *Glomus rubiformis* (40x10)



G. *Glomus rubiformis* (40x10)



H. *Glomus rubiformis* (100x10)

PLATE – 21
Morpho-diversity of *Scutellospora* species



A. *Glomus tenerum* (40x10)



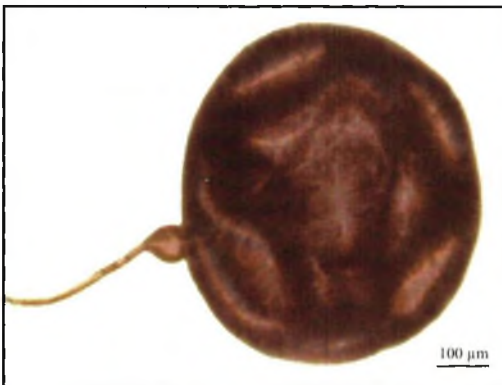
B. *Scutellospora auriglobosa* (40x10)



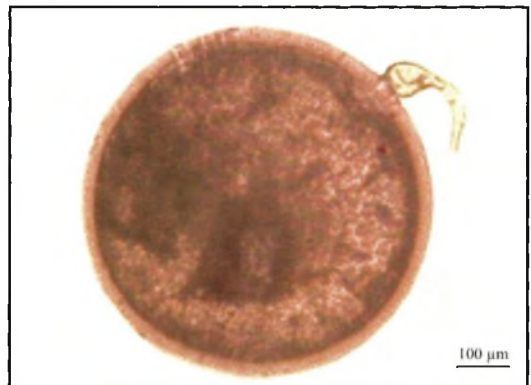
C. *Scutellospora pellucida* (40x10)



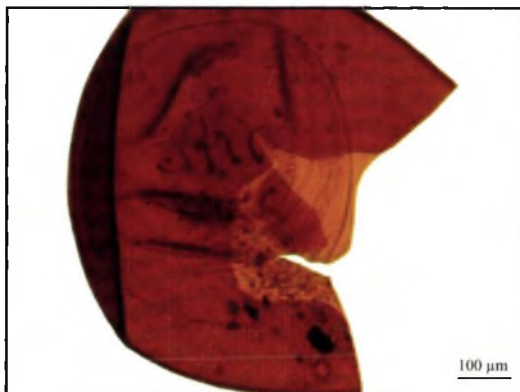
D. *Scutellospora pellucida* (40x10)



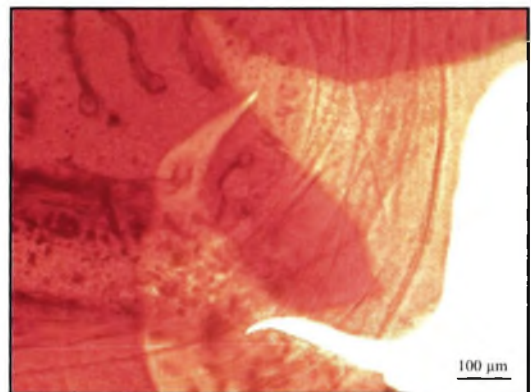
E. *Scutellospora persica* (10x10)



F. *Scutellospora tricalypta* (10x10)



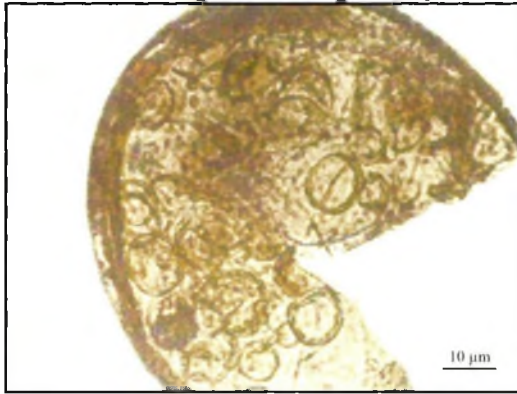
G. *Scutellospora weresubi* (10x10)



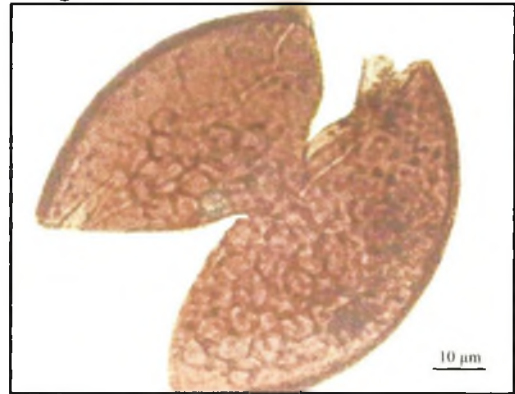
H. *Scutellospora weresubi* (40x10)

PLATE – 22

Spores in spore syndrome and germination shields



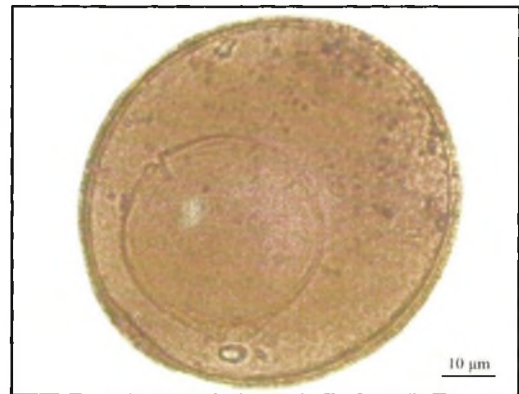
A. Spores in spore syndrome (40x10)



B. Spores in spore syndrome (40x10)



C. Spores in spore syndrome (40x10)



D. Spores in spore syndrome (40x10)



E. Spores in spore syndrome (40x10)



F. Spore germination shield (40x10)



G. Spore germination shield (40x10)

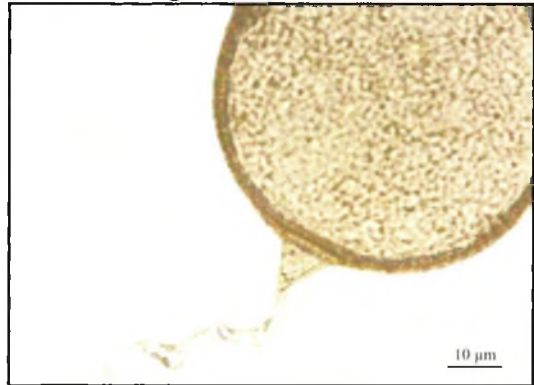


H. Spore germination shield (40x10)

PLATE – 23
Morphological aspects of *Glomus* at genus level



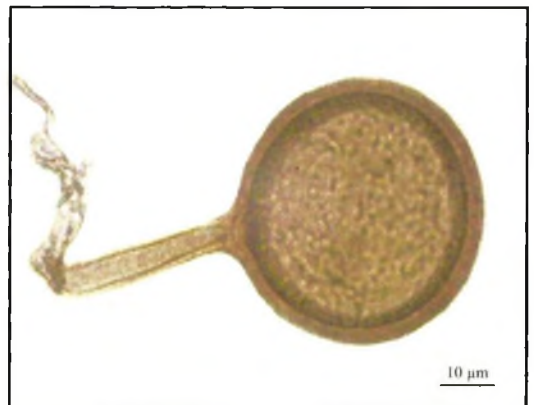
A. *Acaulospora* sp. (40x10)



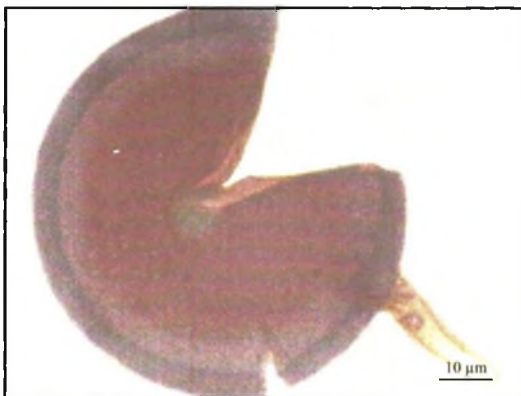
B. *Gigaspora* sp. (40x10)



C. *Glomus* sp. (40x10)



D. *Glomus* sp. (40x10)



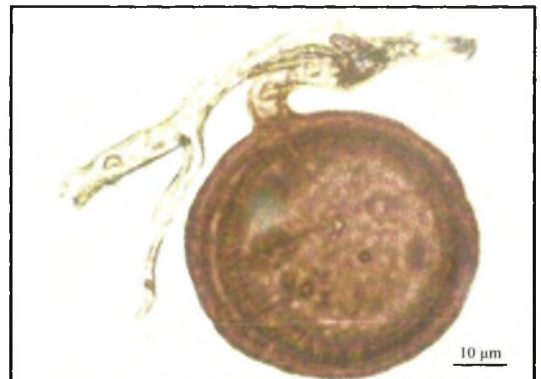
E. *Glomus* sp. (40x10)



F. *Glomus* sp. (40x10)

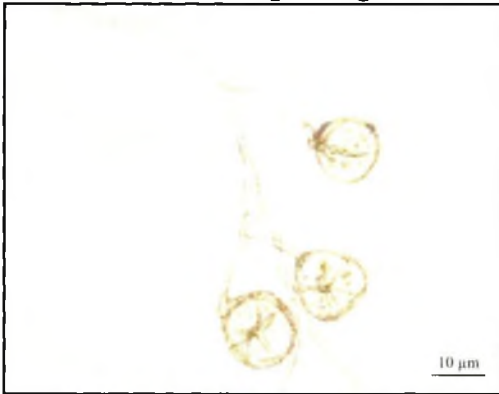


G. *Glomus* sp. (40x10)

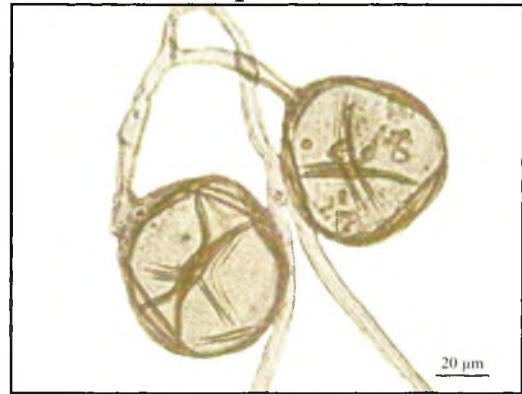


H. *Glomus* sp. (40x10)

PLATE – 24
Morphological studies of unidentified spores



A. Unidentified spore (10x10)



B. Unidentified spore (40x10)



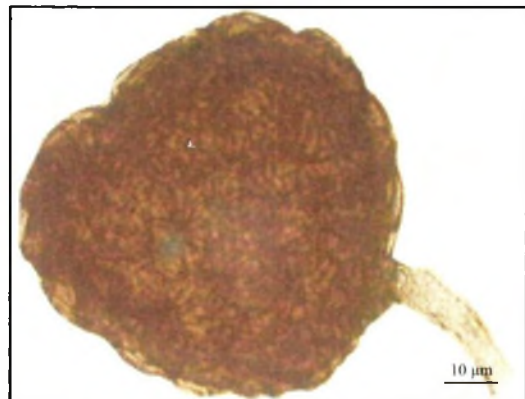
C. Unidentified spore (100x10)



D. Unidentified spore (40x10)



E. Unidentified spore (40x10)



F. Unidentified spore (40x10)



G. Unidentified spore (10x10)



H. Unidentified spore (40x10)

PLATE – 25

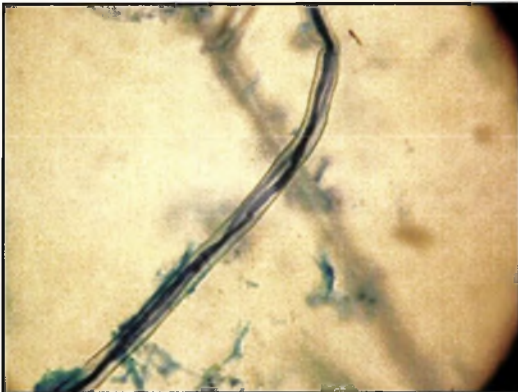
Trypan blue stained hyphal infections among rhizoids



A) *Plagiochasma* rhizoidal infection



B) *Plagiochasma* rhizoidal infection



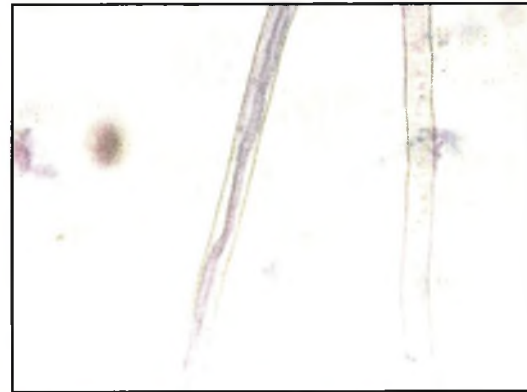
C) *Asterella* rhizoidal infection



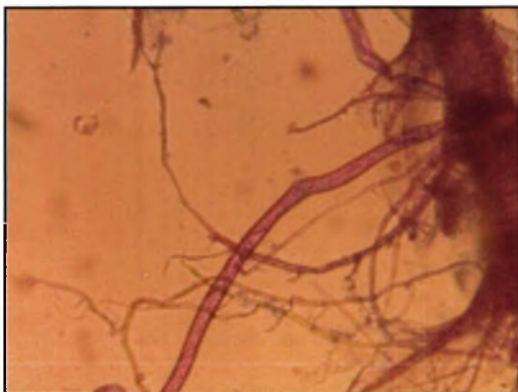
D) *Reboulia* rhizoidal infection



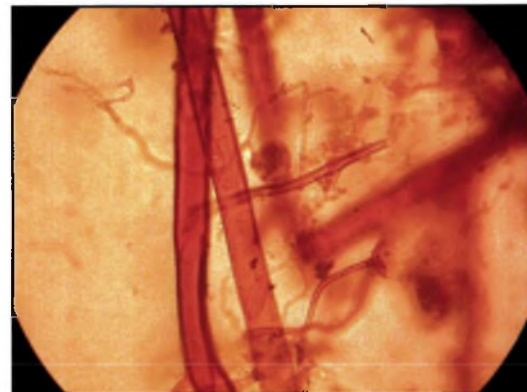
E) Swollen infected rhizoids



F) *Reboulia* rhizoidal infection



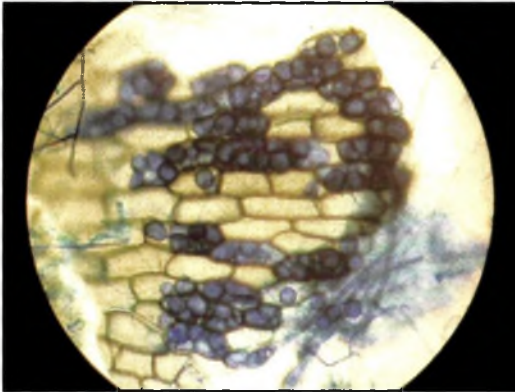
G) *Funaria* swollen rhizoids



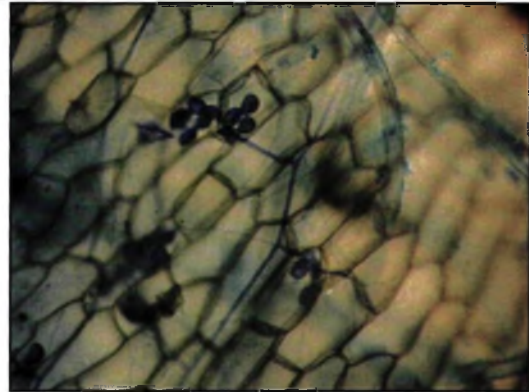
H) *Bryum* swollen rhizoids

PLATE – 26

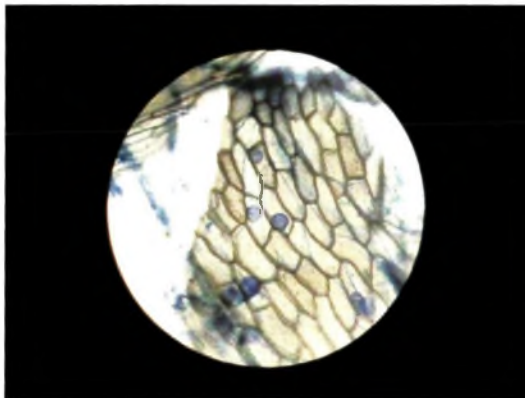
Trypan blue stained large vesicles in rhizoids



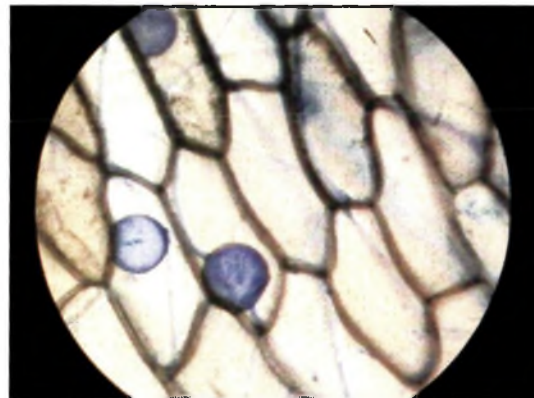
A) Heavy vesicles in *Plagiochasma*



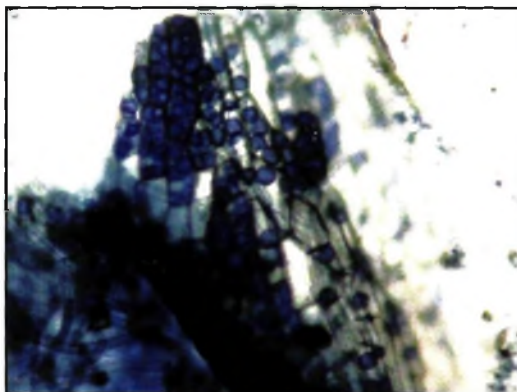
B) Hyphal network in *Plagiochasma*



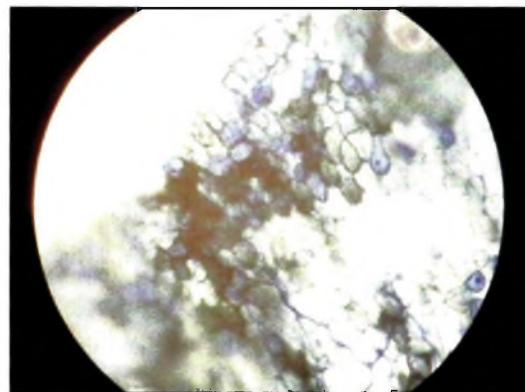
C) Vesicles present among scales



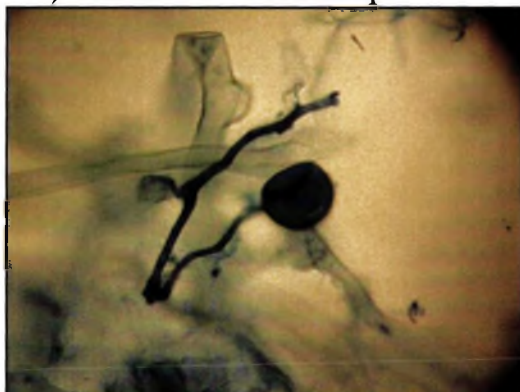
D) Large vesicles and hyphal ramify



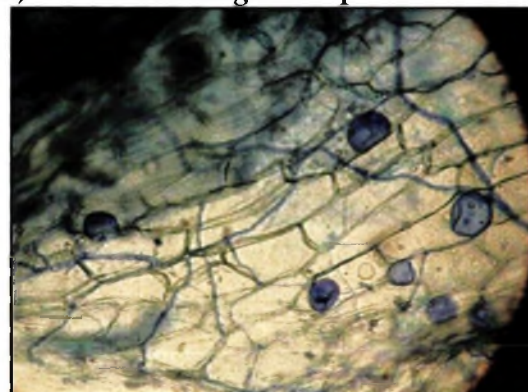
E) Vesicles in *Reboulia* sp.



F) Vesicles in *Targionia* sp.

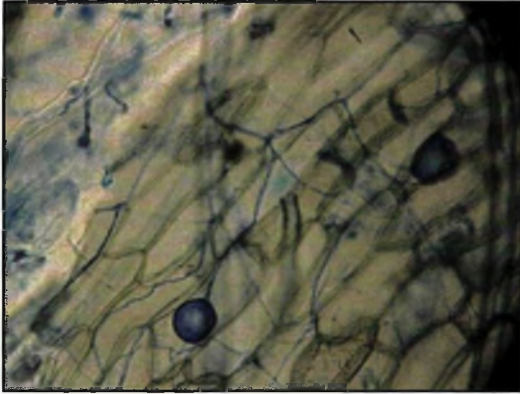


G) Vesicles in *Anthoceros* sp.



H) Vesicles in *Riccia* sp.

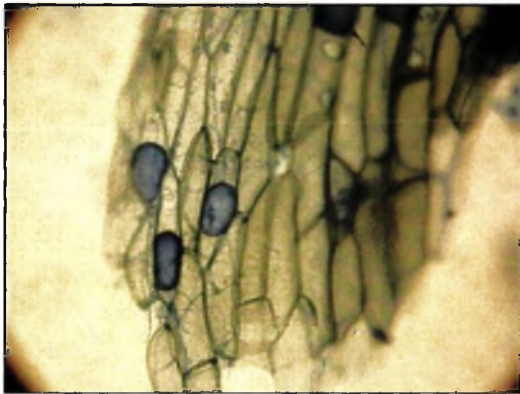
Abundant vesicles formation and mycorrhization



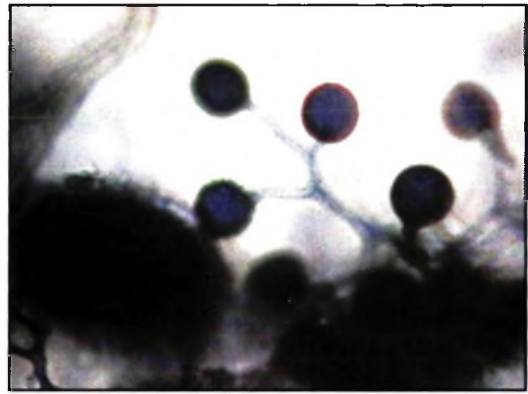
A. Hyphal network in *Reboulia* sp.



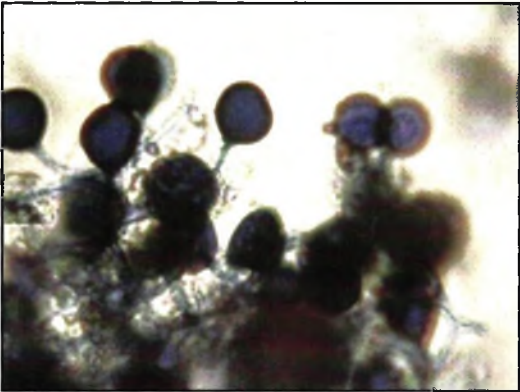
B. High degree of vesicles formation



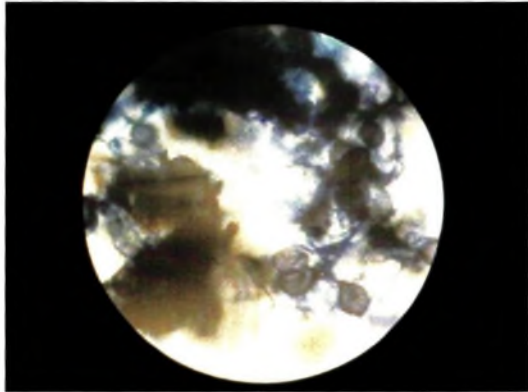
C. Vesicles in *Asterella* sp.



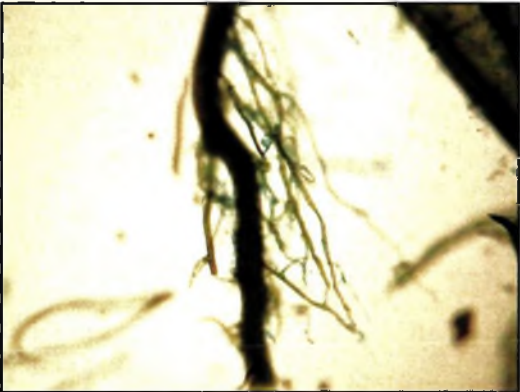
D. Vesicles in *Anthoceros* sp.



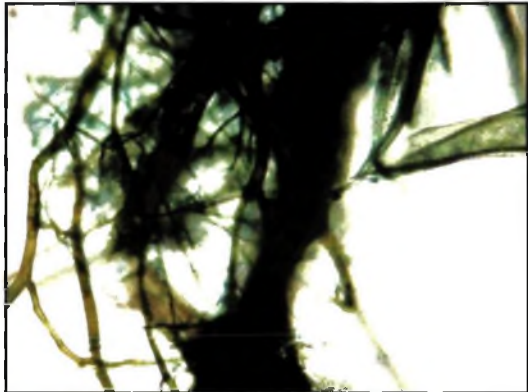
E. Vesicles in *Phaeoceros* sp.



F. Vesicles in *Folioceros* sp.

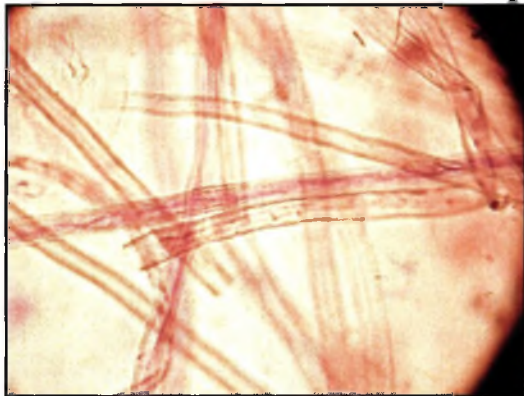


G. Rhizoids of *Hymenostylium* sp.

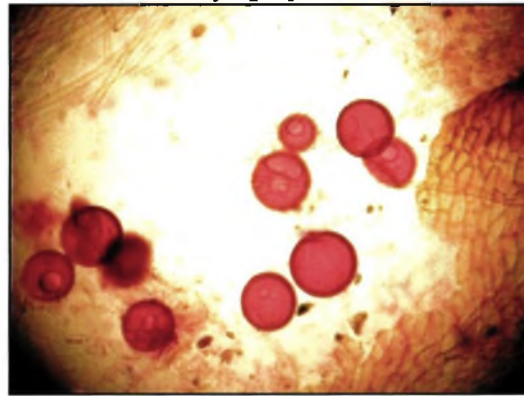


H. Rhizoids of *Hyophila* sp.

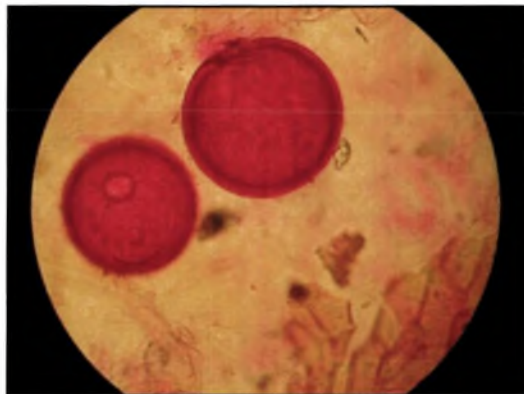
PLATE – 28
Acid Fuchsin stained pink vesicles in bryophytes



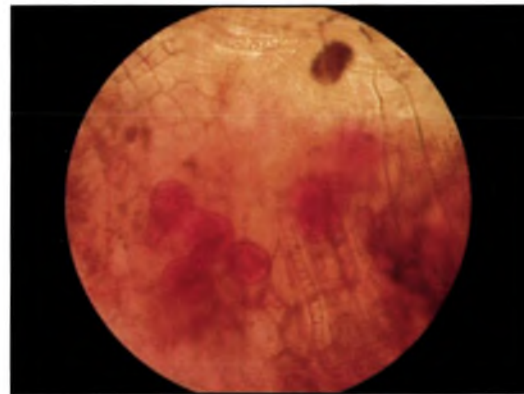
A) Rhizoidal infection in *Plagiochasma* sp.



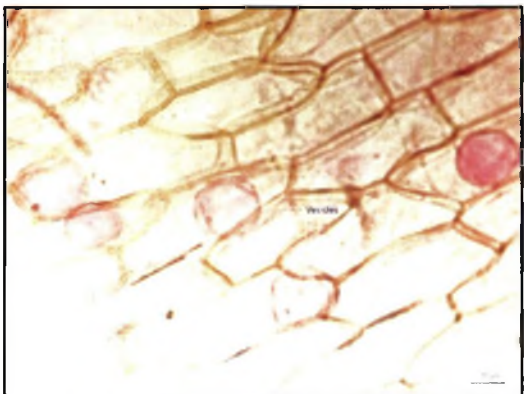
B) Spores in *Funaria* sp.



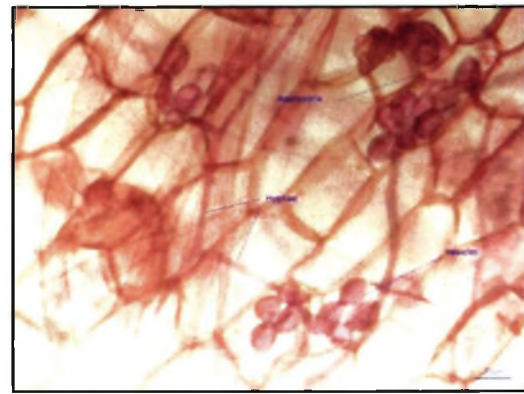
C) *Glomus* spores in *Funaria* sp.



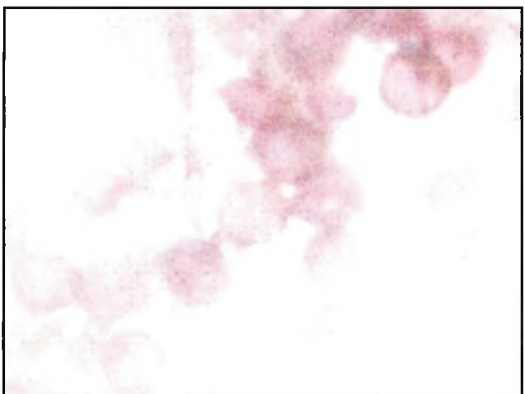
D. Spores in *Targionia* sp.



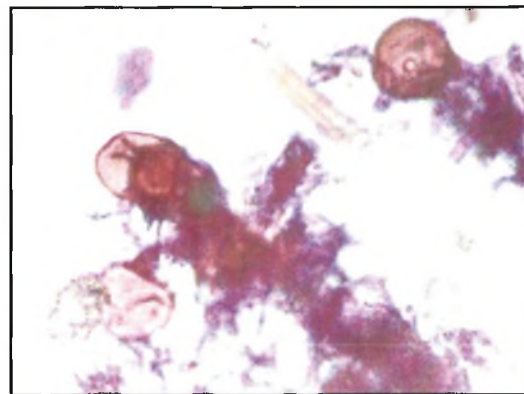
F) Acid fuchsin stained vesicles



G) Hyphal ramification



H) Vesicles in *Anthoceros* sp.



I. Vesicles in *Targionia* sp

4.4 Antimicrobial activity of bryophytes

When an organism liberates antimicrobial substances in nature, it may imply that the organism is at advantage, ecologically over the susceptible microbes at the respective micro-niche. Antibiosis in the real sense of the term has been previously demonstrated in bryophytes like moss *Sphagnum* either *in-vivo* or *in vitro* culture.

In present investigation, the occurrence of antimicrobial substances in experimental bryophyte species like, *Plagiochasma appendiculatum* Lehm. et. Lindenb., *Targionia hypophylla* L., *Cyathodium tuberosum* Kash, *Reboulia hemispherica* (L) Radii, *Riccia discolor* Lehm. et. Lindenb., *Anthoceros erectus* Kash., *Funaria hygrometrica* Hedw. and *Hyophila involuta* was carried out by the author. These plants were selected based on their common and abundant occurrence in the region and considering their importance in conservation point of view. Fresh and dried plants were extracted with different solvents like distilled water, petroleum ether, ethanol, chloroform, methanol and acetone and made bioassay. The test microorganisms used for the antimicrobial activity were selected based on their pathogenicity against human beings. The test organisms selected are *Escherichia coli* (MTCC-729), *Proteus vulgaris* (MTCC-744), *Klebsiella pneumoniae* (MTCC-661), *Shigella flexneri* (MTCC-1457), *Staphylococcus aureus* (MTCC-96), *Pseudomonas aeruginosa* (MTCC-424), *Salmonella typhimurium* (MTCC-98), *Aspergillus niger* (MTCC-281), *Candida albicans* (MTCC-227) and *Rhizopus oryzae* (MTCC-554). The microbial cultures were revived and maintained in ideal lab conditions for time-to-time use. The antibacterial activity was carried out using different plant extracts against different microorganisms by disc-diffusion method.

Most of the extracts have shown positive result against at least one of the test organisms i.e. bacteria or fungi. The effect of crude extracts may be due to single compound or cumulative activity of two or more compounds (Banerjee and Sen, 1979). It is also noteworthy that the antimicrobial activities detected in plant depends upon its age, sex, physiological state, season of collection and its ecological niche. However, The plants species like *Cyathodium tuberosum* has not showed any significant results in all the tested extracts against any bacterial or fungal pathogens. No activity was recorded during the investigations. The susceptibility of liverworts was found more as compared to other hornworts and mosses and their antimicrobial sensitivity is described as below in the description.

4.4.1 Antimicrobial sensitivity test of *Plagiochasma appendiculatum*

(Plate: 29, A-J)

The various extracts of the liverwort *Plagiochasma appendiculatum* was found effective against most of the selected microorganisms with positive results. This plant showed the highest degree of sensitivity as compared to other species and forms of bryophytes collected from Melghat forest (Table: 4.4.1).

The aqueous extract of the plant was positive against the microorganisms like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungus *Aspergillus niger* and *Candida albicans* (Fig: 4.4.2-C). In petroleum ether extract no any zone of inhibition was observed among all test organisms (Fig: 4.4.2-A). The ethanol crude extract was the most sensitive to *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Shigella flexneri* and fungus *Aspergillus niger* and *Candida albicans* except fungus *Rhizopus oryzae* (Fig: 4.4.2-E). The chloroform extracts were interactive with *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Aspergillus niger* and *Candida albicans* (Fig: 4.4.2-B). Methanol extracts also showed sound microbial zone of inhibition against *Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*, *Aspergillus niger* and *Candida albicans* (Fig: 4.4.2-D).

The microorganisms like *Pseudomonas aeruginosa* and *Aspergillus niger* were sensitive to only acetone extract (Fig: 4.4.2-F). The ethanol, chloroform and methanol extracts were more sensitive as compared to other extracts like aqueous and acetone extracts (Fig: 4.4.2-A). Most reactive microorganisms to all crude extracts were found as *Escherichia coli* and *Aspergillus niger*. However, the microorganisms like *Shigella flexneri*, *Salmonella typhimurium*, and *Klebsiella pneumoniae* were less reactive to all the extracts (Table: 4.4.1).

The variations and diversity in results can be clearly configured from the present investigation. Most of the bacteria found sensitive to the different plant extracts against gram-negative bacteria rather than gram-positive bacteria. The antimicrobial sensitivity results compared with other test organisms and tabulated by representing graphically as below. This graphical representation showed that the extracts were sensitive to at least one test organisms. The extracts of this plant found more dark and saturated during extraction process by Soxhlet method and crude extraction method. (Plate: 29, A-J).

Table: 4.4.1 Antimicrobial sensitivity test of *Plagiochasma appendiculatum*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO
<i>Plagiochasma appendiculatum</i>	Aqueous	04	0	0	0	5	7	0	11	08	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	13	08	06	05	09	06	0	11	10	0
	Chloroform	10	0	11	0	0	0	08	09	09	0
	Methanol	07	04	05	07	0	0	0	07	06	0
	Acetone	0	0	0	0	0	08	0	11	0	0
Antibiotics	Tetracycline	18	16	21	19	22	21	19	-	-	-
	Nystatin	-	-	-	-	-	-	-	21	19	20

* Data represented in mean of three replicates.

EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744],

KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457],

SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424],

ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281],

CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

Fig: 4.4.1 Analysis of antimicrobial sensitivity of *Plagiochasma appendiculatum*

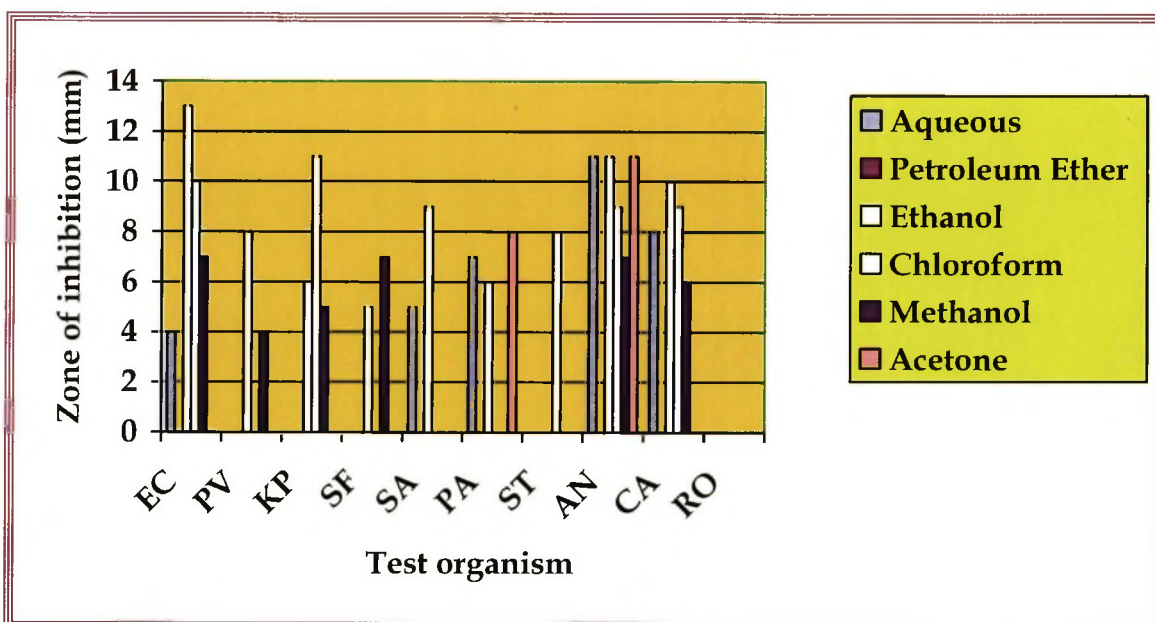
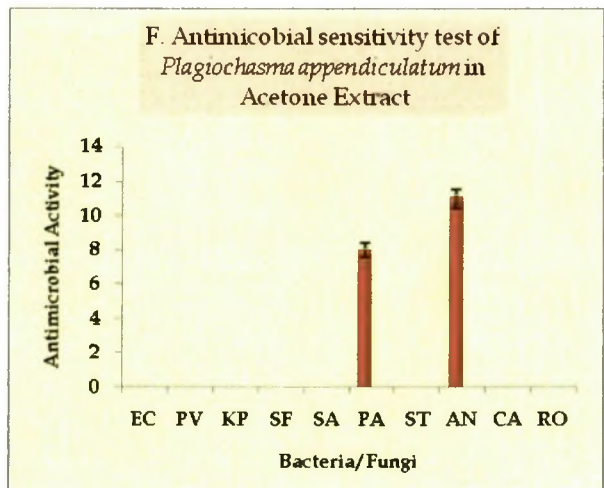
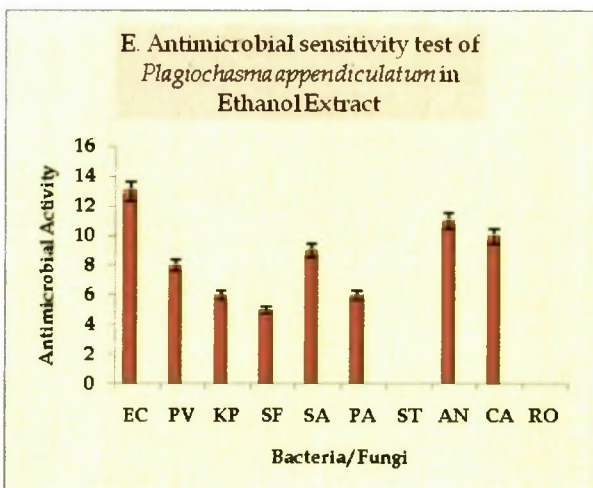
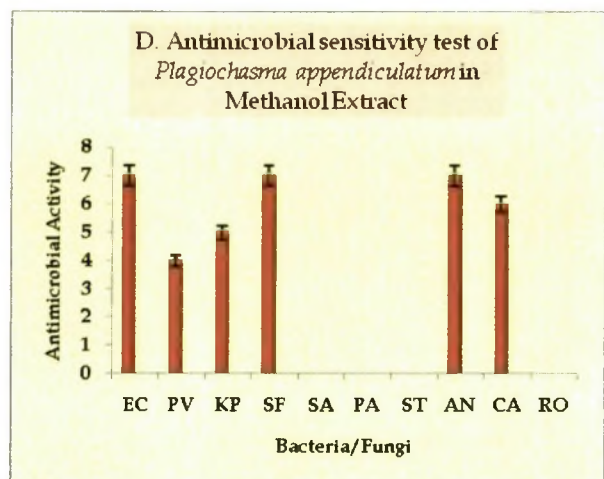
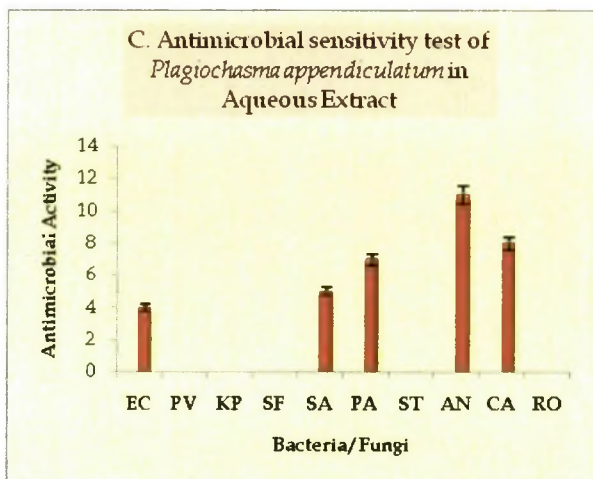
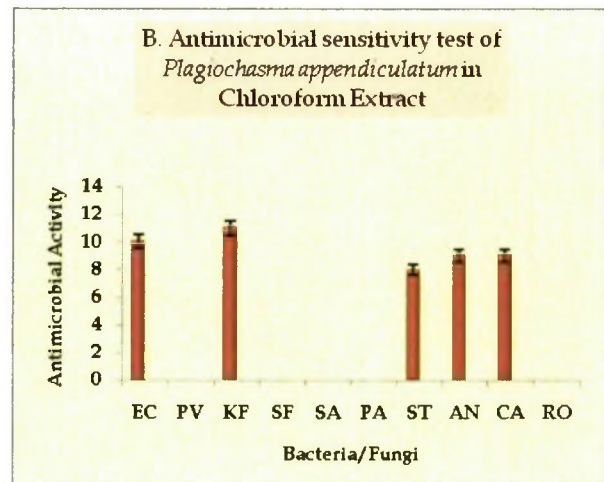
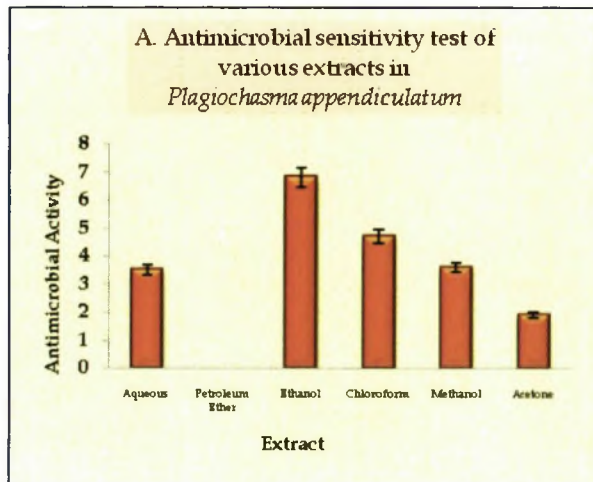


Fig: 4.4.2 Extracts analysis of the plant *Plagiochasma appendiculatum*



EC = *Escherichia coli* [MTCC-729], PV = *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA = *Staphylococcus aureus* [MTCC-96], PA = *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA = *Candida albicans* [MTCC-227], RO = *Rhizopus oryzae* [MTCC-554]

4.4.2 Antimicrobial sensitivity test of *Targionia hypophylla*

(Plate: 30, A-J)

The plant extracts of another liverwort *T. hypophylla* showed significant activity of antibiosis against at least one test microorganism (Fig: 4.4.3). The aqueous extract of the plants were inhibitory against *E. coli*, *S. aureus* and fungus *A. niger* (Fig: 4.4.4-C). However, the *petroleum ether* extracts showed no any response to the all microorganisms and reactions were nullified (Fig: 4.4.4-A). The ethanol extracts were more effective with broad spectrum of antibiosis against most of the microorganisms except the *S. flexneri* and *R. oryzae* (Fig: 4.4.4-E). The chloroform extracts of the plant showed positive result against six microorganisms i.e. *E. coli*, *K. pneumoniae*, *S. flexneri*, *S. typhimurium*, *A. niger* and *C. albicans* (Fig: 4.4.4-B). However, the methanol extracts of the plant was found positive against five selected microorganisms like *E. coli*, *K. pneumoniae*, *S. flexneri*, *S. typhimurium*, and *C. albicans* (Fig: 4.4.4-D). The acetone extracts showed very promising response of action in all the extracts except *K. pneumoniae* and *R. oryzae* (Fig: 4.4.4-F).

Among all the extracts, the petroleum ether extract was found non-reactive against the entire microorganism. However, slight induction was observed in some petri plates rarely but non-recordable. The aqueous extract is less reactive than the other extracts but ethanol, chloroform, methanol and acetone extracts were more responsive to most of the pathogens (Table: 4.4.2)

The maximum responses to all the extracts were found in microorganism *E. coli*, *S. typhimurium*, *A. niger* and *C. albicans* and less in *K. pneumoniae*, *S. flexneri*, and *S. aureus* or very less in *P. vulgaris*. Most of the extracts bear light green, yellow green, dark green and blackish green colour in crude form (Plate: 30, A-J).

Table: 4.4.2 Antimicrobial sensitivity test of *Targionia hypophylla*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]										AVERAGE
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO	
<i>Targionia hypophylla</i>	Aqueous	3	0	0	0	4	0	0	4	0	0	
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0	
	Ethanol	07	04	06	0	08	09	09	07	09	0	
	Chloroform	05	0	08	03	0	0	06	07	06	0	
	Methanol	06	0	05	06	0	0	07	0	08	0	
	Acetone	05	09	0	04	06	07	03	08	03	0	
	Ampicillin	22	20	27	29	34	31	30	-	-	-	
	Nystatin	-	-	-	-	-	-	-	31	29	28	

* Data represented in mean of three replicates.

***EC** = *Escherichia coli* [MTCC-729], **PV**= *Proteus vulgaris* [MTCC-744], **KP** = *Klebsiella pneumoniae* [MTCC-661], **SF** = *Shigella flexneri* [MTCC-1457], **SA**= *Staphylococcus aureus* [MTCC-96] , **PA**= *Pseudomonas aeruginosa* [MTCC-424], **ST** = *Salmonella typhimurium* [MTCC-98], **AN** = *Aspergillus niger* [MTCC-281], **CA**= *Candida albicans* [MTCC-227], **RO**= *Rhizopus oryzae* [MTCC-554]

Fig 4.4.3 Analysis of antimicrobial sensitivity of *Targionia hypophylla*

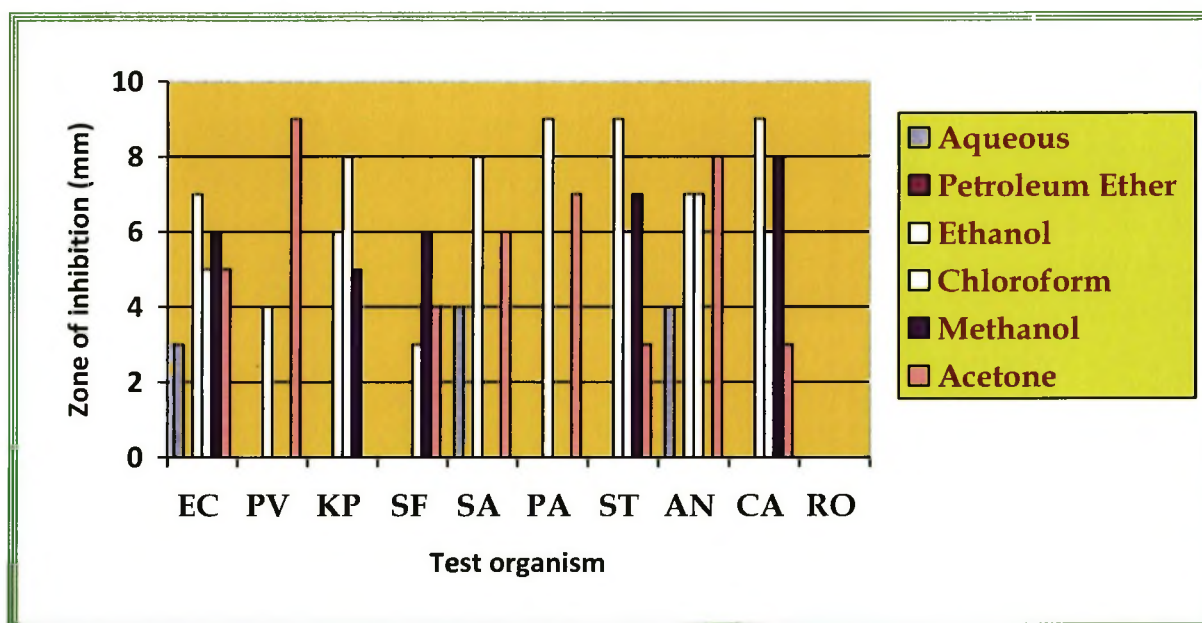
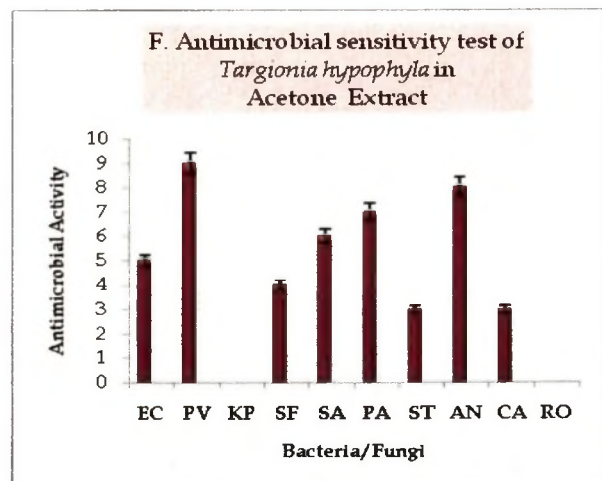
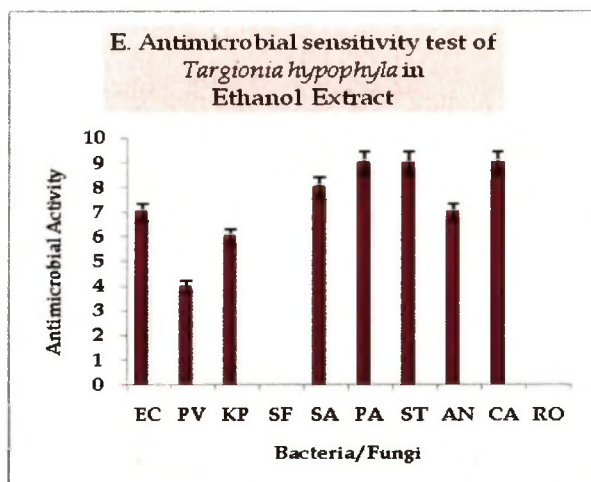
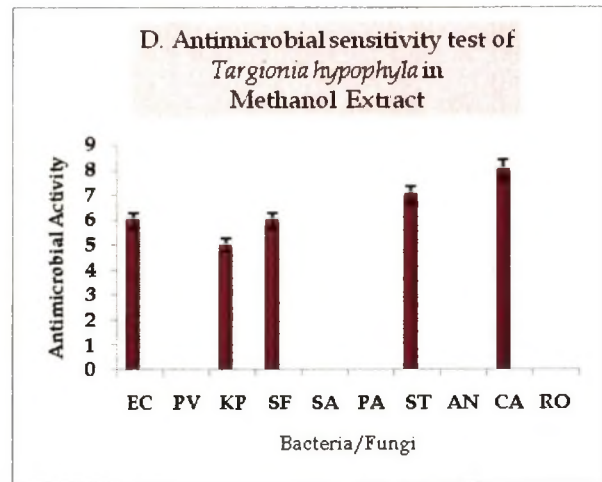
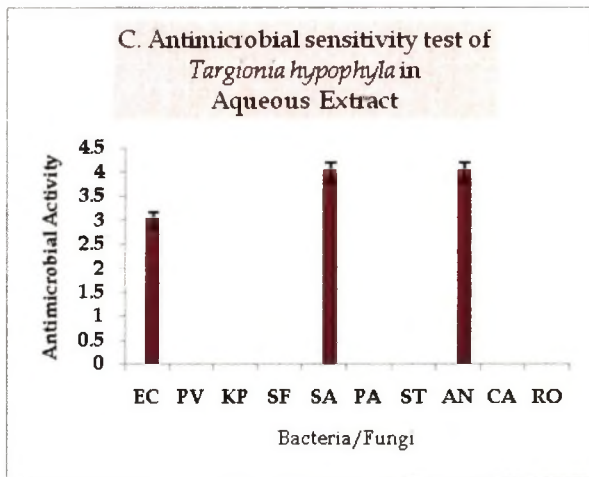
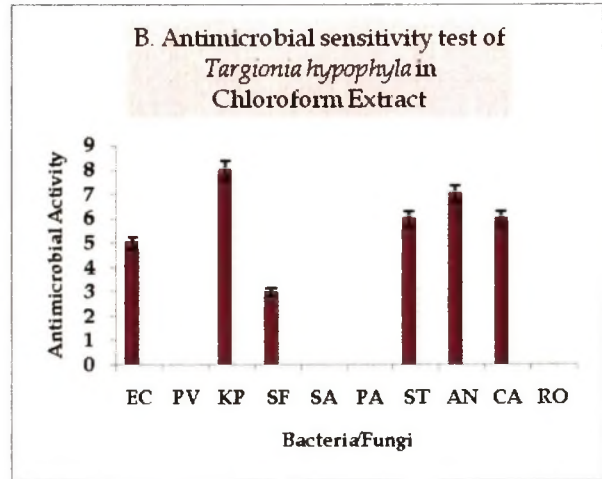
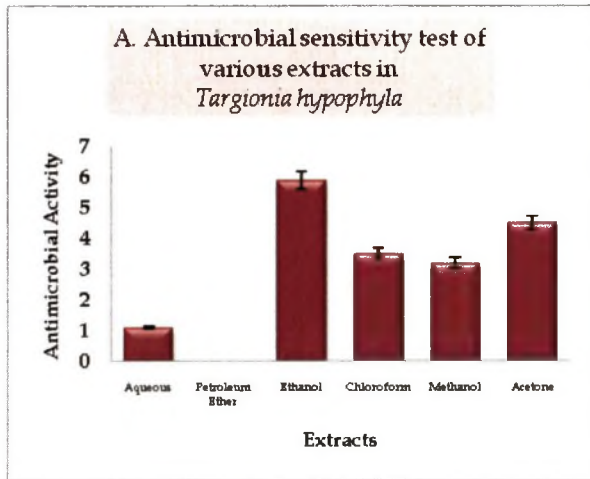


Fig : 4.4.4 Extracts analysis of the plant *Targionia hypophylla*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

4.4.3 Antimicrobial sensitivity test of *Riccia discolor*

(Plate: 31, A-J)

Riccia discolor, a thalloid liverwort showed antimicrobial response in various extracts (Fig: 4.4.5).

The aqueous extracts of the plant were interactive against three pathogens like *E. coli*, *S. aureus* and *A. niger* (Fig: 4.4.6-C). However, the petroleum ether extract was found non-reactive to almost all the microorganisms (Fig: 4.4.6-A). The ethanol extracts of the plant was found more responsive to the bacterial pathogens like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and fungus *C. albicans* (Fig: 4.4.6-E). The black green chloroform extracts were observed much reactive against micro organisms like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *S. typhimurium*, *A. niger* and *C. albicans* (Fig: 4.4.6-B). The acetone extract was found less reactive and observed the responses in pathogens like *P. vulgaris*, *S. flexneri*, and *A. niger* (Fig: 4.4.6-F). The methanol extract is of greenish colour and becomes dark on evaporation showing positive result in most of the microorganisms except *K. pneumoniae*, *P. aeruginosa* and *R. oryzae* (Fig: 4.4.6-D).

Among all the extracts, the aqueous and acetone extracts were found less reactive against pathogens. However, the promising results have been observed in ethanol, methanol and chloroform extract. The petroleum ether extract remained nullified (Fig: 4.4.6-A). The microbial zone of inhibition were observed more in microorganisms like *E. coli*, *P. vulgaris* and *A. niger*, followed by *S. aureus* and *A. niger*. Less zone of inhibition was found in the activity of *K. pneumoniae*, *S. flexneri*, *P. aeruginosa*, and *S. typhimurium*. The maximum zone of inhibition of 13 mm was observed in ethanol extract against pathogen *Candida albicans* (Table: 4.4.3).

Table: 4.4.3 Antimicrobial sensitivity test of *Riccia discolor*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO
<i>Riccia discolor</i> .	Aqueous	04	0	0	0	03	0	0	07	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	09	07	05	0	09	09	0	0	13	0
	Chloroform	07	06	03	0	04	0	07	04	09	0
	Methanol	07	08	0	05	0	06	07	06	07	0
	Acetone	0	03	0	07	0	0	0	04	0	0
	Penicillin	22	21	27	23	25	30	29	-	-	-
	Nystatin	-	-	-	-	-	-	-	30	28	31

* Data represented in mean of three replicates.

EC = *Escherichia coli* [MTCC-729], **PV**= *Proteus vulgaris* [MTCC-744], **KP** = *Klebsiella pneumoniae* [MTCC-661], **SF** = *Shigella flexneri* [MTCC-1457], **SA**= *Staphylococcus aureus* [MTCC-96] , **PA**= *Pseudomonas aeruginosa* [MTCC-424], **ST** = *Salmonella typhimurium* [MTCC-98], **AN** = *Aspergillus niger* [MTCC-281], **CA**= *Candida albicans* [MTCC-227], **RO**= *Rhizopus oryzae* [MTCC-554]

Fig: 4.4.5 Analysis of antimicrobial sensitivity of *Riccia discolor*

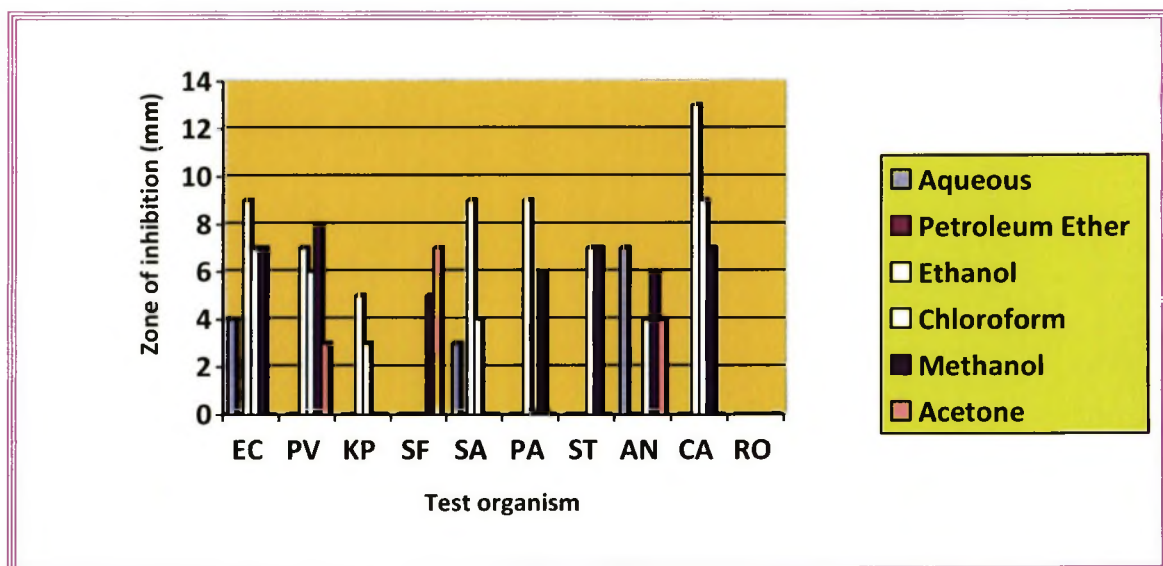
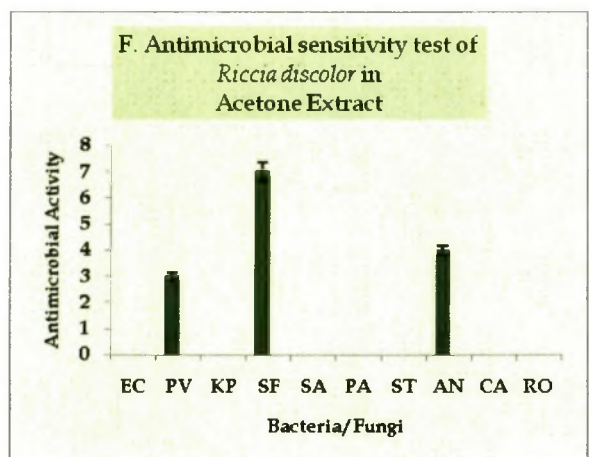
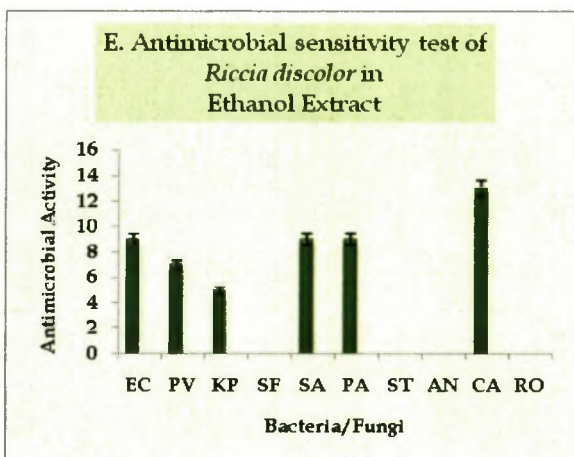
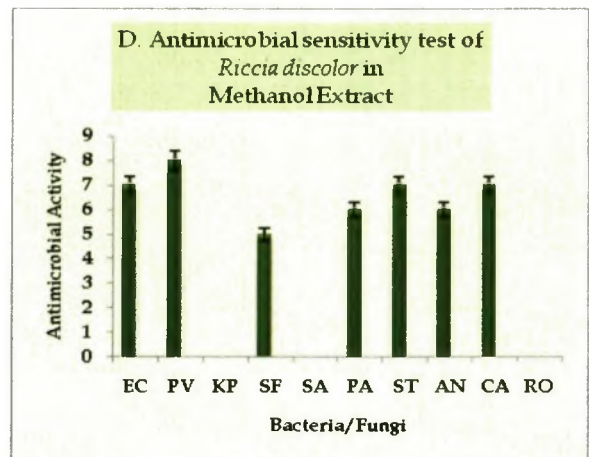
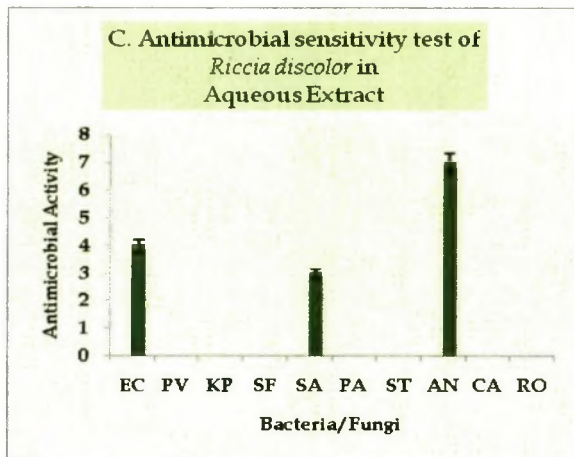
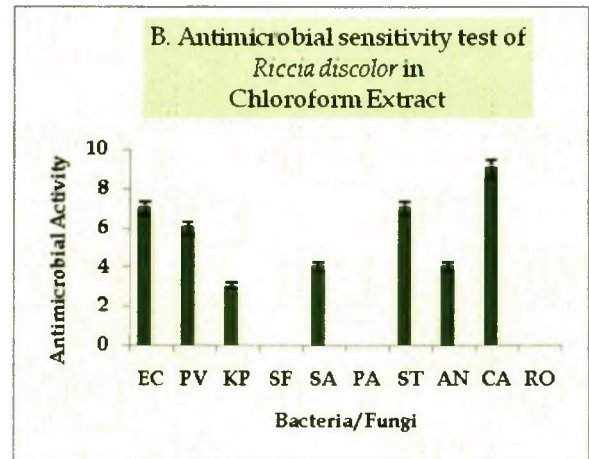
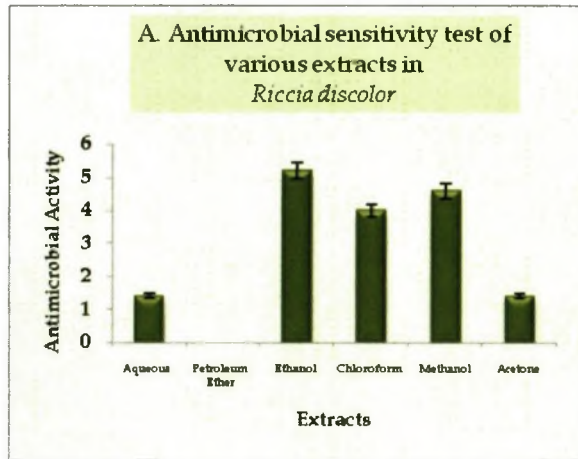


Fig : 4.4.6 Extracts analysis of the plant *Riccia discolor*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

4.4.4. Antimicrobial sensitivity test of *Reboulia hemisphaerica*

(Plate: 32, A-J)

Reboulia hemisphaerica a liverwort showed a very significant antibacterial activity against most of the microorganisms (Table: 4.4.4).

Aqueous extracts of the plant found effective against pathogens like *E. coli*, *S. aureus*, *P. aeruginosa*, *A. niger* and *C. albicans* (Fig: 4.4.8-C). The petroleum ether extract was almost non-reactive to the all microorganisms selected for activity (Fig: 4.4.8-A). The plant extract in solvent ethanol showed very promising results against microbial pathogens like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. flexneri*, *S. aureus*, *P. aeruginosa*, *A. niger* and *C. albicans* while null in bacteria *S. typhimurium* and fungus *R. oryzae* (Fig: 4.4.8-E). The extracts of chloroform showed positive antimicrobial response against six micro-organisms like *E. coli*, *P. vulgaris*, *S. aureus*, *S. typhimurium*, *A. niger* and *C. albicans* while non-reactive to the pathogens like *K. pneumoniae*, *S. flexneri.*, *P. aeruginosa* and *R. oryzae* (Fig: 4.4.8-B). The methanol extracts were observed effective against six test organisms like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *A. niger* and *C. albicans* while non reactive to *S. flexneri*, *P. aeruginosa*, *S. typhimurium.*, *A. niger* and *C. albicans* (Fig: 4.4.8-D). The green coloured acetone extract of the plant showed positive result against *S. flexneri*, *S. aureus*, *P. aeruginosa*, and *A. niger* mainly (Fig: 4.4.8-F).

Among all the extracts, the ethanol, methanol, and chloroform extracts were more effective as compared to the aqueous and acetone extract (Table: 4.4.4). However, petroleum ether extract was non-reactive to almost all microorganisms (Fig: 4.4.8-A). The microorganism *S. aureus* was not sensitive to all the extracts except petroleum ether, while *S. typhimurium* was least sensitive to all the extracts. The ethanol extract showed the maximum zone of inhibition of 13 mm against pathogen *E. coli* while least of 3 mm in aqueous extract. No effect was observed in fungus *Rhizopus oryzae* (Fig: 4.4.7)

Table: 4.4.4 Antimicrobial sensitivity test of *Reboulia hemisphaerica*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO
<i>Reboulia hemisphaerica</i> .	Aqueous	03	0	0	0	6	8	0	10	07	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	13	07	05	06	09	08	0	11	10	0
	Chloroform	08	03	0	0	5	0	07	09	08	0
	Methanol	08	05	04	0	09	0	0	07	06	0
	Acetone	0	0	0	0	06	08	0	09	0	0
	Tetracycline	22	23	26	24	25	28	27	-	-	-
	Nystatin	-	-	-	-	-	-	-	27	30	31

* Data represented in mean of three replicates.

EC = *Escherichia coli* [MTCC-729], **PV**= *Proteus vulgaris* [MTCC-744], **KP** = *Klebsiella pneumoniae* [MTCC-661], **SF** = *Shigella flexneri* [MTCC-1457], **SA**= *Staphylococcus aureus* [MTCC-96] , **PA**= *Pseudomonas aeruginosa* [MTCC-424], **ST** = *Salmonella typhimurium* [MTCC-98], **AN** = *Aspergillus niger* [MTCC-281], **CA**= *Candida albicans* [MTCC-227], **RO**= *Rhizopus oryzae* [MTCC-554]

Fig: 4.4.7 Analysis of antimicrobial sensitivity of *Reboulia hemisphaerica*

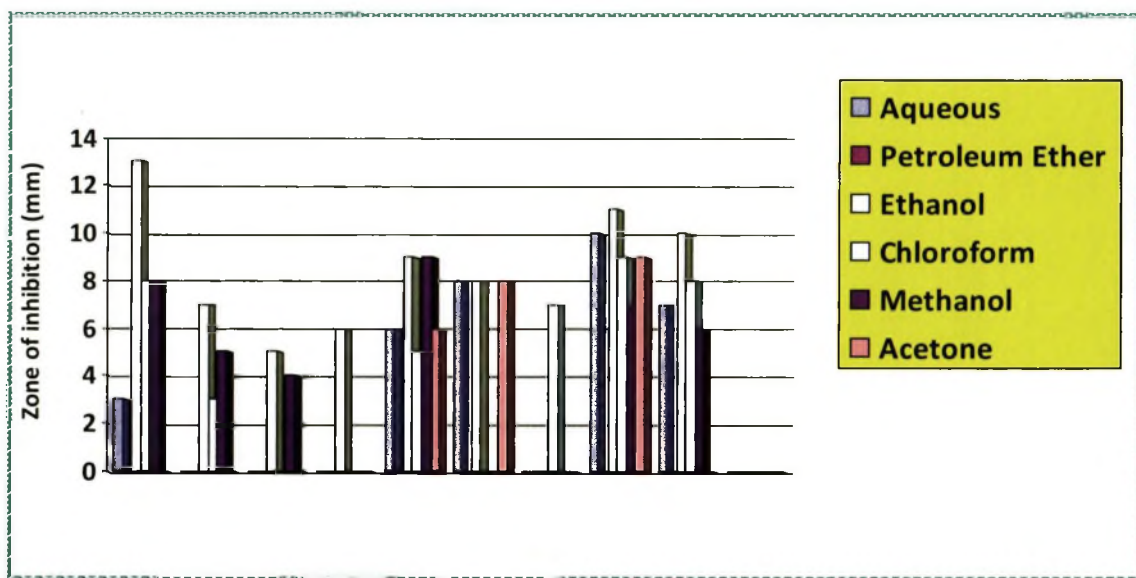
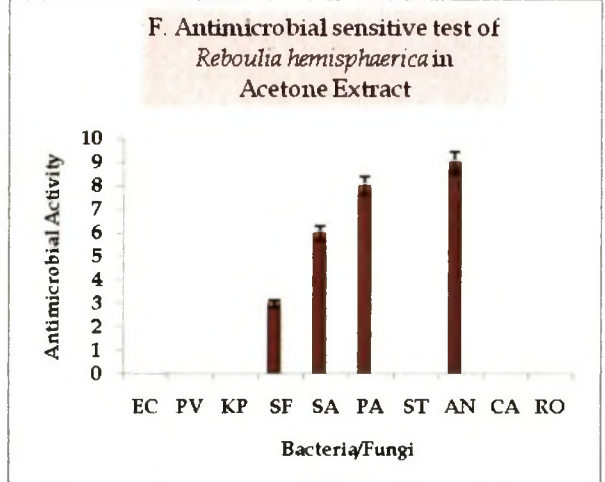
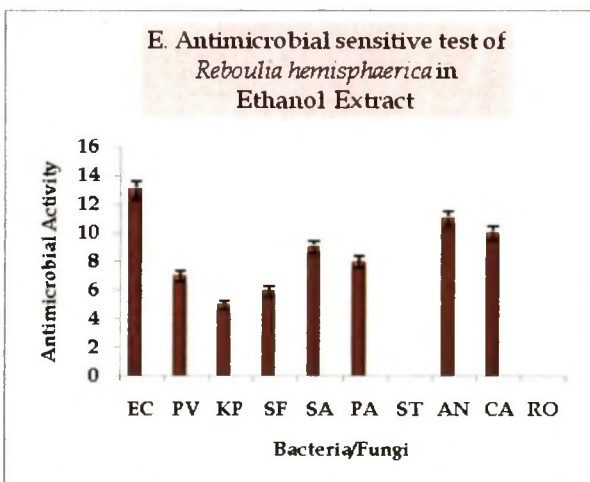
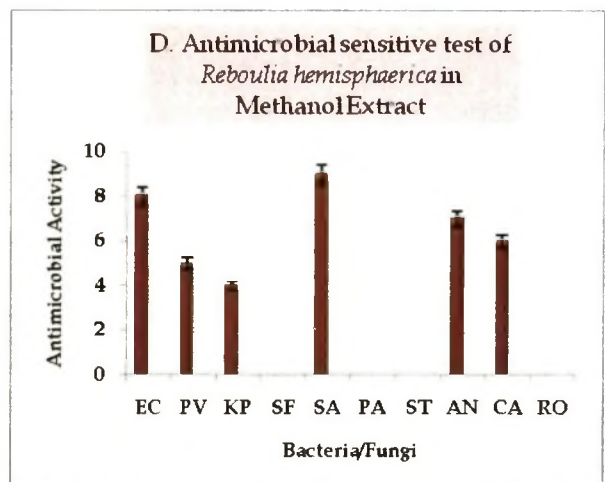
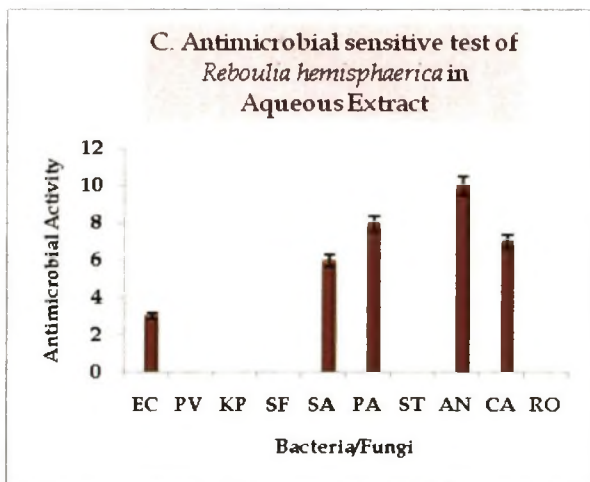
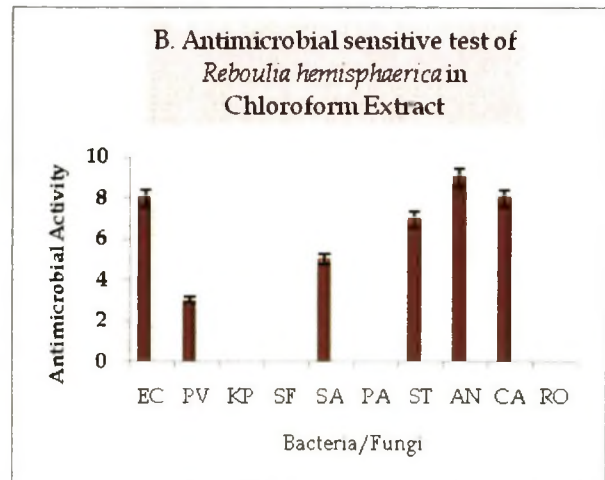
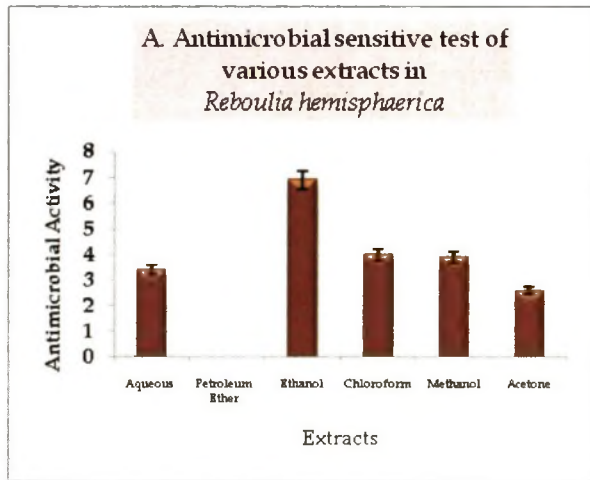


Fig: 4.4.8 Extracts analysis of the plant *Reboulia hemisphaerica*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

4.4.5. Antimicrobial sensitivity test of *Anthoceros erectus*

(Plate: 33, A-J)

Anthoceros erectus is a small, green liverwort on extraction in different solvents showed broad spectrum of antimicrobial activity (Table: 4.4.5).

The aqueous extract showed positive interaction with pathogens like *E. coli*, *S. aureus* and *A. niger* while rest pathogens remained as null (Fig: 4.4.8-C). However, the petroleum ether extract of the plant exhibited no significant interaction with any of the pathogens (Fig: 4.4.8-A). Subsequently, the ethanol extract found much sensitive with positive results against micro organisms like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *A. niger* and *C. albicans*, while the other microorganisms like *S. flexneri*, *S. typhimurium* and *R. oryzae* showed no any positive effect (Fig: 4.4.8-E). The greenish black coloured extract of chloroform was found sensitive to various pathogens with positive interaction like *E. coli*, *P. vulgaris*, *S. flexneri*, *S. aureus*, *S. typhimurium*, *A. niger* and *C. albicans* however, it did not show any interaction with the microorganisms like *K. pneumoniae*, *P. aeruginosa* and *R. oryzae* (Fig: 4.4.8-B). The methanolic extract of the plant was sensitive to microorganisms, like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, *A. niger* and *C. albicans* except *S. flexneri* and *R. oryzae* (Fig: 4.4.8-D). The acetonetic extract of the plant showed least response against microorganisms like *P. vulgaris*, *S. flexneri* and *A. niger* while non-reactive to other pathogens (Fig: 4.4.8-F).

The aqueous and acetone extract of the plant found less sensitive to all the pathogens as compared to the ethanol, chloroform and methanol extracts of the selected plant (Fig: 4.4.8-A). The ethanolic extract showed highest zone of inhibition of 8 mm against *E. coli* and lowest zone of inhibition of 3 mm against pathogen *P. vulgaris* in acetone extract. The ethanolic extract showed consistently positive result against maximum microbial pathogens (Fig: 4.4.9).

Table: 4.4.5 Antimicrobial sensitivity test of *Anthoceros erectus*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO
<i>Anthoceros erectus</i> .	Aqueous	05	0	0	0	04	0	0	08	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	08	06	04	0	07	05	0	07	10	0
	Chloroform	04	05	0	03	04	0	06	07	06	0
	Methanol	06	07	6	0	6	04	07	06	08	0
	Acetone	0	03	0	3	0	0	0	04	0	0
	Tetracycline	21	26	23	27	29	30	29	-	-	-
	Nystatin	-	-	-	-	-	-	-	27	30	34

* Data represented in mean of three replicates.

*EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

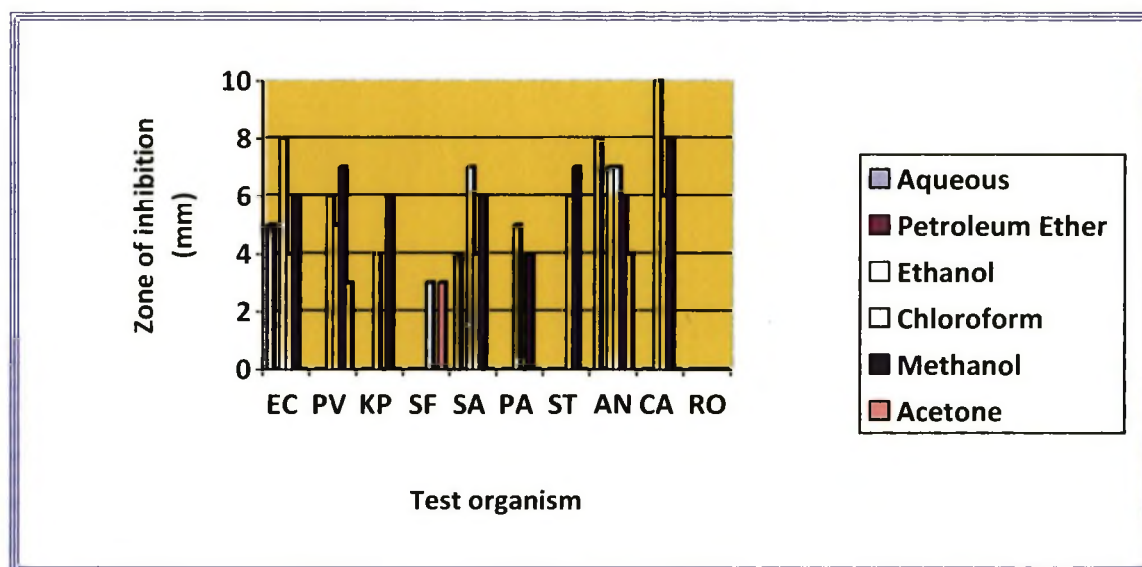
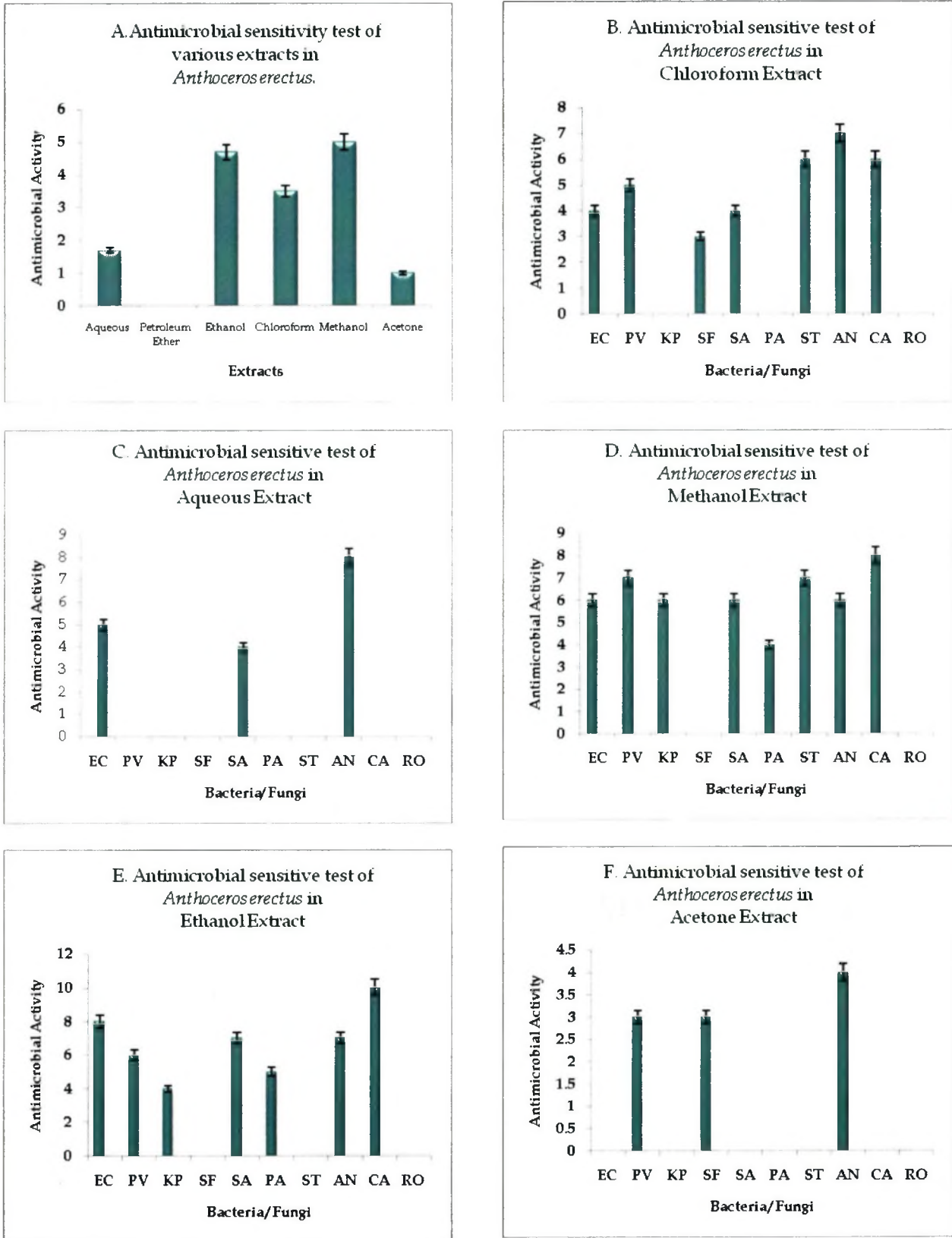
Fig: 4.4.9 Analysis of antimicrobial sensitivity of *Anthoceros erectus*

Fig: 4.4.10 Extracts analysis of the plant *Anthoceros erectus*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

4.4.6 Antimicrobial sensitivity test of *Funaria hygrometrica*

(Plate: 34, A-J)

Funaria hygrometrica, a moss plant extracts obtained in different solvents by testing against microorganisms have shown positive and promising results (Table: 4.4.6).

The aqueous extracts of the plant found sensitive to microbial pathogens like *E. coli* and *S. aureus* only (Fig: 4.4.12-C). However, the petroleum ether extract not showed any significant interaction with other microorganisms, and the reactions found null (Fig: 4.4.12-A). The ethanolic extract of the plant showed positive results with the microorganisms like *E. coli*, *K. pneumoniae*, *S. flexneri*, *P. aeruginosa*, *A. niger*, and *C. albicans*. The greenish coloured chloroform extract showed positive interaction with bacterial pathogens like *E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, *A. niger* and *C. albicans* while no result was observed with pathogens like *K. pneumoniae*, *S. flexneri* and *R. oryzae* (Fig: 4.4.12-B). The another promising results were observed in methanolic extracts of the plant with positive interaction against microorganisms like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. flexneri*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, *A. niger* and *C. albicans*. No interaction was observed in the fungus *Rhizopus oryzae* (Fig: 4.4.12-E). Acetone extract was found least interactive against most pathogens but found positive with the pathogens like *P. vulgaris*, *S. flexneri* and *A. niger* only (Fig: 4.4.12-F).

Analyzing all the extracts it was found that the aqueous and acetone extracts were less sensitive for representing zone of inhibition. However, the ethanolic, chloroform and methanol extracts showed significant zone of inhibition against maximum microorganisms (Fig: 4.4.12-A).

The ethanolic extract of the plant showed maximum zone of inhibition of 9 mm against fungal pathogen *Candida albicans* and least zone of inhibition was found against *P. vulgaris* in acetone extract as well as in *K. pneumoniae* in methanol extract along with aqueous extract against *S. aureus* (Fig: 4.4.11).

Table: 4.4.6 Antimicrobial sensitivity test of *Funaria hygrometrica*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO
<i>Funaria hygrometrica</i>	Aqueous	06	0	0	0	03	0	0	0	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	07	0	04	05	07	04	0	07	09	0
	Chloroform	06	04	0	0	06	08	07	07	06	0
	Methanol	04	05	03	4	6	05	07	06	07	0
	Acetone	0	03	0	3	0	0	0	04	0	0
	Tetracycline	24	26	21	27	24	24	21	-	-	-
	Nystatin	-	-	-	-	-	-	-	28	27	30

* Data represented in mean of three replicates.

EC = *Escherichia coli* [MTCC-729], **PV**= *Proteus vulgaris* [MTCC-744], **KP** = *Klebsiella pneumoniae* [MTCC-661], **SF** = *Shigella flexneri* [MTCC-1457], **SA**= *Staphylococcus aureus* [MTCC-96], **PA**= *Pseudomonas aeruginosa* [MTCC-424], **ST** = *Salmonella typhimurium* [MTCC-98], **AN** = *Aspergillus niger* [MTCC-281], **CA**= *Candida albicans* [MTCC-227], **RO**= *Rhizopus oryzae* [MTCC-554]

Fig 4.4.11 Analysis of antimicrobial sensitivity of *Funaria hygrometrica*

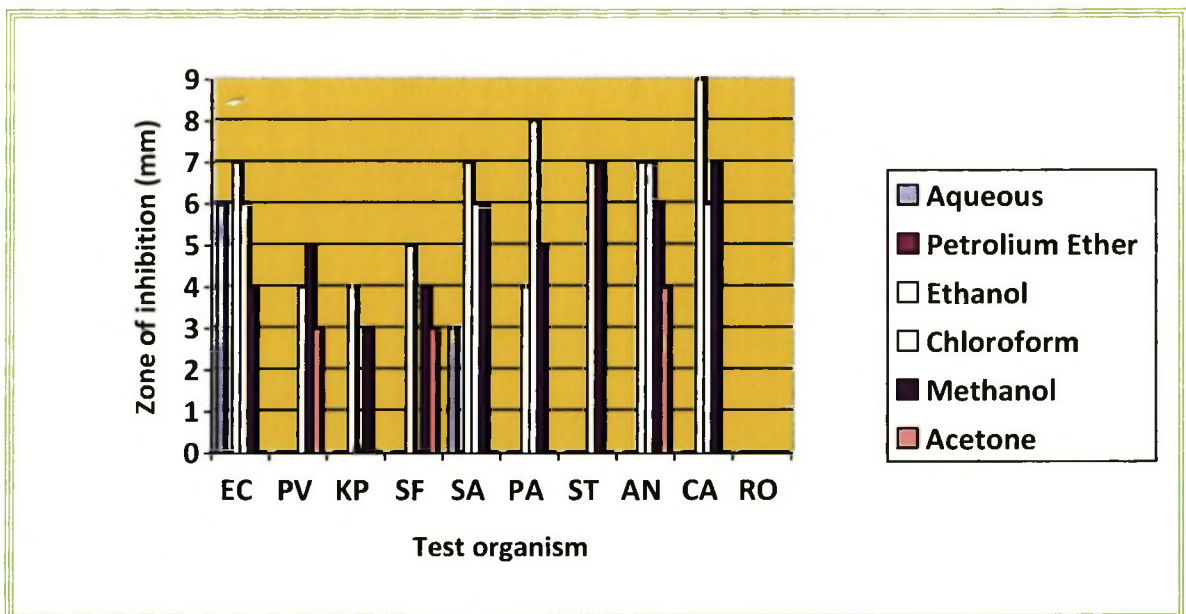
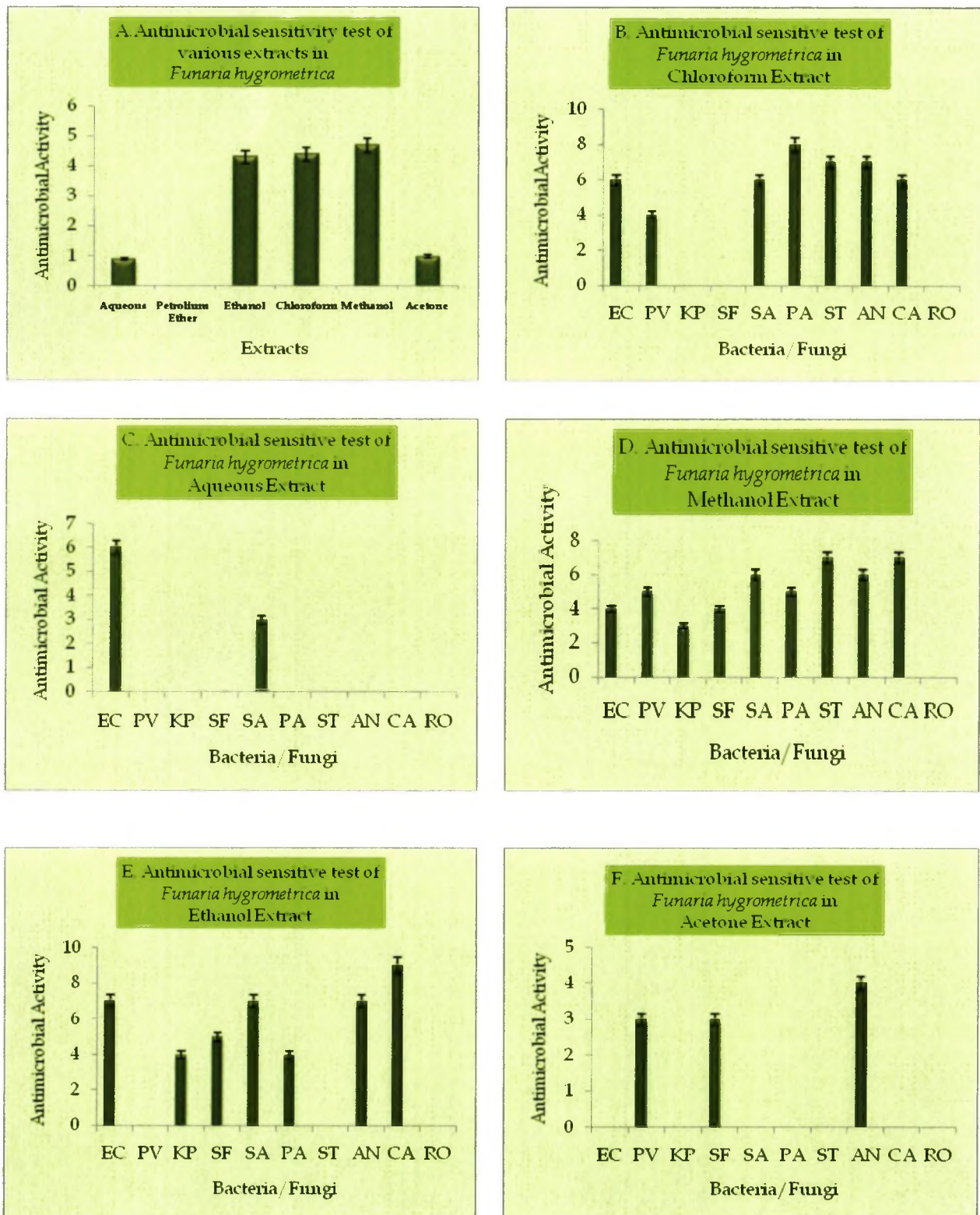


Fig: 4.4.12 Extracts analysis of the plant *Funaria hygrometrica*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

4.4.7 Antimicrobial sensitivity test of *Hyophila involuta*

(Plate: 35, A-J)

Hyophila involuta a moss, extracts obtained in different solvents and tested against various pathogens showing positive results (Table: 4.4.7).

The aqueous extract of the plant in distilled water showed least interaction against *E. coli* and *S. aureus* and no activity against other bacterial or fungal test pathogens (Fig: 4.4.14-C). The petroleum ether extract showed null effect against all the pathogens under interaction (Fig: 4.4.14-A). However, the ethanolic extract of the plant was found highly interactive with most of pathogens under study and exhibited sensitivity against microorganisms like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. flexneri*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, *A. niger* and *C. albicans*. No reaction noticed against the pathogenic fungi *R. oryzae* (Fig: 4.4.14-E). The dark green chloroform extract was found to be effective against the pathogens like *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *A. niger* and *C. albicans* however, no interaction was noticed in pathogens like *S. flexneri*, *P. aeruginosa*, *S. typhimurium* and *R. oryzae* (Fig: 4.4.14-B). Subsequently, the methanolic extract of the plant revealed promising results against microorganisms such as *E. coli*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *A. niger* and *C. albicans* while negative results were found against *S. flexneri*, *S. typhimurium* and *R. oryzae* (Fig: 4.4.14-D). The acetone extract showed significant results against pathogen *K. pneumoniae*, *S. flexneri*, *P. aeruginosa*, *S. typhimurium*, *A. niger* and *C. albicans* while no specific interaction was observed against bacteria *E. coli*, *P. vulgaris*, *S. aureus* and the fungus *R. oryzae* (Fig: 4.4.14-C).

It is interesting and intended to put the results of antimicrobial activity on the canvas of present investigation that during course of activity the aqueous and acetone extracts were less interactive as compared to the other extract like ethanol, chloroform and methanol (Fig: 4.4.14-A). The test organism *Rhizopus oryzae* remained negative against all the extracts. The highest zone of inhibition of 8 mm was found in ethanol extract against fungus *Aspergillus niger* and least in aqueous extract against *E. coli* and *C. aureus* with 3 mm and above same as in acetone extract against *S. flexneri* (Fig: 4.4.13).

Table: 4.4.7 Antimicrobial sensitivity test of *Hyophila involuta*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RA
<i>Bryum coronatum</i>	Aqueous	03	0	0	0	03	0	0	0	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	07	05	04	06	04	0	06	08	07	0
	Chloroform	05	04	06	0	06	0	0	07	06	0
	Methanol	04	05	03	0	06	04	0	04	05	0
	Acetone	0	0	04	03	0	05	06	07	06	0
	Tetracycline	27	24	23	21	22	26	27	-	-	-
	Nystatin	-	-	-	-	-	-	-	27	26	30

* Data represented in mean of three replicates.

*EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96] , PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281] , CA= *Candida albicans* [MTCC-227], RA= *Rhizopus oryzae* [MTCC-554]

Fig 4.4.13 Analysis of antimicrobial sensitivity of *Hyophila involuta*

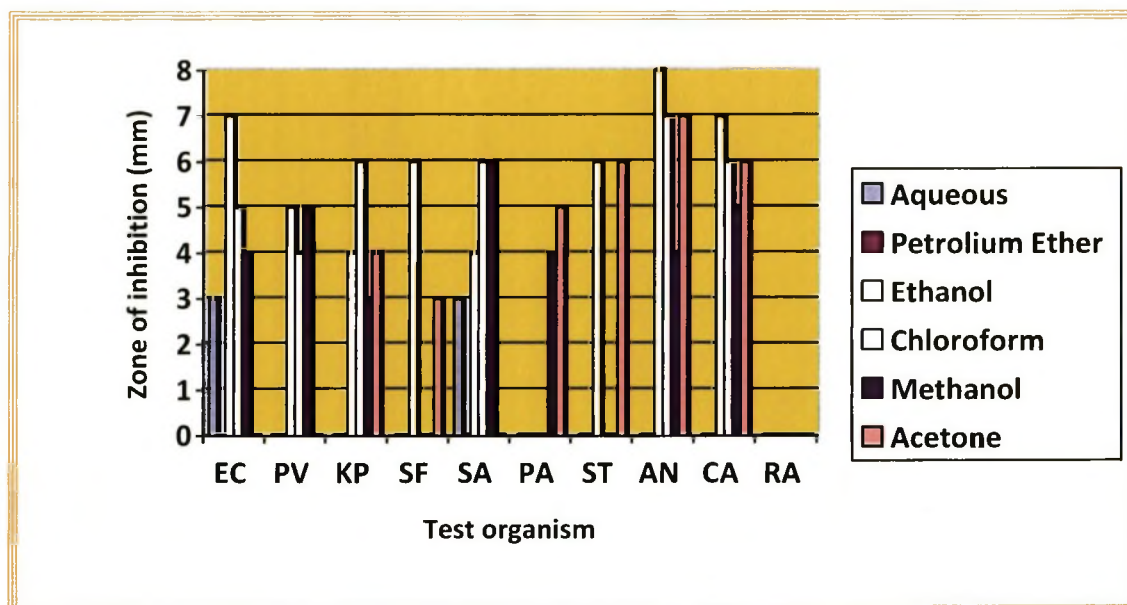
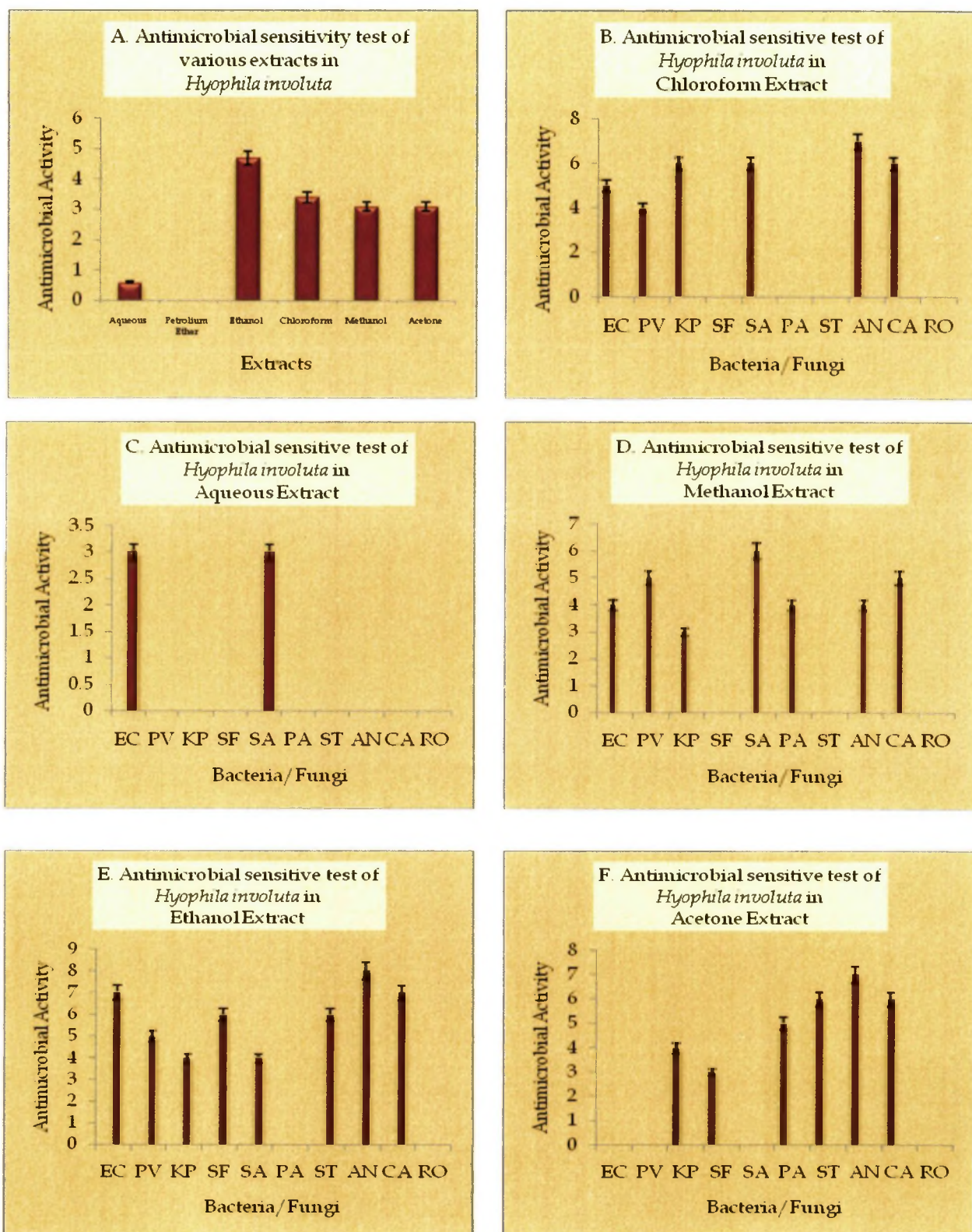


Fig: 4.4.14 Extracts analysis of the plant *Hyophila involuta*



EC = *Escherichia coli* [MTCC-729], PV= *Proteus vulgaris* [MTCC-744], KP = *Klebsiella pneumoniae* [MTCC-661], SF = *Shigella flexneri* [MTCC-1457], SA= *Staphylococcus aureus* [MTCC-96], PA= *Pseudomonas aeruginosa* [MTCC-424], ST = *Salmonella typhimurium* [MTCC-98], AN = *Aspergillus niger* [MTCC-281], CA= *Candida albicans* [MTCC-227], RO= *Rhizopus oryzae* [MTCC-554]

PLATE – 29

Antimicrobial sensitivity test of *Plagiochasma appendiculatum*



A. *Escherichia coli*



B. *Proteus vulgaris*



C. *Klebsiella pneumoniae*



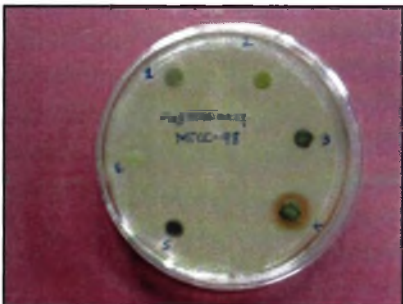
D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*



J) *Rhizopus oryzae*

Extract 1. Aqueous 2. P.Ether 3. Ethanol 4. Chloroform 5. Methanol 6. Acetone

PLATE – 30

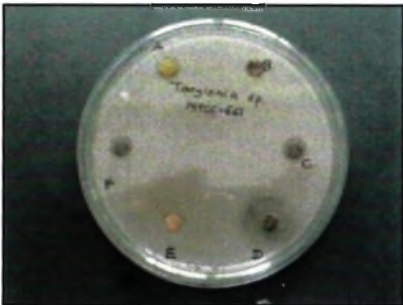
Antimicrobial sensitivity test of *Targionia hypophylla*



A. *Escherichia coli*



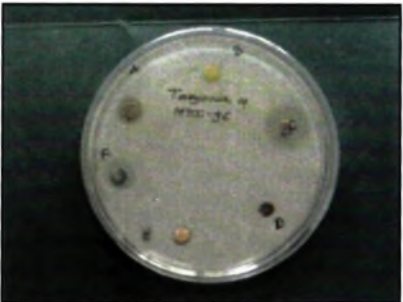
B. *Proteus vulgaris*



C. *Klebsiella pneumoniae*



D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



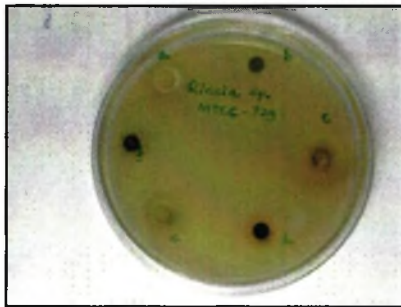
I) *Candida albicans*



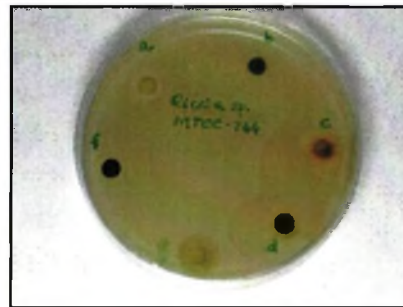
J) *Rhizopus oryzae*

Extract I-Aqueous, II- P. Ether, III-Ethanol, IV-Chloroform, V-Methanol, VI-Acetone

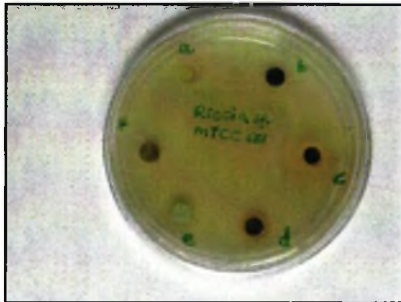
PLATE – 31
Antimicrobial sensitivity test of *Riccia discolor*



A. *Escherichia coli*



B. *Proteus vulgaris*



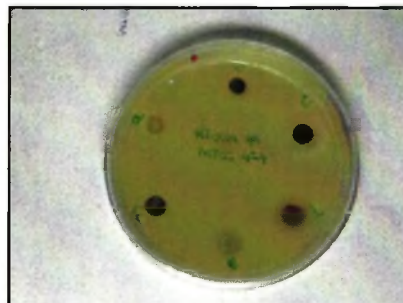
C. *Klebsiella pneumoniae*



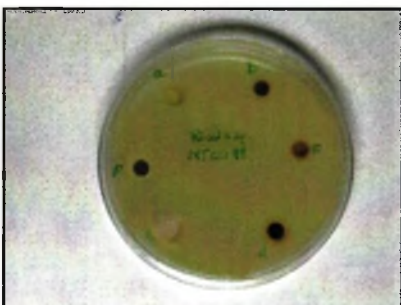
D. *Shigella flexneri*



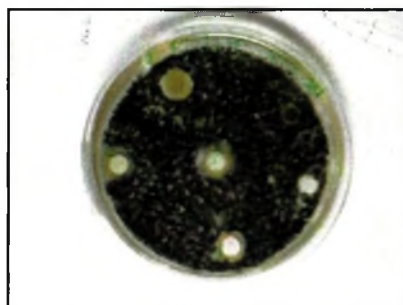
E) *Staphylococcus aureus*



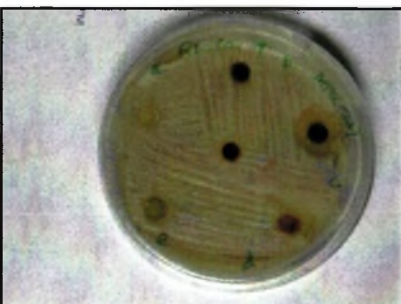
F) *Pseudomonas aeruginosa*



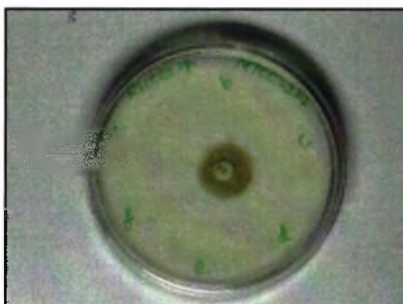
G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*



J) *Rhizopus oryzae*

Extract a-Aqueous. b- P. Ether. c-Ethanol. d-Chloroform. e-Methanol. f-Acetone

PLATE -- 32

Antimicrobial sensitivity test of *Reboulia hemisphaerica*



A. *Escherichia coli*



B. *Proteus vulgaris*



C. *Klebsiella pneumoniae*



D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*



J) *Rhizopus oryzae*

Extract I-Aqueous, II- P. Ether, III-Ethanol, IV-Chloroform, V-Methanol, VI-Acetone

PLATE – 33

Antimicrobial sensitivity test of *Anthoceros erectus*



A. *Escherichia coli*



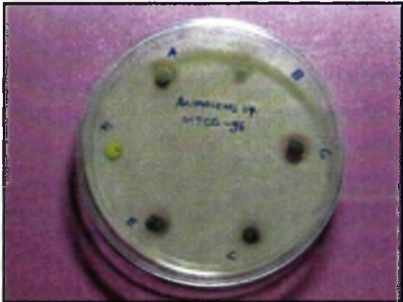
B. *Proteus vulgaris*



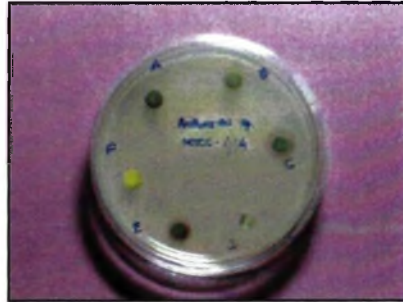
C. *Klebsiella pneumoniae*



D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*



J) *Rhizopus oryzae*

Extract A-Aqueous, B- P. Ether, C-Ethanol, D-Chloroform, E-Methanol, F-Acetone

PLATE – 34

Antimicrobial sensitivity test of *Funaria hygrometrica*



A. *Escherichia coli*



B. *Proteus vulgaris*



C. *Klebsiella pneumoniae*



D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*

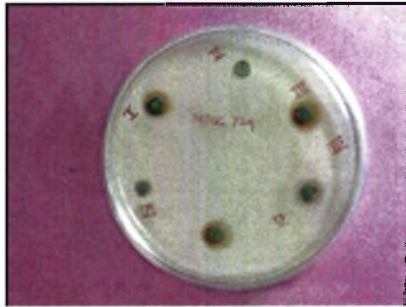


J) *Rhizopus oryzae*

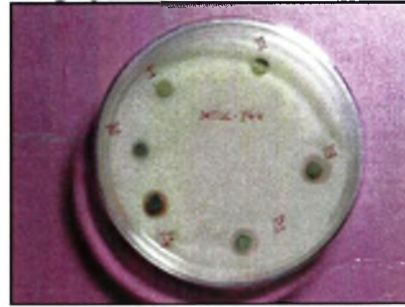
Extract a-Aqueous, b- P. Ether, c-Ethanol, d-Chloroform, e-Methanol, f-Acetone

PLATE – 35

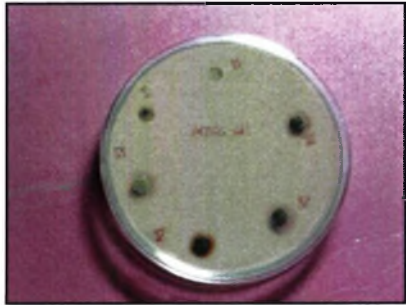
Antimicrobial sensitivity test of *Hyophila involuta*



A. *Escherichia coli*



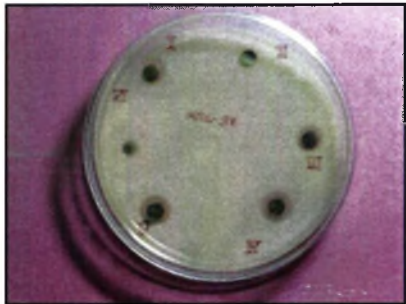
B. *Proteus vulgaris*



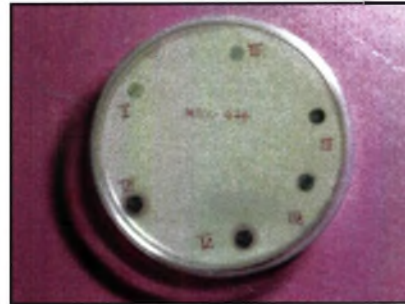
C. *Klebsiella pneumoniae*



D. *Shigella flexneri*



E) *Staphylococcus aureus*



F) *Pseudomonas aeruginosa*



G) *Salmonella typhimurium*



H) *Aspergillus niger*



I) *Candida albicans*



J) *Rhizopus oryzae*

Extract I-Aqueous. II- P. Ether. III-Ethanol. IV-Chloroform. V-Methanol. VI-Acetone

4.5. Phytochemical analysis of bryophytes

During present investigation, preliminary phytochemical analysis was done and it was found that, the most of the liverworts like *P. appendiculatum*, *T. hypophylla*, *R. discolor*, *R. hemisphaerica*, *P. intermedium* and *A. angusta* possess positive results mostly for terpenoids, flavonoids, glycosides and other compounds like alkaloids, tannins, saponins, sterols found in random. The hornwort *Anthoceros erectus* showed presence of terpenoids, flavonoids, glycosides, alkaloids, tannins and not found positive for saponins and sterols. It is interesting that the green fluorescent, small liverwort *Cyathodium tuberosum* did not show any positive or reactive results against all phytochemical tests. The delicate mosses also showed positive results to certain phytochemical tests. The mosses like *Funaria hygrometrica* showed mostly presence of alkaloids, flavonoids, sterols, glycosides and less reactive to test for tannins and saponins. However, the other mosses like *Hyophila involuta*, *Stereophyllum decorum* and *Hymenostylium recurvirostre* also showed positive results for the test of terpenoids, flavonoids, alkaloids and glycosides mostly and less for the saponins and sterols. Moreover, it is notable that, the chemical composition of bryophytes may vary due to collection time, seasons and other environmental conditions in response to substrate and surroundings (Table: 4.5.1).

Table: 4.5.1 Preliminary qualitative phytochemical analysis of the bryophytes

Sr. No.	NAME OF THE PLANT	PHYTOCHEMICAL TESTS						
		Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Glycosides	Terpenoids
1.	<i>P. appendiculatum</i>	+	+	-	+	+	+	+
2.	<i>T. hypophylla</i>	+	+	+	-	+	-	+
3.	<i>R. discolor</i>	-	+	-	+	-	+	+
4.	<i>R. hemisphaerica</i>	-	+	+	+	+	+	+
5.	<i>A. erectus</i>	+	+	+	-	-	+	+
6.	<i>F. hygrometrica</i>	+	+	-	-	+	+	+
7.	<i>H. involuta</i>	-	+	-	+	-	-	+
8.	<i>A. angusta</i>	-	+	-	+	+	+	+
9.	<i>P. intermedium</i>	+	+	+	+	+	+	+
10.	<i>C. tuberosum</i>	-	-	-	-	-	-	-
11.	<i>S. decorum</i>	+	+	-	-	-	-	+
12.	<i>H. recurvirostre</i>	-	+	+	-	-	+	+

4.5.1 GC-MS analysis of bryophytes

Most of the bryophytes are being used as medicinal plants in China, Europe, North America and rest of the world. Bryophytes have been applied as decoctions to cure various kinds of diseases. They have potential antibiotic activity owing to the presence of typical chemical constituents. The present study emphasizes to elicit out probable phyto-constituents from the thalli of selected bryophytes. About 12 plant species of the experimental bryophytes were subjected for the Phytochemical and GC-MS analysis. Preliminary phytochemical test with qualitative aspects done and presence of alkaloids, flavonoids, sterols, saponins, tannins, terpenoids, carbohydrates etc. were studied and found variably among all selected plants.

The GC-MS analysis of the liverwort *Plagiochasma appendiculatum* (Table: 4.5.2) revealed the presence of compounds like Caryophyllene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Phytol, Hexacosane and Heneicosane (Fig: 4.5.1). The phyto-constituents recorded in the hornwort *Anthoceros erectus* (Table: 4.5.3) were Caryophyllene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Phytol, Nonacosane, Tetratriacontane and Stigmasterol (Fig: 4.5.2). The liverwort *Targionia hypophylla* (Table: 4.5.4) also showed the presence of different and diversified compounds like, Longifolene, Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl, Patchouli alcohol, n-Hexadecanoic acid and 9-Octadecenoic acid (Fig: 4.5.3).

The moss *Funaria hygrometrica* (Table: 4.5.5) in GC-MS analysis showed wide range of phyto-constituents like 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-,methyl ester,7,10,13-Hexadecatrienoic acid, methyl ester, Heptadecane, 2,6,10,15-tetramethyl, Nonacosane, Hexacosane and Nonadecane (Fig: 4.5.4). The another species of liverwort *Plagiochasma intermedium* (Table: 4.5.6) also exhibited remarkable presence of some new phyto-constituents like Caryophyllene, 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene, Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene -1- (1-methylethyl), Cycloisolongifolene, 8, 9-dehydro, Spathulenol, 1,4-Methanoazulene,decahydro-4,8,8-trimethyl-9-methylene, Gamma-Gurjunenepoxide-(2), 7-Tetracyclo [6.2.1.0(3.8) 0 (3.9)] undecanol, 4,4,11,11-tetramethyl and n-Hexadecanoic acid and 9-Octadecenoic acid (Fig: 4.5.5).

Another exciting liverwort *Reboulia hemisphaerica* (Table: 4.5.7) have shown the presence of certain chemical compounds like Thujopsene, Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl), Longifolenaldehyde, 4a, 7- Methano – 4 aH-naphth [1,8a-b] oxirene, octahydro-4,4,8, 8-tetramethyl, 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl,5.alpha.-Androstan-17.beta.-ol, 2.beta., 3.beta.-epithio, 1R, 4s, 7s, 11R -2, 2, 4, 8-Tetramethyltricyclo [5.3.1.0 (4,11)] undec-8-ene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 2-[4-methyl-6-(2,6,6-trimethyl cyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde (Fig: 4.5.8).

The terrestrial and saxicolous moss *Hyophila involuta* (Table: 4.5.8) grow luxuriantly across the Melghat revealed showed the presence of different chemical constituents like 2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 9-Hexadecenoic acid, methyl ester, Hexadecanoic acid, methyl ester, Dibutyl phthalate, n-Hexadecanoic acid, 9,12 Octadecadienoic acid (Z,Z)-, methyl ester and Tricyclo [8.6.0.0(2,9)] hexadeca-3,15-diene, trans-2,9-transoid-9,10-cis-1,10 (Fig: 4.5.7).

The green fluorescent plant *Cyathodium tuberosum* (Table: 4.5.9) with small thallus did not show any positive result and no data could be recorded from GC-MS analysis (Fig: 4.5.8).

In the moss plant *Stereophyllum decorum* (Table: 4.5.10) the presences of only one chemical compound like 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was observed (Fig: 4.5.9). The liverwort *Asterella angusta* (Table: 4.5.11) also revealed the wide range of different phyto-constituents like Humulen, Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl), Germacrene D §§ 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, Patchouli alcohol, 14-Methyl-8-hexadecyn-1-ol, Phenol, 4-(1,1,3,3-tetramethylbutyl) n-Hexadecanoic acid (Fig: 4.5.10).

The common moss occurring in saxicolous and terrestrial habitat *Hymenostylium recurvirostre* (Table: 4.5.12) exhibited very interesting chemical constituents like Cyclooctasiloxane, hexadecamethyl, Cyclononasiloxane octadecamethyl, Heptacosane and Tetracosane that were totally found different from others (Fig: 4.5.11).

The rosette and terrestrial green liverwort *Riccia discolor* (Table: 4.5.12) also showed another interesting and novel chemical phyto constituents like Longifolene, Thujopsene; Cyclopropa [d] naphthalene, 1,1a,4,4a, 5,6,7,8-octahydro-2, 4a,8, 8-

tetramethyl, Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene, Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl), Patchouli alcohol, Longifolenaldehyde, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, methyl ester, Dibutyl phthalate, n-Hexadecanoic acid and Phytol(Fig: 4.5.11).

Fig: 4.5.1 GC-MS chromatogram of *Plagiochasma appendiculatum*

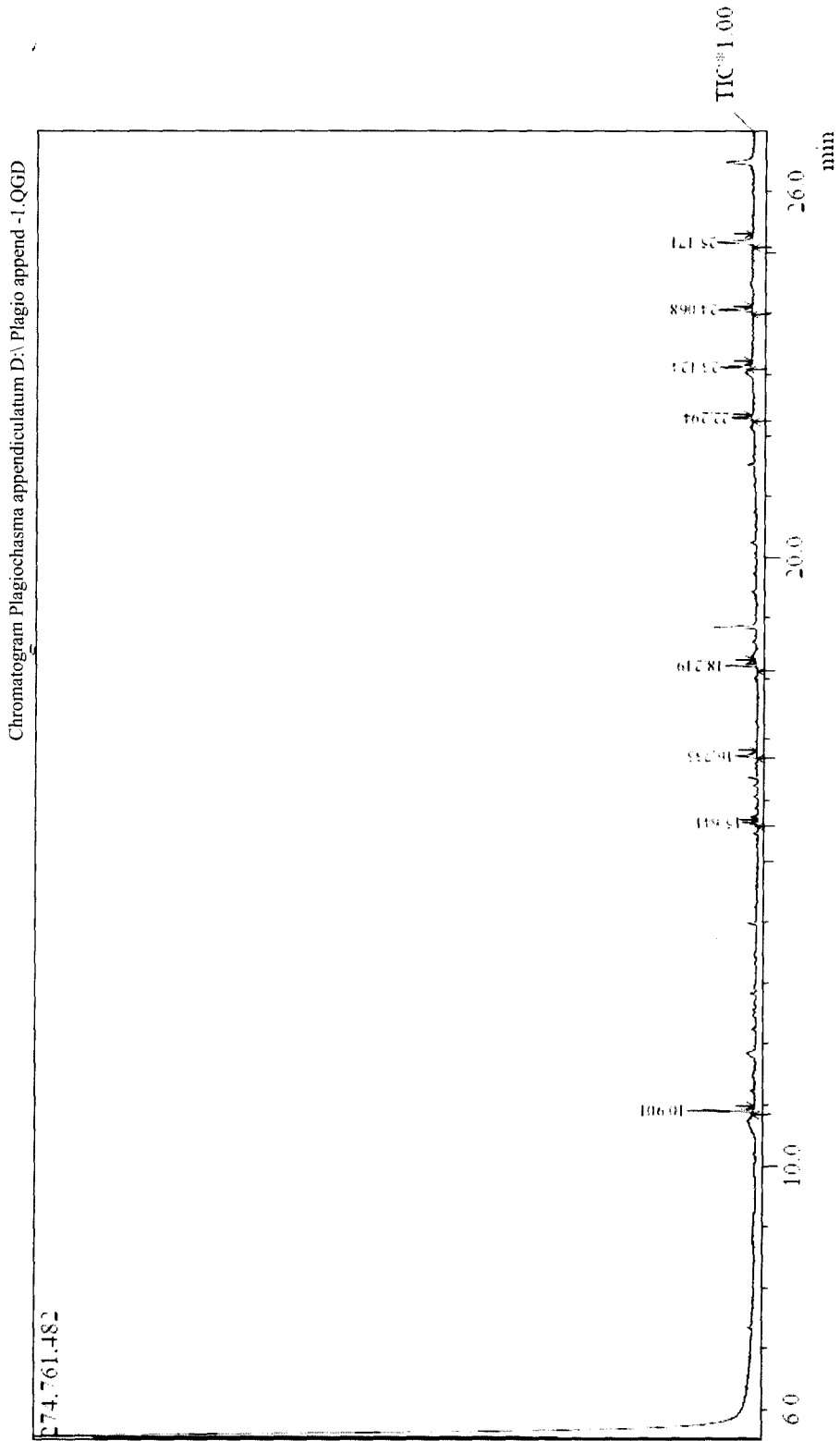


Table: 4.5.2 GC-MS analysis of *Plagiochasma appendiculatum*


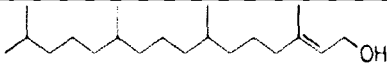

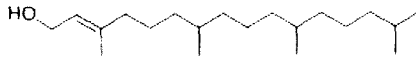

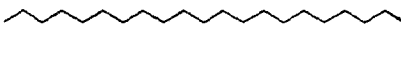
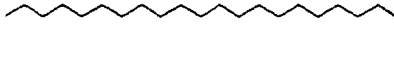

Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.90	20.70	Caryophyllene	C ₁₅ H ₂₄	204	Sesquiterpene		Antibacterial, Antifungal and Cytotoxicity
2	15.64	5.19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory Antibacterial, and Antifungal
3	16.73	9.80	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Palmitic acid		Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant,
4	18.21	10.15	Phytol	C ₂₀ H ₄₀ O	296	Diterpene		Antimicrobial Anti-inflammatory Anti-cancer, Diuretic
5	22.29	7.72	Hexacosane	C ₂₆ H ₅₄	366	Alkane		Anti-inflammatory
6	23.12	10.44	Heneicosane	C ₂₁ H ₄₄	296	Alkane		Anti-inflammatory
7	24.06	14.89	Heneicosane	C ₂₁ H ₄₄	296	Alkane		Anti-inflammatory
8	25.17	21.11	Hexacosane	C ₂₆ H ₅₄	366	Alkane		Anti-inflammatory

Fig: 4.5.2 GC-MS chromatogram of *Anthoceros erectus*

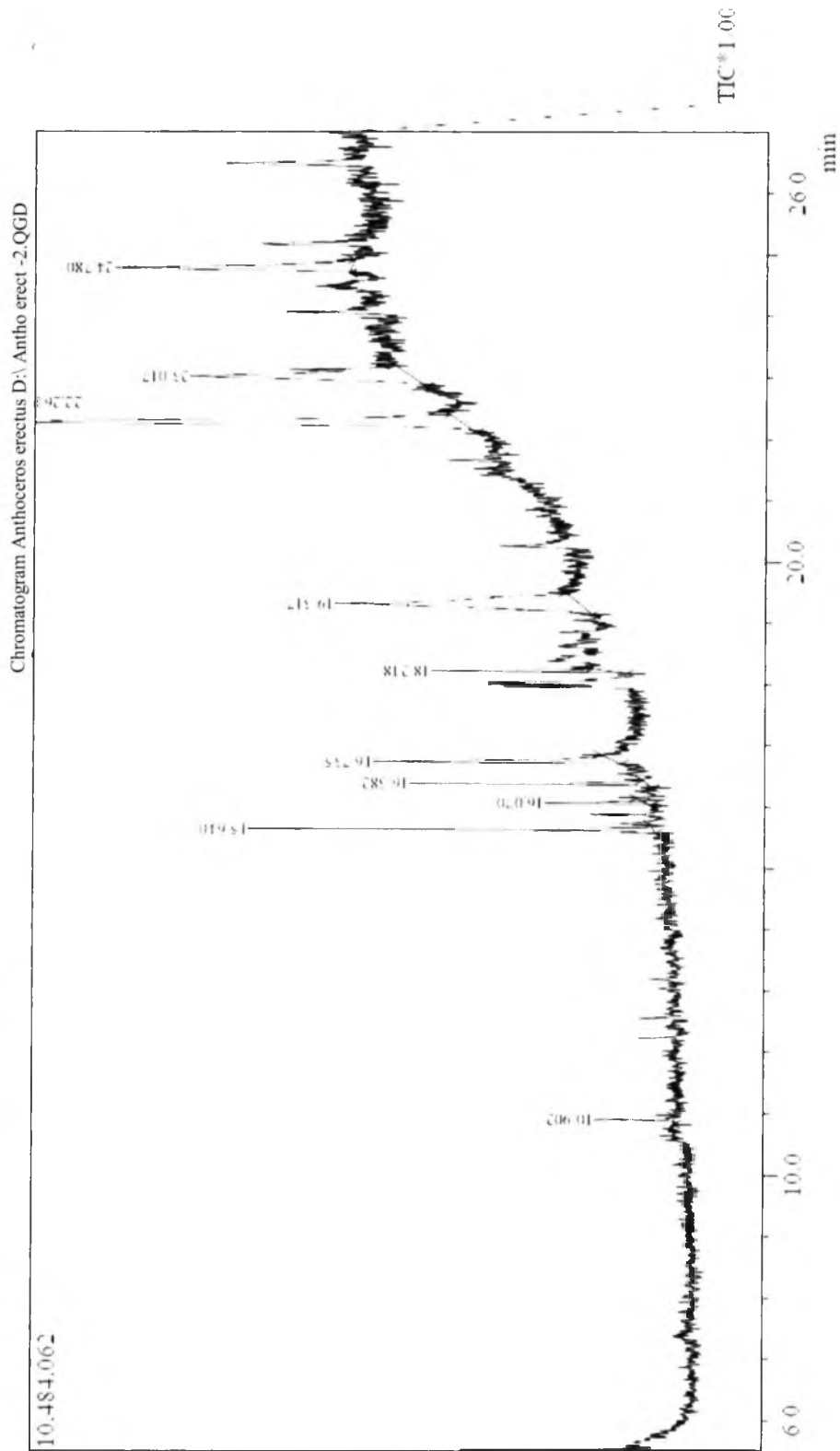


Table: 4.5.3 GC-MS analysis of *Anthoceros erectus*


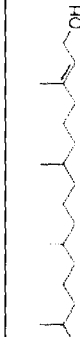
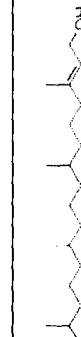




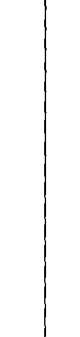


Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.90	1.11	Caryophyllene	C ₁₅ H ₂₄	204	Sesquiterpene		Antibacterial, Antifungal and Cytotoxicity
2	15.64	6.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory, Antibacterial, and Antifungal
3	16.07	1.29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory, Antibacterial, and Antifungal
4	16.38	4.67	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	Palmitic acid ester		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
5	16.73	6.90	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Palmitic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
6	18.25	3.40	Phytol	C ₂₀ H ₄₀ O	296	Diterpene		Antimicrobial, Anti-inflammatory, Anti-cancer, Diuretic
7	19.31	20.58	Nonacosane	C ₂₉ H ₆₀	408	Alkane		Antibacterial, waxy paraffin, Insect pheromonal
8	22.26	26.86	Tetratriacontane	C ₃₄ H ₇₀	478	Alkane		Antibacterial, Antifungal
9	23.01	17.12	Stigmasterol	C ₂₉ H ₄₈ O	412	Sterol		Antifungal and Antibacterial
10	24.78	11.20	Nonacosane	C ₂₉ H ₆₀	408	Alkane		Antibacterial, waxy paraffin, Insect pheromonal

Fig: 4.5.3 GC-MS chromatogram of *Targionia hypophylla*

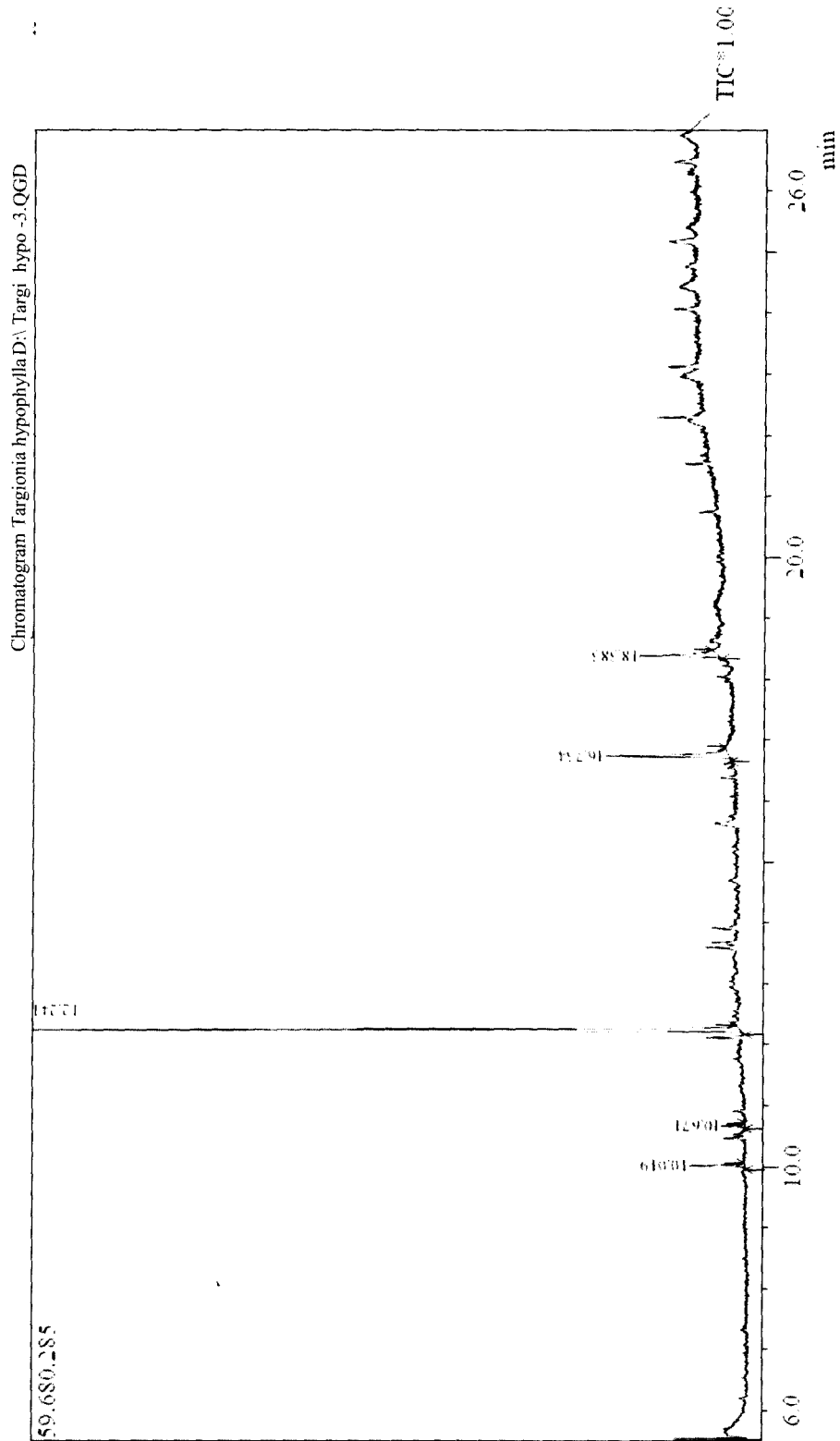


Table: 4.5.4 GC-MS analysis of *Targionia hypophylla*

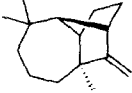
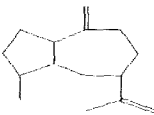
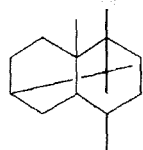
Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.01	4.95	Longifolene	C ₁₅ H ₂₄	204	Sesquiterpene		Acne vulgaris treatment, Antibacterial and Antifungal
2	10.66	2.17	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	C ₁₅ H ₂₄	204	Alkene		Anticancerous
3	12.24	65.25	Patchouli alcohol	C ₁₅ H ₂₆ O	222	Terpene		Antibacterial
4	16.73	16.37	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Palmitic acid		Antioxidant, Hypocholesterolemic Nematicide, Pesticide
5	18.38	11.26	9-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	Oleic acid		Antitumor and antibacterial activity.

Fig: 4.5.4 GC-MS chromatogram of *Funaria hygrometrica*

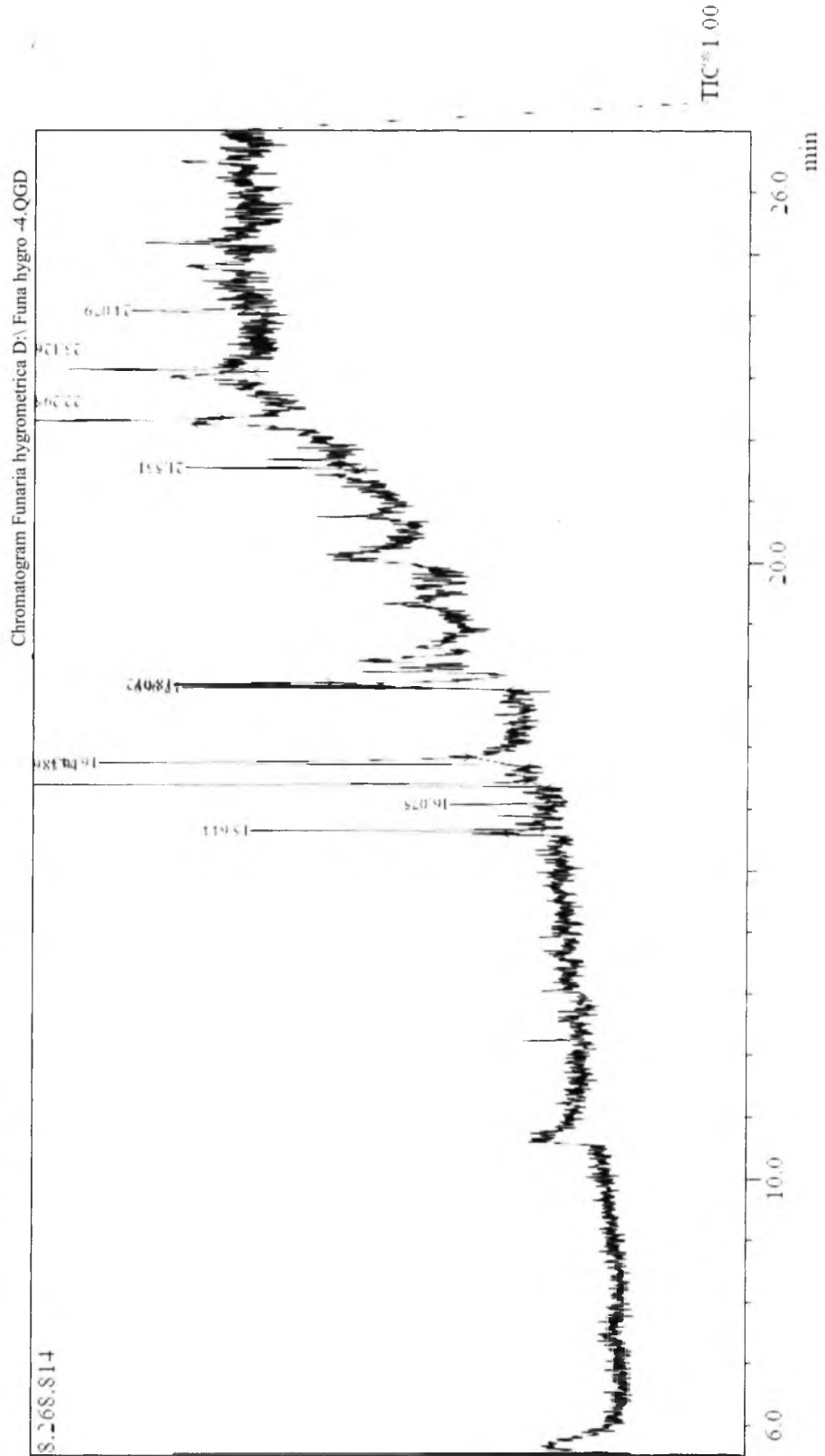


Table 4.5.5 GC-MS analysis of *Funaria hygrometrica*

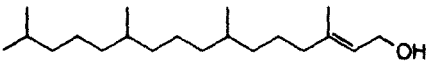
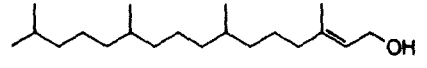


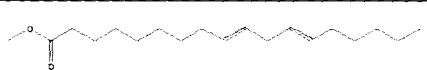

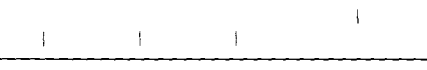


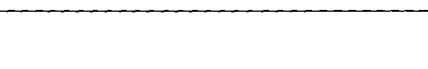
Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	15.64	8.18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpen alcohol		Anti-inflammatory Antibacterial, and Antifungal
2	16.07	3.59	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpen alcohol		Anti-inflammatory Antibacterial, and Antifungal
3	16.38	20.49	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	Palmitic acid ester		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
4	16.74	26.23	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Palmitic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
5	17.96	7.29	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	Linoleic acid		Antioxidant Antibacterial, Anticancerous
6	18.01	7.01	7,10,13-Hexadecatrienoic acid, methyl ester	C ₁₇ H ₂₈ O ₂	264	Gamma-Linoleic acid		Antibacterial
7	21.53	5.49	Heptadecane, 2,6,10,15-tetramethyl	C ₂₁ H ₄₄	296	Alkane		Antibacterial Antifungal
8	22.29	6.90	Nonacosane	C ₂₉ H ₆₀	408	alkane		Antibacterial, waxy parafin Insect pheromonal
9	23.12	7.83	Hexacosane	C ₂₆ H ₅₄	366	alkane		Anti-inflammatory
10	24.79	6.98	Nonadecane	C ₁₉ H ₄₀	268	alkane		Antibacterial

Fig: 4.5.5 GC-MS chromatogram of *Plagiochasma intermedium*

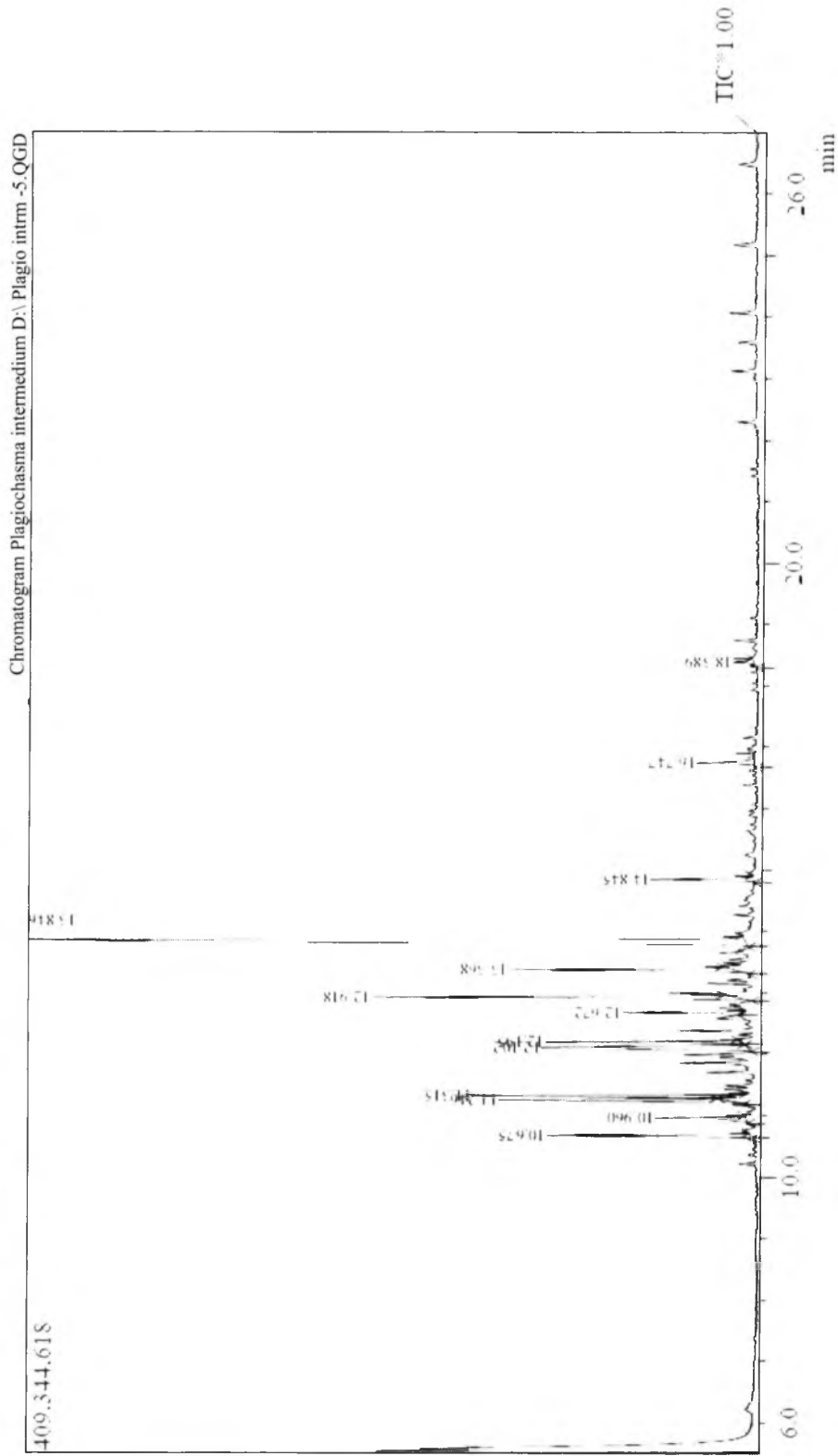

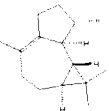
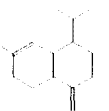
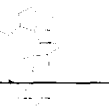





Table: 4.5.6 GC-MS analysis of *Plagiochasma intermedium*

Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.67	5.97	Caryophyllene	C ₁₅ H ₂₄	204	Sesquiterpene		Antibacterial, Antifungal and Cytotoxicity
2	10.96	2.96	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	C ₁₅ H ₂₄	204	Terpenoid		Antibacterial, Blue colour pigmentation
3	11.24	6.95	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	C ₁₅ H ₂₄	204	Terpenoid		Antibacterial, Blue colour pigmentation
4	11.31	6.99	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	C ₁₅ H ₂₄	204	Terpenoid		Antibacterial, Blue colour pigmentation
5	12.10	9.34	Cycloisolongifolene, 8,9-dehydro	C ₁₅ H ₂₂	202	Sesquiterpene		Antibacterial, Antifungal
6	12.19	5.27	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	C ₁₅ H ₂₄	204	Sesquiterpene		Antibacterial, Blue colour pigmentation
7	12.67	3.09	Spathulenol	C ₁₅ H ₂₄ O	220	Sesquiterpene		Antibacterial, natural flavour agent.

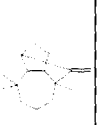




8	12.91	10.42	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene (longifolene)	$C_{15}H_{24}$	204		Sesquiterpene	Anticancerous
9	13.36	6.42	gamma-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	220		Epoxide	-----
10	14.84	2.83	7-Tetracyclo [6.2.1.0(3.8) 0 (3.9)] undecanol, 4,4,1,1,1-tetramethyl	$C_{15}H_{24}O$	220		Aromatic compound	Anticancerous
11	16.74	1.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256		Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
12	18.38	0.87	9-Octadecenoic acid,	$C_{18}H_{34}O_2$	282		Oleic acid	Antitumor and antibacterial activity.

Fig: 4.5.6 GC-MS chromatogram of *Reboulia hemispherica*

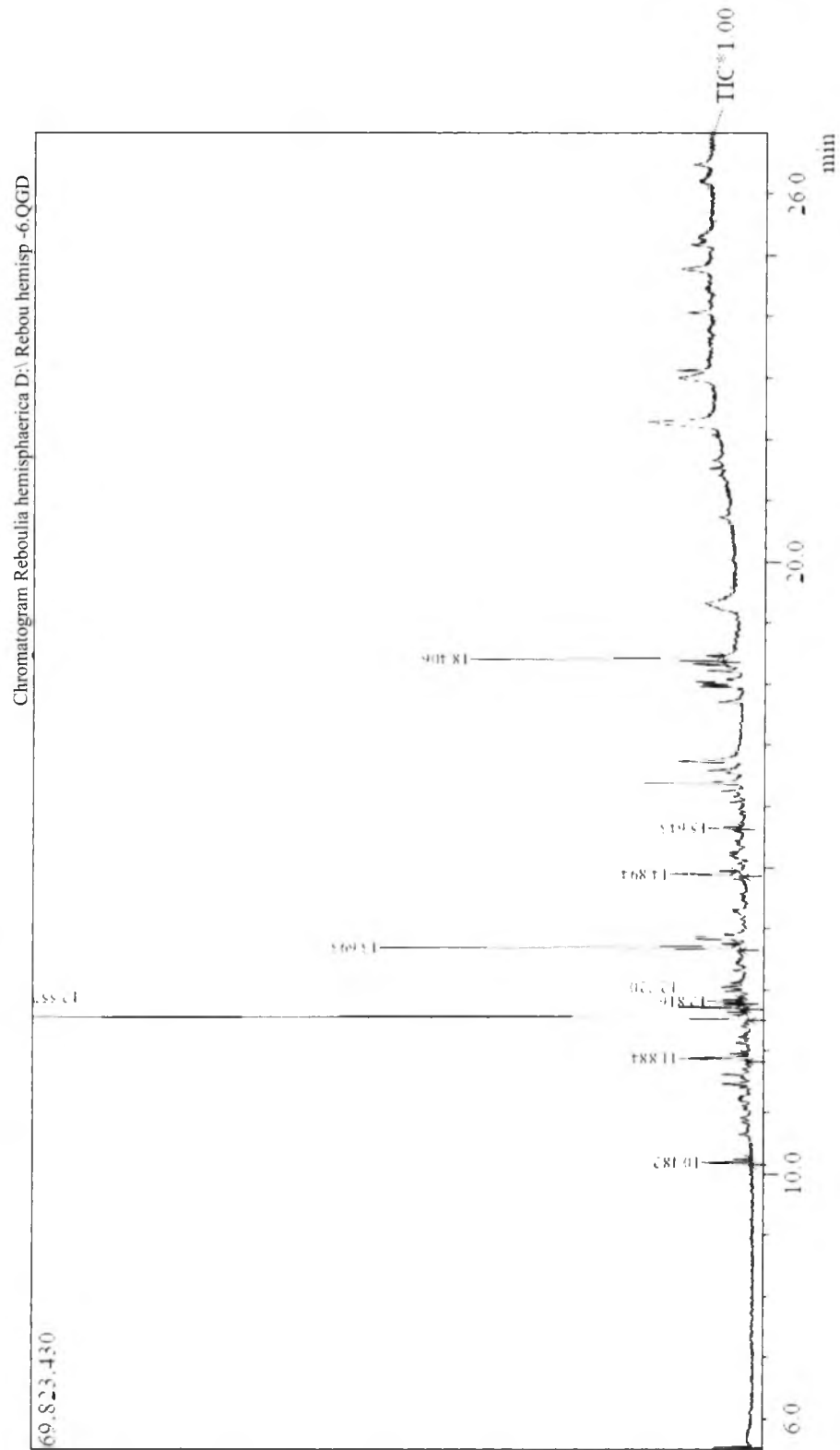


Table: 4.5.7 GC-MS analysis of *Reboulia hemisphaerica*










Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.18	2.63	Thujopsene	C ₁₅ H ₂₄	204	Sesquiterpene		Antifungal
2	11.88	4.29	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)	C ₁₅ H ₂₂	202	Aromatic compound		-----
3	12.55	43.12	Longifolenaldehyde	C ₁₅ H ₂₄ O	220	Sesquiterpene		Antibacterial, Antifungal
4	12.12	4.08	4a,7-Methano-4aH-naphth[1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl	C ₁₅ H ₂₄ O	220	Aromatic compound		-----
5	12.81	2.26	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	C ₁₅ H ₂₆ O	222	Aromatic compound		-----
6	13.69	21.17	5.alpha.-Androstan-17.beta.-ol, 2.beta.,3.beta.-epithio	C ₁₉ H ₃₀ OS	306	Aromatic compound		-----
7	14.89	4.59	1R,4s,7s,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	C ₁₅ H ₂₄	204	Aromatic compound		-----
8	15.64	1.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory Antibacterial, and Antifungal
9	18.40	16.38	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C ₂₃ H ₃₂ O	324	Aldehyde		Antibacterial

Fig: 4.5.7 GC-MS chromatogram of *Hyophila involuta*

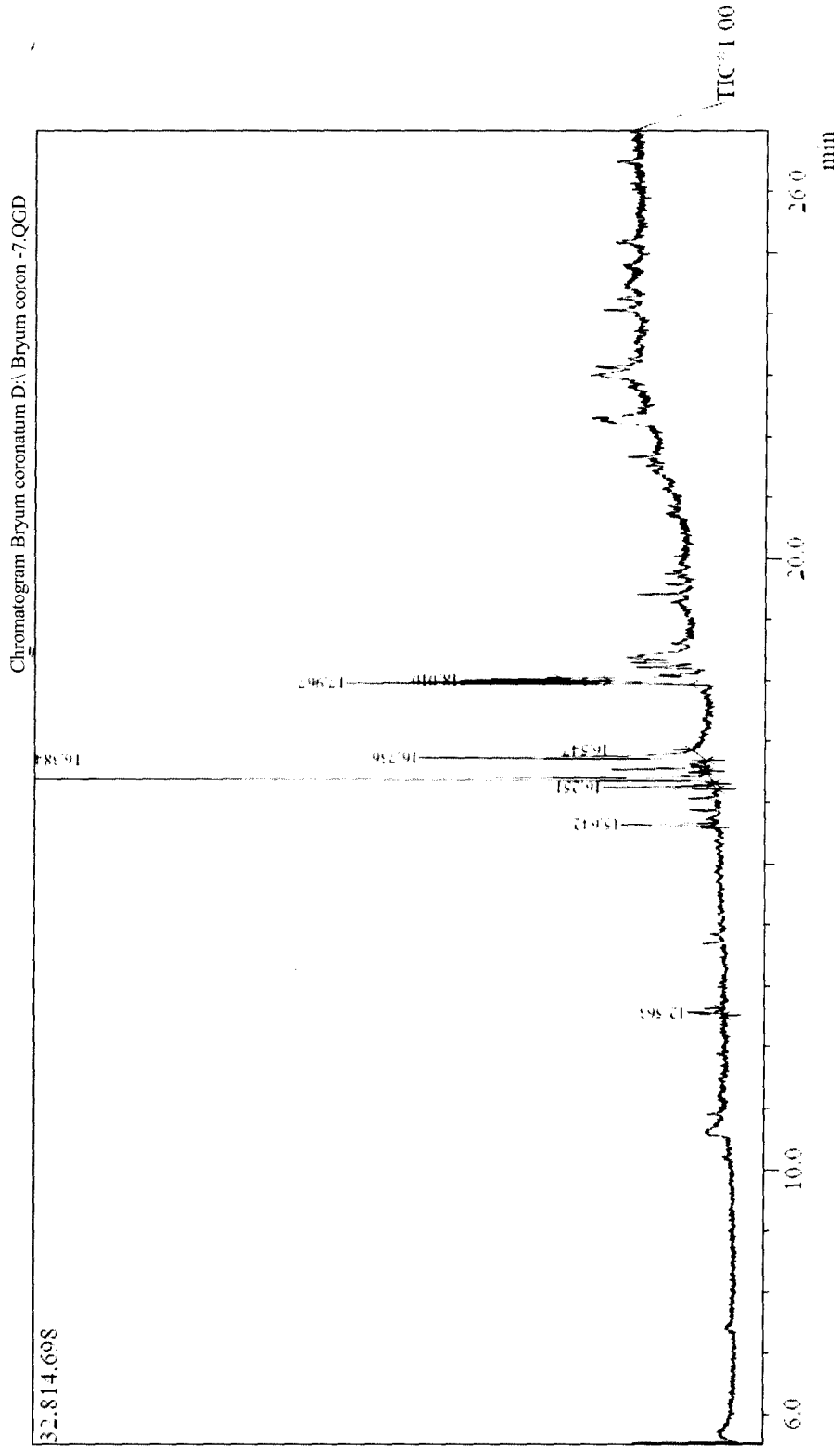


Table: 4.5.8 GC-MS analysis of *Hyophila involuta*

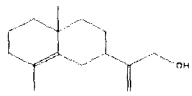
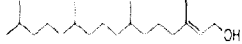




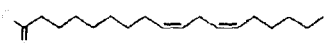
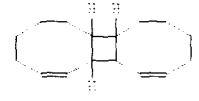
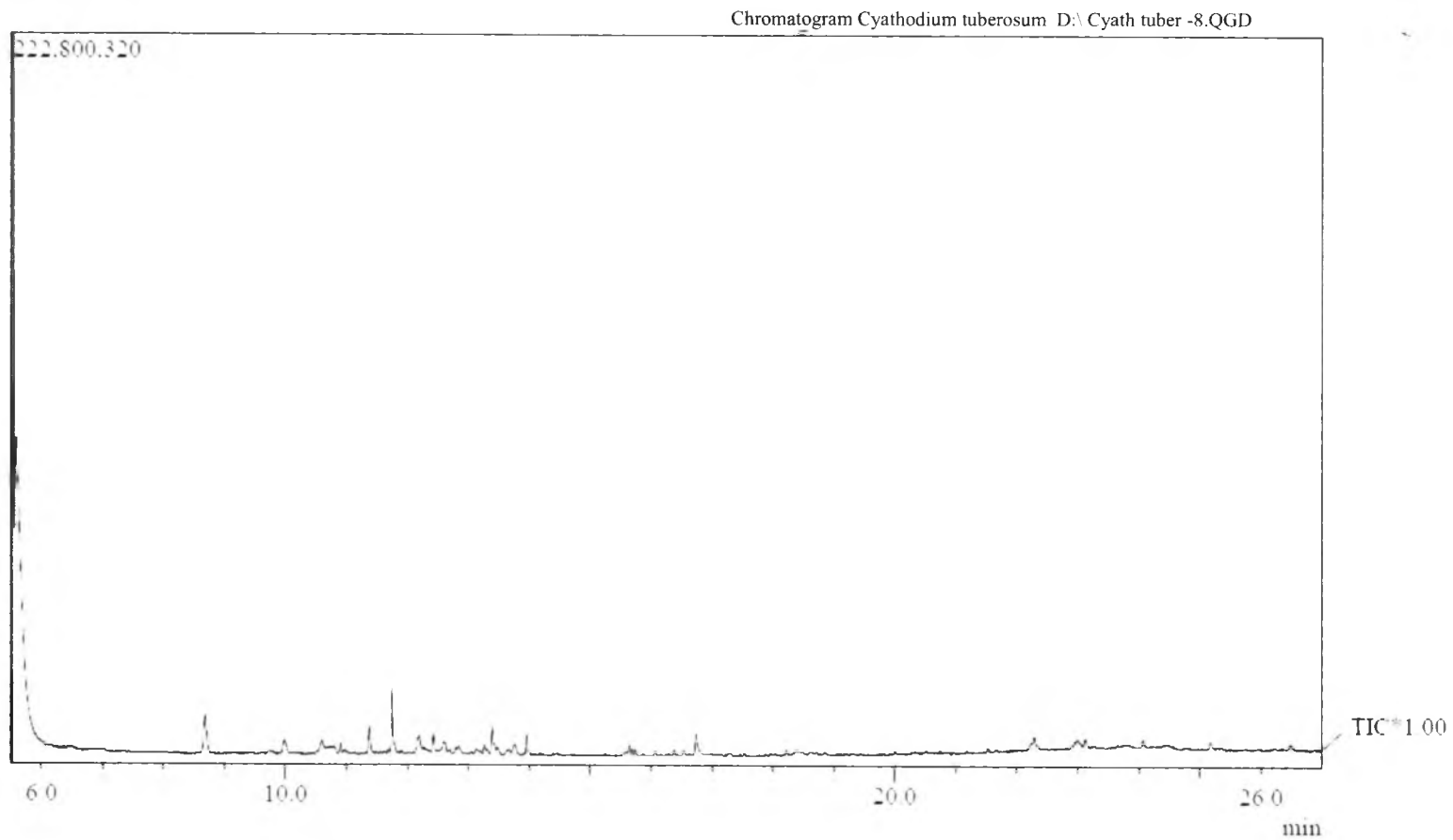
Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	12.56	1.88	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	C ₁₅ H ₂₄ O	220	Alcohol		Anticancerous Antibacterial
2	15.64	4.70	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Antimicrobial
3	16.25	6.18	9-Hexadecenoic acid, methyl ester,	C ₁₇ H ₃₂ O ₂	268	Linolenic acid		Antioxidant, Nematicide, Pesticide
4	16.38	38.35	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	Linolenic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
5	16.54	6.14	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	Ester		Antimicrobial
6	16.37	24.38	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Linolenic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
7	17.96	14.77	9,12 Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	Linolenic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
8	18.01	3.60	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-transoid-9,10-cis-1,10	C ₁₆ H ₂₄	216	--		-----

Fig: 4.5.8 GC-MS chromatogram of *Cyathodium tuberosum*



GC-MS analysis of *Cyathodium tuberosum* : No data found

Fig: 4.5.9 GC-MS chromatogram of *Stereophyllum decorum*

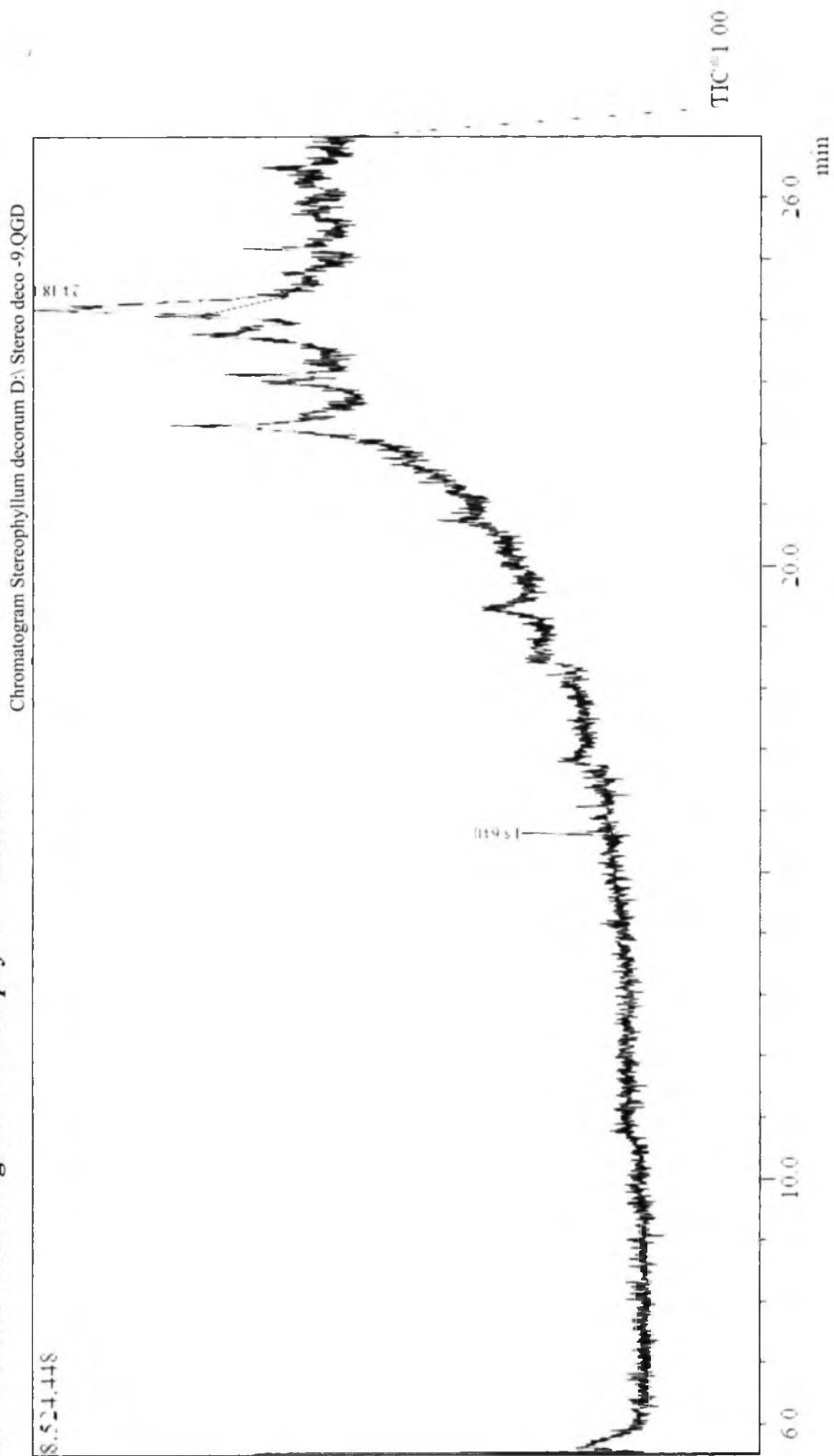


Table: 4.5.9 GC-MS analysis of *Stereophyllum decorum*

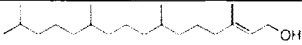
Sr No	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	15.64	9.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory Antibacterial, Antifungal
2	24.18	90.63	No data found					

Fig: 4.5.10 GC-MS chromatogram of *Asterella angusta*

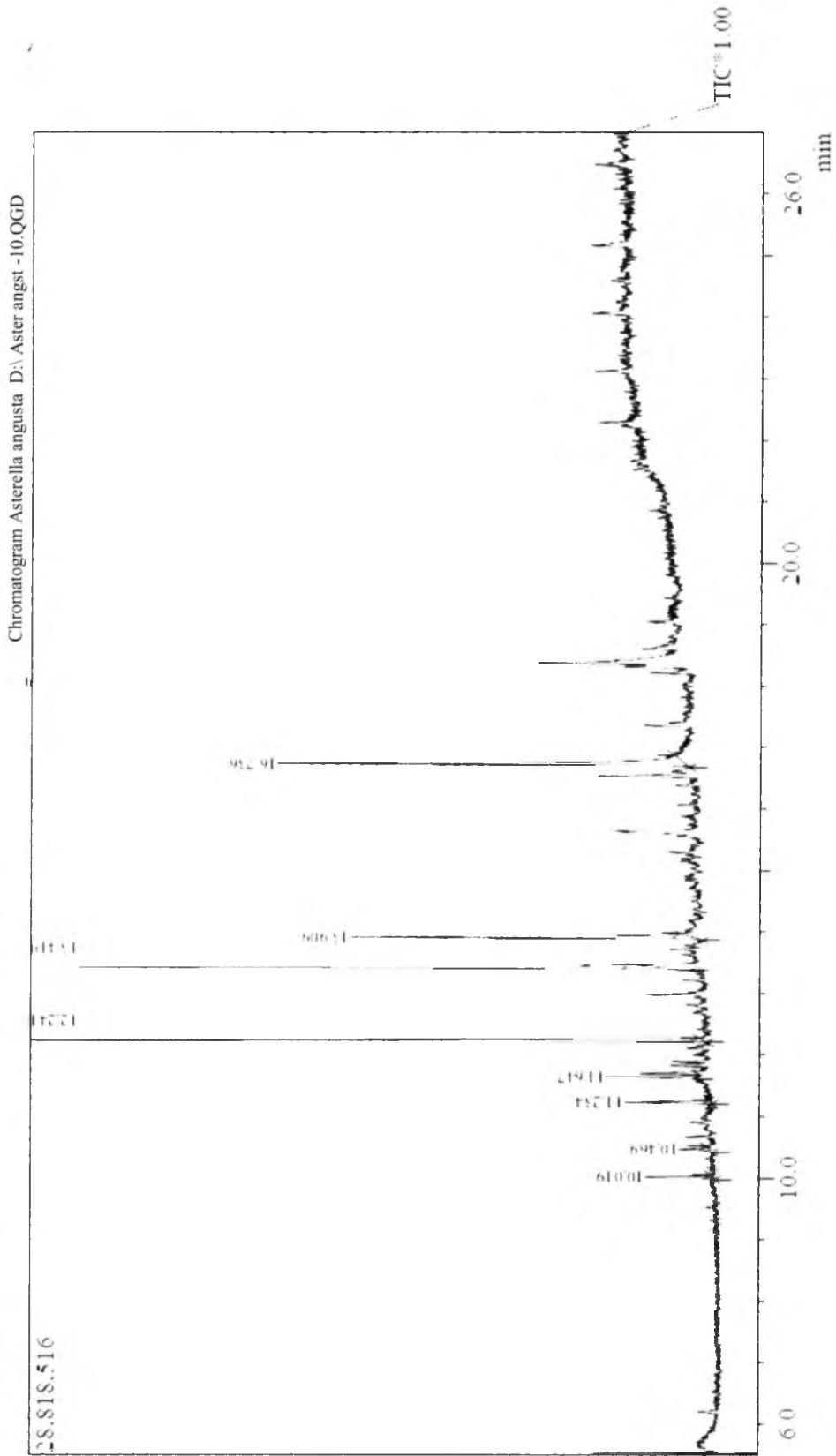


Table: 4.5.10 GC-MS analysis of *Asterella angusta*

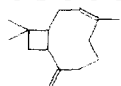
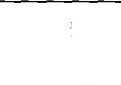
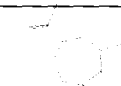
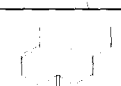
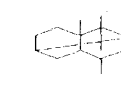
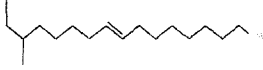
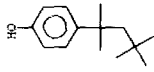
Sr. No.	Retenti on time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.01	2.66	Humulen	C ₁₅ H ₂₄	204	<u>Sesquiterpene</u>		Anti-inflammatory Antibacterial, Anifungal
2	10.46	1.29	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	C ₁₅ H ₂₄	204	Alkene		Anticancerous
3	11.23	3.51	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)	C ₁₅ H ₂₄	204	Alkene		Antibacterial, Blue colour pigmentation
4	11.64	3.74	Germacrene D 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-	C ₁₅ H ₂₄	204	<u>Sesquiterpene</u>		Antibacterial, Nematocide, Insect Pheromones
5	12.24	25.85	Patchouli alcohol	C ₁₅ H ₂₆ O	222	Terpene		Antibacterial
6	13.41	24.76	14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252	Alcohol		Antioxidant
7	13.90	15.46	Phenol, 4-(1,1,3,3-tetramethylbutyl)	C ₁₄ H ₂₂ O	206	Alcohol		-----
8	16.73	22.71	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Linolenic acid		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide

Fig: 4.5.11 GC-MS chromatogram of *Hymenostylium recurvirostre*

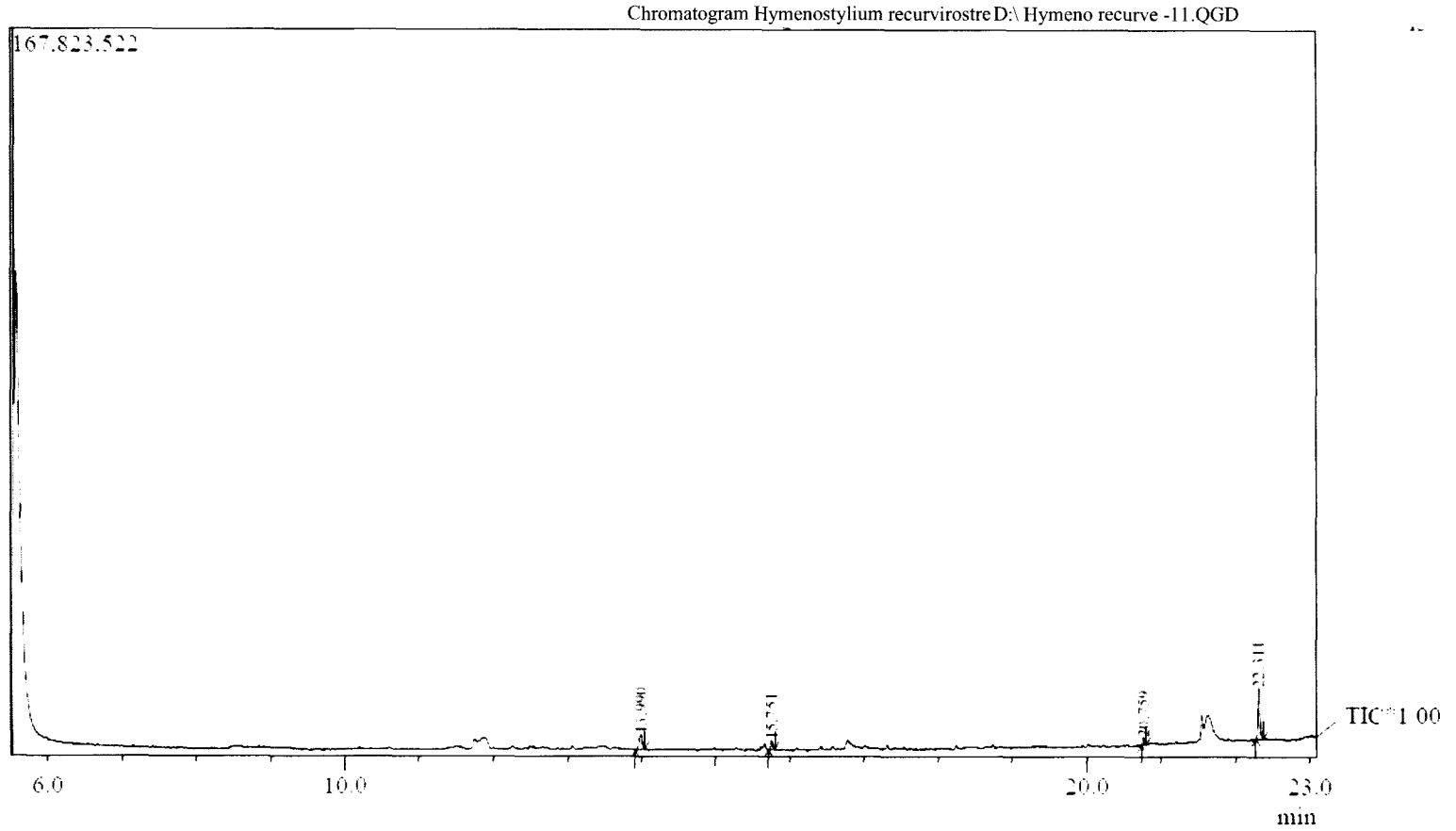


Table: 4.5.11 GC-MS analysis of *Hymenostylium recurvirostre*

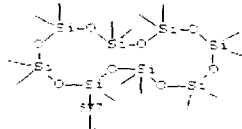
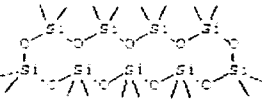


Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	13.99	28.05	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	---		---
2	15.75	8.31	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	---		---
3	20.75	7.02	Heptacosane	$C_{27}H_{56}$	380	Alkane		Antibacterial
4	22.31	56.62	Tetracosane	$C_{24}H_{50}$	338	Alkane		Antibacterial Antioxidant

Fig: 4.5.12 GC-MS chromatogram of *Riccia discolor*

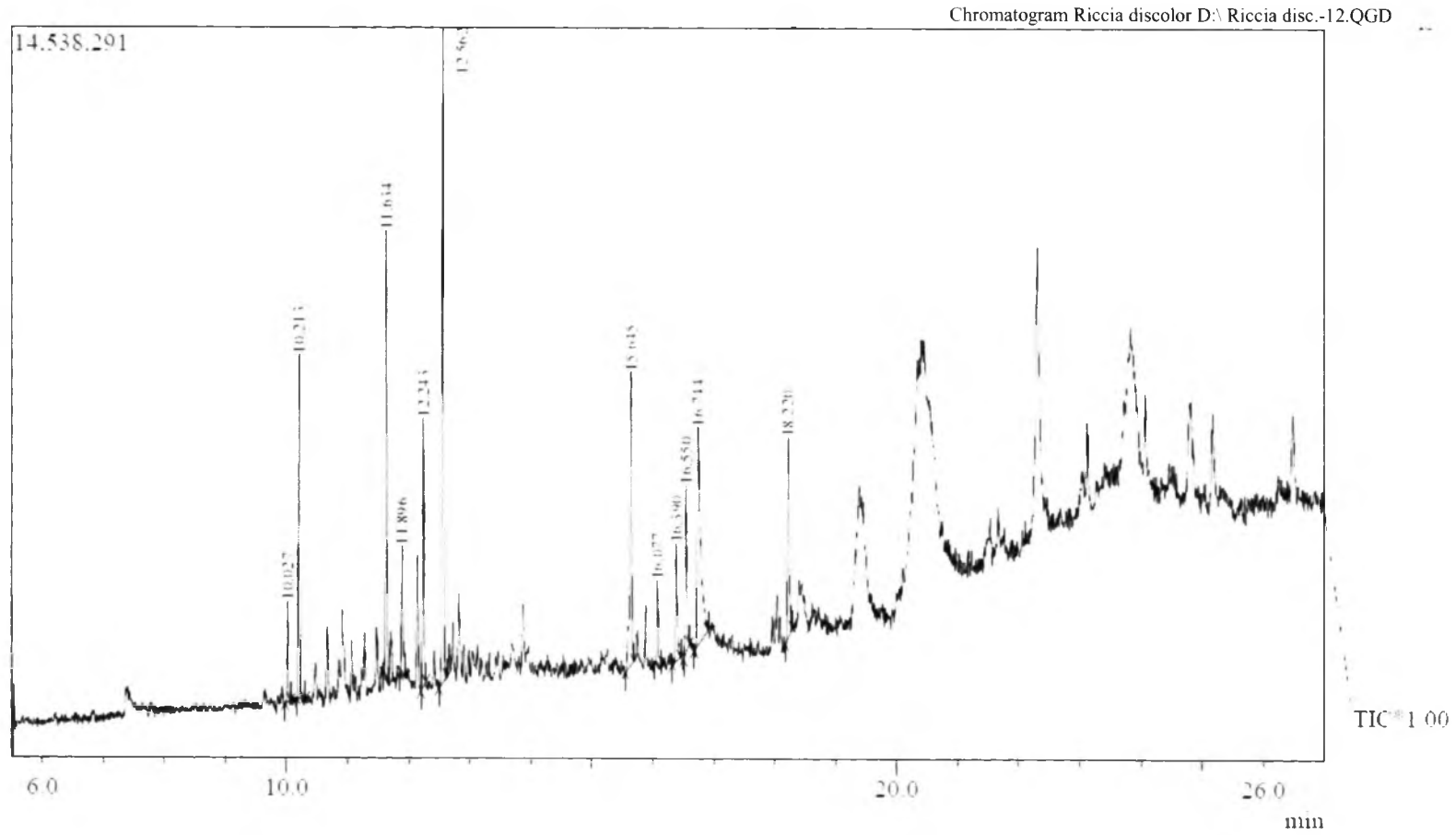






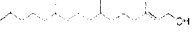
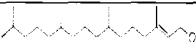




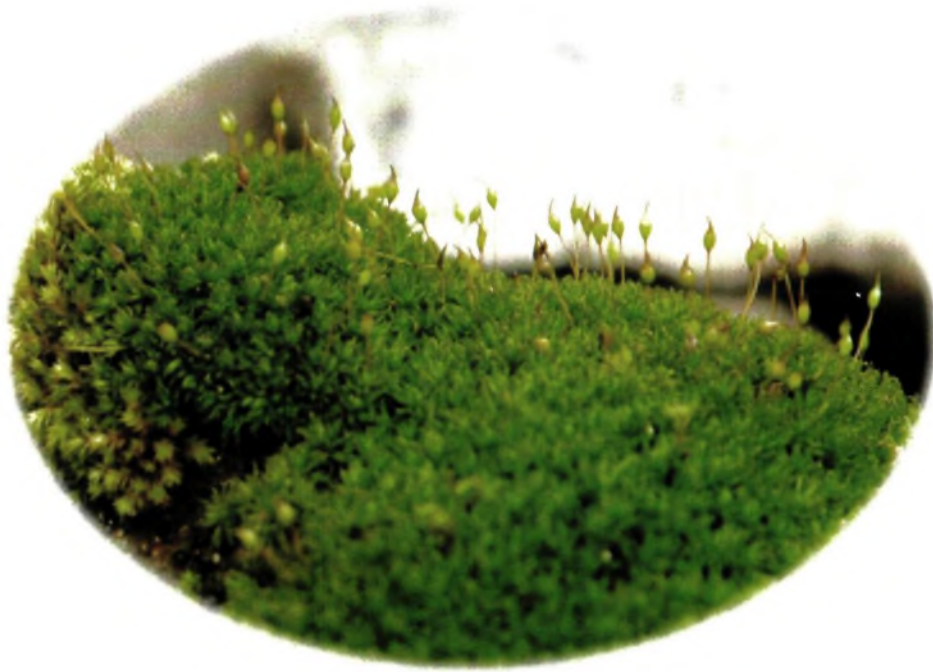


Table : 4.5.12 GC-MS analysis of *Riccia discolor*

Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Probable Structural formula	Activity reported
1	10.02	2.58	Longifolene	C ₁₅ H ₂₄	204	Sesquiterpene		Acne vulgaris treatment, Antibacterial and Antifungal
2	10.21	9.71	Thujopsene Cyclopropa[d]naphthalene, 1,1a,4,4a,5,6,7,8-octahydro-2,4a,8,8-tetramethyl	C ₁₅ H ₂₄	204	Sesquiterpene		Antifungal
3	11.63	11.85	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene	C ₁₅ H ₂₄	204	alkene		-----
4	11.89	4.29	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)	C ₁₅ H ₂₂	202	alkene		-----
5	12.24	7.77	Patchouli alcohol	C ₁₅ H ₂₆ O	222	Terpene		Antibacterial
6	12.54	20.75	Longifolenaldehyde	C ₁₅ H ₂₄ O	220	Sesquiterpene		Antibacterial, Antifungal
7	15.64	10.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory Antibacterial, and Antifungal
8	16.07	2.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Terpene alcohol		Anti-inflammatory Antibacterial, and Antifungal
9	16.39	4.03	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	Palmitic acid		Antioxidant, Nematicide, Pesticide
10	16.55	5.09	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	ester		Antibacterial
11	16.74	14.78	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Palmitic acid		Antioxidant, Nematicide, Pesticide, Antimicrobial
12	18.22	6.65	Phytol	C ₂₀ H ₄₀ O	296	Diterpene		Anti-inflammatory Anti-cancer, Diuretic



CHAPTER FIVE
DISCUSSION

5. DISCUSSION

5.1 Soil dynamics

Natural environments are extremely diverse and mostly contain a wide range of microorganisms which reflect the nature of habitat and the ability of individual members to compete successfully and coexist within given ecosystem. Hence, the greater heterogeneity of the environment represents more diverse and complex microflora. Soil is the complex, natural, dynamic entity having its own physical, chemical and biological properties. The soil biota depends upon various characteristics of soil whereas these properties in turn are continuously modified by the activities of biological population. The physico-chemical properties of soil are the indicator of the soil (Kennedy and Papendick, 1975) nature, quality of soil and vegetation.

5.1.1 Physical characters

Patel, (1968) described the soil of Melghat as tropical, lateritic type with red-brown colour arose from Deccan trap. The colour of soil depends upon the constituents like iron, manganese compound and organic matter in the soil. Dhore, (2002) reaffirmed the characteristics of Melghat soil as Lateritic, Clay, Alluvium and Bouldery of Murrum types. The regions like Chikhaldara plateau, Semadoh, Ghatang road represents red bouldery soil in patches showing less fertility, nutrient deficiency and less water holding capacity. Certain saxicolous habitat of bryophytes like *Funaria hygrometrica*, *Targionia hypophylla* observed commonly on this soil. Nutrient deficient bryophytes can easily attach to the substratum of such soil. However, the regions like Amazari, Belkund, Tarubanda, Kolkhas and Semadoh show red brown lateritic type of soil. This represents high content of ferric oxides. Hornworts like *Anthoceros erectus*, *Folioceros udarii* and mosses like *Hyophila involuta*, *Funaria hygrometrica* were found on such type of soil. Frequent black soil clay patches found at the riversides of Sipna and Dollar at Semadoh, Kolkhas and near small creeks or water bodies at Chikhaldara and allied regions. The blackish clay or alluvium soil found at Semadoh, Churani, Madaki and on the plains show rich fertility and water holding capacity. The terricolous plants like *Riccia gangetica*, *Riccia discolor*, *Anthoceros erectus*, *Notothylas indica*, *Phaeoceros laevis* and sometimes *Plagiochasma appendiculatum* were found on this type of soil.

The bright coloured, yellow green, fluorescent *Cyathodium tuberosum* also found on forest soil in shady

conditions during rainy seasons. The brownish colour of soil observed at Semadoh, Gugamal and Chikhaldara region showing plants like *Targionia hypophylla*, *Hyophila involuta*, *Bryum coronatum* etc. in terricolous and rupicolous habitat. The colour of soil may be due to presence of humus (Ratnaparkhi, 2007). The mosses like *Brachythecium turgidum* and *Stereophyllum decorum* are of corticolous or epixylic in habitat found on the trees like *Mangifera indica* or *Ficus virens* due to rigid cortical cells and high moisture content in the region. The ability of mosses helps and enables them to occur on various habitats due to nutrient deficient trophic levels. This confirms that bryophytes can occur in wide range of different habitats (Slack, 1976).

Kapoor *et al.* (2002) reaffirmed that VAM (Vesicular Arbuscular Mycorrhiza) fungi, like microorganisms occurs globally in broad range of dissimilar environment in response to soil characteristics. The various soil microbes, VAM fungi found abundantly in the black, alluvium, lateritic and among bouldery soils of Melghat forest. The *Glomus* sp. and *Acaulospora* sp. found dominant due to their interactive and symbiotic association with the host plants like bryophytes.

Hence, soil texture affect drainage condition, water holding capacity, amount and size of pores, plant root development, aeration and soil fertility and influence on vegetation growth.

5.1.2 Soil pH

pH is a good measure of the intensity of acidity and alkalinity of the soil-water suspension and provides appropriate information about the chemical nature of soil. pH of soil depends upon relative amounts of adsorbed hydrogen and metallic ions. The soils from the Semadoh, Ghatang found slight acidic in nature while the Chikhaldara plateau, Amazari, Churani, Vairat, Khongada and Gugamal forest showed neutral type of soil. Whereas, soils at few locations like Belkund, Tarubanda, and Gawilgarh revealed slightly basic nature. Most of the bryophytes species occurs at neutral nature of soil and hence represents good biological indicator or ecological indicators in Melghat forest. (Table 4.1.1)

pH of soil influences the distribution of various VAM fungi with relation to edaphic factor (Mosse, 1973). *Glomus* sp. and *Acaulospora* sp. mostly occurred in

the soils of Melghat forest as they are found commonly in neutral or slight basic or slight acidic soils (Wang *et al.*, 1985).

Powel and Bhagyaraj (1984) affirmed the VAM fungal spore germination occurs within a range of pH that is acceptable to plant growth. The bryophytes soil pH ranges from 6.73 - 8.06 (Fig: 4.1.1) and significantly influences the soil microflora favouring germination of VAM spores like *Glomus fasciculatum*. It is noteworthy that VA mycorrhiza formation decreases with decrease in soil pH but they are tolerant to higher broad soil pH (Hayman and Tavares, 1985). Hence, the relation between soil pH and mycorrhization may be complex but depends upon the host plant species and type of soil within niche along with its nutrient status or mycotrophic habitat.

5.1.3 Soil temperature

The temperature of soil is important in promoting chemical or biological activities. Soil temperature also controls the growth of plants, microflora and distribution of vegetation on the surface of the earth (Table: 4.1.1). At low temperature, the speed of chemical reaction get reduced but hindered at high temperature. The bryophytes occurring at low soil temperature showed maximum growth favouring prostrate or rosette type growth at Belkund, Amazari, and Chikhaldara with plants like *Riccia* sp. and *Plagiochasma* sp. The soil temperature also depends upon slope, latitude of land, distribution of land and water, vegetation cover, nature of the soil and height of the location from mean sea level (MSL). As bryophytes are shade and moisture loving plants, their distribution favours the low temperature, high moisture and availability of water sources (Wang *et al.*, 1985).

VA mycorrhizal formation and functions are potentially sensitive to temperature and maximum mycorrhization occurs at 18 - 26⁰C in soil temperature, while moderate at 7.5⁰C and reduced or inhibited above 30⁰C (Cooper and Tinker, 1981). The Melghat forest soil with bryophytic vegetation showed soil temperature variation of range of lowest 16.2⁰C at Chikhaldara and highest 29.1⁰C at Madaki location (Fig: 4.1.2). However, moderate range of 16 - 22⁰C soil temperature found in the region.

Increase in soil temperature also stimulates VAM colonization and spores germination. The *Glomus* sp., *Acaulospora* sp. found abundantly among bryophytes of Melghat forest favours germination in between 20-26⁰C and *Gigaspora* sp. around

30°C (Daniel and Trappe, 1980). Hence, maximum diversity of VAM fungi occurred in the soils associated with the bryophytic thalli of Melghat region.

5.1.4 Total Dissolved Solids

The total dissolved solids (TDS) offers an idea about presence of nutrients or salts present in soil with respect to concentration. Nutrient salts are important resources that limit the plant growth in tropical soil (Jordan and Herrera, 1981). The TDS range found in bryophytes of Melghat soil ranges from 008 to 031 mg/L (Table: 4.1.1). The plants like *Anthoceros* sp. and *Folioceros* sp. at Semadoh found in less TDS range while plants at Belkund and Koha like *Asterella* sp. and *Plagiochasma* sp. possess high TDS range as compared to others (Fig: 4.1.3). The *Glomus* sp., *Acaulospora* sp. and *Gigaspora* sp. represents the diversity of mycorrhization with respect to TDS values in the soils of Melghat forest. This sensitivity of VAM fungi depends upon soil characteristics or soil environmental factors by promoting colonization, spore germination and influence of the physiology or growth of plants. Hence, soil biota interacts with the specificity of edaphic factors to establish for growth and development. However, the capacity of VAM fungi to supply nutrients to the host plant varies with the species of fungus as well as the host plants (Kapoor *et al.*, 2002).

5.1.5 Electrical conductivity

The electrical conductivity of the soil sample is the measure of current carrying capacity explaining the value of soluble salts present in it. The soil nutrient status in the rhizosphere will affect change in soil biological community, which ultimately has consequences on plant growth. The rhizosphere is a partnership between the plant, soil and soil organisms. Plants provide carbon and food source for soil organisms that bind the soil particles into aggregates and recycle soil nutrients while, the soil provides the habitat, water and mineral nutrients for both soil organisms and plants (Carling *et al.*, 1979). The region of Churani showed maximum electrical conductivity of 0.35 Mmhos/cm and abundance of soluble salts along with regions like Khongada, Kolkhas, Semadoh and few regions of Chikhaldara (Table: 4.1.1). Hence, these locations showed more density of vegetation of plants and bryophytes as compared to other sites. The bryophytes thalli attached to the soil surface in Melghat forest also favours the diverse soil microflora like VA

mycorrhization and provide shelter to vertebrates and insects. The plants like *Plagiochasma appendiculatum*, *Targionia hypophylla* and other liverworts found in the soil of high electrical conductivity (Fig: 4.1.4) and reciprocate more diversity of VAM spores in the soil than other plants. This may be due to high saturation level of colonization of VAM fungi in response to soil physical characteristics of the Melghat forest. Hence, (EC) electrical conductivity of all soil samples indicates that all soils were good for growth and development of any type of plants.

5.1.6 Nitrogen status

Plants absorb combined nitrogen in the form of NH_4^+ and NO_3^- ions from the soil solutions. The main source of nitrogen is organic matter. Generally, about 2-3% nitrogen present in organic matter is available to plants. The rest is present in complex organic contents, which are ordinarily resistant to decomposition. Nitrogen is an important macro-element in plant physiology and metabolism. Nitrogen is essential constituent of chlorophyll, amino acid, proteins, protoplasm, nucleotides and alkaloids. It is also present in many vitamins, enzymes and plant growth hormones, and favours the photosynthesis and carbohydrates assimilation.

The nitrogen content in the soil of Melghat forest and among bryophytic thalli ranges from 134-501 kg/ha (Table: 4.1.1). The nitrogen content found more in Belkund, Koha, Tarubanda region and few sites at Semadoh and Chikhaldara region. The plants like *Plagiochasma* sp., *Asterella* sp., *Reboulia* sp. and *Anthoceros* sp. in the region occurred in nitrogen rich soils of the forest with increase in vegetative growth (Fig: 4.1.5). The organic matter formed from the decomposition of fallen debris is the most important constituent of the soil and the apex region of maximum biological activity. It is a primary source of plant nutrients, especially nitrogen and also determines the physical properties of the soil, like porosity and aeration. The regions like Semadoh, Tarubanda, Koha, Belkund and few sites at Chikhaldara exhibits more vegetation development than other regions (Ratnaparkhi, 2007).

The role of mycorrhiza in improving nitrogen fixation capacity has been well known and documented. Nitrogen can suppress or enhance root colonization and can be easily used by VAM fungi. However, excess nitrogen contents reduce mycorrhizal development where NO_3^- being more inhibitory than NH_4^+ (Menge, 1984). High concentration of nitrogen lower downs the mycorrhizal growth or damaging effects on

mycorrhizal colonization. Low to moderate nitrogen contents found in the soils of Churani, Amazari, Gugamal and Ghatang showing good covering of bryophytes carpets.

5.1.7 Phosphorus uptake

The phosphorus is one of the important macro-nutrient for plants. In most of the tropical soil, phosphate is present predominantly as inorganic compound of calcium under neutral and alkaline condition, while under acidic condition, iron and aluminium phosphates predominate phosphate. Availability of phosphorus in early stages benefits the host plant by producing deeper and abundant roots. However, the deficiency of phosphates causes purple colour of leaves, stunted growth and development and senescence. The phosphorus content among bryophytes soil varies from moderate to high contents (Table: 4.1.1) but deficient in the plants like *Plagiochasma intermedium* and among mosses growing on saxicolous habitats like *Funaria* sp., *Hyophila* sp. and *Hymenostylium* sp. due to epilithic substratum and nutrient deficient condition (Fig: 4.1.6). However, the Chikhaldara and Semadoh exhibits high phosphate concentration in soil and the regions found rich in vegetation cover with maximum density of plants (Ratnaparkhi, 2007).

In nature, most of the plants possess integral association with VAM fungi for phosphorus uptake by roots. This is largely due to external hyphae absorbing phosphorus beyond the depletion zones around roots and root hairs and transporting it to root tissues (Koide, 1991). Nevertheless, on other hand some reports have shown that increasing concentration of soluble phosphate in soils can decrease fungal colonization of roots. This reduction could result from direct inhibition of external hyphal growth or from indirect effects including changes in endomycorrhizal infections (Plenchette *et al.*, 1983). In present investigations, moderate amount of phosphorus was found to be present among soils of the bryophytes of Melghat forest with diverse distribution and VAM fungi association.

5.1.8 Potassium contents

The potassium contents among the soil of bryophytic thalli showed high value as compared to nitrogen and phosphorous (Table: 4.1.1). The regions like Koha, Belkund, Semadoh and Ghatang showed high concentration of potassium while Chikhaldara and other allied areas showed moderate value of potassium (Fig: 4.1.7).

Potassium is major contributor to osmotic potential of plant cells by balancing the charge of both diffusible and non-diffusible ions. It also acts as an activator of many enzymes in physiological processes like photosynthesis, respiration, carbohydrates metabolism, protein synthesis and stomatal movements (Jain, 2006). Hence, bryophytes are capable of taking nutrients from weak solutions by permitting them to grow in situations that may be limiting to tracheophyte (Glime, 2006).

5.1.8 Percentage Carbon

The percentage of organic carbon in bryophytes of Melghat forest found in moderate range of 0.29% - 0.53% (Table: 4.1.1). The regions like Semadoh, Chikhaldara, Khongada and Ghatang exhibits high organic content in the soil as compared to other regions (Fig: 4.1.8). Hence, these regions show good quality of vegetation and forest cover.

5.1.9 Cation Exchange Capacity (CEC)

Bryophytes use their rhizoids to gather nutrients by penetrating and attaching to the soil surface. The source of nutrients also includes precipitation, rainfall and the substrate where bryophytes absorb mineral nutrients over their entire surface. The ability of bryophytes to take up nutrients from any substrate permits them to grow in situation that may not be favourable for existence (Babb and Whitfield, 1977).

Bryophytes possess a typical cation exchange capacity (CEC) due to high concentrations of non-esterified pectates i.e. polyuronic acids called galacturonic acid within primary cell wall, than any of the other land plants (Clymo, 1963). These galacturonic acids have a carboxyl group (COOH^+) protruding on the outer surface of the wall. This carboxyl group freely exchanges its H^+ for other cations in its surroundings. K^+ ions often filter through the bryophyte layer and mostly bound on the bryophytes (Bates, 1982). Bryophytes have many exchange sites permitting differential binding of ions. Hence, the present investigations correlate with the findings of Glime (2006). From above discussion, it is noteworthy that fungi are often associated with rhizoids of bryophytes because large numbers of bryophytes are afforded the advantages of fungal partner relationships providing them with considerably more surface area for acquiring nutrients.

5.2. Bryophytes of Melghat

The Indian subcontinent divided into six bryo-geographical regions viz., Western Himalayan territory, Gangetic plains, Eastern Himalayan territory, Central Indian zone, Punjab plains and Rajasthan and South Indian zone (Pande, 1958). According to Singh (2001), the Melghat forest comes under Central Indian zone and near to South Indian zone. Patel (1968) considered the Melghat forest as a tropical deciduous forest as per geographical condition however, present author correlated the distribution of bryophytes in relation to environmental conditions. The Melghat forest can be divided into some different bryoecological zones. These zones based on the form and species composition of the vegetation cover as well as one or more characteristics of physical environment.

Melghat forest core can be divided into three major bryoecological regions based on climatic conditions, forest types and the soil. Each area having distinct climatic conditions resulting in variation at vegetation types and number of species.

- 1) Chikhaldara Plateau
- 2) Semadoh Corridor
- 3) Belkund Sector

I) Chikhaldara Plateau

This region includes Vairat, Churani, Gawilgarh, Salona, Amazari, Madaki, part of Gugamal forest and Bhimkund Valleys. The region dominantly influenced by physiography of Satpura Mountains, hilly ranges, deep valleys called "Khoras" and flat tops. The average elevation ranges from 750 m to 1100 m above MSL. The soil composition mainly of bouldery, lateritic red and grey in colour dominantly. The region receives high rainfall of more than 1500-2000 mm (Table: 1.1) and humidity above 85 % or more (Table: 1.3). The rich bryophytes vegetation occurs in the region. Tropical moist deciduous and tropical semi evergreen forest occurs in humid-hilly regions. Here the climate is more humid with ample moisture favourable for rich vegetation and xeric forms are replaced by the species, which grow in places that are more humid. The number of hornworts, mosses and liverworts suddenly increases along with their wider distribution. The common bryophytes are *Riccia* sp., *Plagiochasma* sp., *Targionia* Sp., *Cyathodium* sp. *Reboulia* sp., *Anthoceros* sp., *Notothylas* sp., *Stereophyllum* sp., *Hyophila* sp., and *Funaria* sp., etc.

II) Semadoh Corridor

The region consists of Ghatang, Kolkhas, Raipur, Makhala and core part of Gugamal forest. The region is ranging between 500-750 m altitudes with mostly Deccan trap. The soil is lateritic red, grey coloured with alluvium along river basins. The region experiences moist condition during Monsoon and received average rainfall mean of 1200 mm (Table: 1.1) with humidity found about 70 % or more (Table: 1.3). The overall topography is highly rugged with hilly ranges with deeply dissected valleys and flat surfaces across region. The region also shows rich diversity and vegetation of bryophytes. The common species of the region are *Bryum coronatum* Schwaegr, *Hyophila involuta* (Hook) Jaeg, *Targionia hypophyla* Linn, *Plagiochasma appendiculatum* Lind et. Lindenb, *Riccia discolor* Lind et. Lindenb, *Cyathodium tuberosum* Kash., *Anthoceros erectus*, *Phaeoceros laevis* Prosk. etc.

III) Belkund Sector

This region covers the area of Tarubanda, Belkund, Koha, Khongada and Parsapur with core part of Gugamal forest. The average elevation of the region ranges from 550 m to 700 m. The annual rainfall received in the region found less than 1000 mm (Table: 1.1) with average humidity of 70 % or more (Table: 1.3). The soil types variable from red, grey and black depending upon locations and habitats. The region shows diverse vegetation of bryophytes but their numbers found reduced. The common species are *Asterella angusta* (Steph) Kachroo, *Riccia gangetica* Ahmad, *Folioceros udarii*. Asthana et. Srivastava, *Bryum Coronatum* Schwaegr. and *Funaria hygrometrica* Hedw.

5.2.1 Distribution in different habitats

In the whole Melghat region, the bryophytes major group viz. liverworts, hornworts and mosses showed unequal distribution (Table: 4.2.1). From Belkund region to Semadoh corridor and towards Chikhaldara plateau, richness in bryo-diversity found due to changes in composition at communities with respect to eco-environmental conditions.

The species which can tolerate wide range of climate are widely distributed in Melghat includes *Riccia gangetica* Ahmad, *Plagiochasma appendiculatum* Lehm et. Lindenb, *Riccia discolor* Lehm. et. Lindenb, *Anthoceros erectus* Kash, *Hyophila involuta* (Hook) Jaeg. etc. Some species like *Stereophyllum decorum* (Mitt) Wijk et.

Marg., *Brachythemium turgidum* Broth ex. Dix. are highly humidity loving species and their occurrence in the limited high altitude places like Chikhaldara and Gawilgarh, Amazari, Chati Bilta etc. indicates adequate moisture condition for bryophytes in the region. The species like *Folioceros udarii* Asthana et. Srivastava found on terricolous habitat but occurs at high moist places near Semadoh in the range of 600-900 m altitude. However, the species like *Riccia*, *Hyophila* and *Funaria* occurs almost in all ranges of elevations. The plant *Cyathodium cavernarum* Kunze in. Lehm., luxuriantly occurs near water bodies especially where water percolations occurs among saxicolous habitats. It seems to be general rule that bryophytes flourish best in crevices where shade and moisture may be more frequently available. In Melghat, bryophytes distributed in diverse habitats (Table: 4.2.2) out of which 09 are found on rocks (Saxicolous) 15 on moist soil floors or on clayey slopes on ditches (Terricolous), 10 on rocky walls, bricks or stones, or pebbles (Rupicolous) and 02 on bark of trees with some rigid base (Corticolous or Phytocolous). The liverworts dominantly occurs on the saxicolous, terricolous and rupicolous habitats due to the availability of substrate and their mode of nutrition (Fig: 4.2.5). However, the hornworts mostly grow on moist soil surface and at shady sites due to their shade loving secondary habit. The mosses are confined to rich terricolous as well as corticolous or phycocolous habitats due to their nutrient absorbing ability from the any substratum like soil or epiphytic base or even from environmental conditions around the habitat. The present findings closely resembles and related with the work of Daniels and Kariyappa, (2007). The Gugamal forest cover is the most core region of the Melghat forest and found more diverse and rich in bryophytes as well as pteridophytes and other floral vegetation.

5.2.3 Factors affecting distribution and species richness or diversity

The occurrence and distribution of bryophyte vegetation in Melghat forest is highly related with the variation in the climate. Nakanishi (1999) correlated the interaction between species diversity and environmental gradients in bryophyte communities in natural conditions and found similar to those in higher plant communities. Jonsgard and Birks (1993) suggested that microclimate is the most important environmental factor but the measured environmental variables accounts for little of the biological variations. The most important factors that affect the distribution of bryophytes are discussed below.

A) Altitudinal Elevation

Regarding the distribution along altitudinal gradient (Fig: 4.2.4), it was recorded that richness of mosses shows maximum diversity at the elevation of 500-750 and 750-1000 m while less at the elevation 250-500 m. The hornworts not found consistent after 1000 m and found decreased. Lloret *et al.*, (1997) also found that maximum species richness decreased after certain altitude. However, the liverworts diversity increases from 0-1000 m altitude and found luxuriant above 750 m in the study area. Andrew *et al.* (2003) stated that altitude have a significant effect on diversity. In the present investigations, bryophytes diversity found rich in altitude above 700 m to 1000 m among liverworts, hornworts and mosses due to substrate diversity and habitat circumstances of vital growth and reproduction. These findings match with the findings of Privitera and Puglisi (2000) who suggested that indices of biodiversity in bryophytes increase with altitude because air pollution is lower at higher altitudes. Here the author also suggests that less human disturbances in higher altitude also favours the bryoflora in the region. The maximum corticolous mosses are found at 750-1000 m altitude in Chikhaldara and Gugamal region due to high moisture or humidity and found similar to the report of Sharma (2002) in Gujarat.

B) Moisture Impact

In Melghat forest, most of the bryophytes dominance found in moist conditions or near water bodies like rivers, lakes, streams or small creeks, and waterfalls. The species like *Cyathodium cavernarum* Kunze. found near region where water percolation and humidity at high range during monsoon at Devipoint and Bhimkund region (Table:1.3). At Gawilgarh, the high humidity and shady condition favours the fluorescent green and yellowish bright species like *Cyathodium tuberosum* Kash. as a distinguished habitat. Although mosses found abundant in moist Gugamal core region but they are also found at Ghatang, Semadoh, Kolkhas, Raipur region where they endure prolonged dry periods. The liverworts and hornworts also found in areas where moisture is higher to complete their life cycle. This finding resembles with the views of Clausen (1952) that the highest level of humidity positively respond to rich bryoflora in the region. As we know that high rainfall in Melghat region ranges from 1000-2500 mm which makes it moist deciduous forest. Hence, the canopy pattern maintains the humidity content and also provides various moist and shady habitats supporting bryophyte vegetation. Eldridge and Tozer (1997) considered

rainfall as primary determinant of plant cover which in turn affects light and nutrient availability and affects bryoflora. Hence, more the humidity (Fig: 1.6) in local microclimate, greater is the richness and diversity of bryophytic communities. During monsoon season heavy showering of rainfall and misty climate makes the Melghat valley as a “Paradise of Bryophytes”.

C) Temperature

The temperature in Melghat varies from below 8⁰C to more than 37⁰C (Table: 1.2) and affects the humidity and moisture. The region becomes pleasant during the monsoon to winter due to low temperature and becomes dry during summer season with higher temperature (Fig: 1.5). In general, mosses generally found where relatively low temperature and high humidity conditions are present. In present study, as we move from Paratwada to Ghatang, Semadoh, Kolkhas, Belkund and Chikhaldara the temperature, humidity and light variations are found due to hilly areas and elevations. In hilly regions, maximum numbers of bryophytic species are found in upward direction and along the interior sides. The liverworts and hornworts distribution positively responds the low temperature and species like *Reboulia hemisphaerica* (L) Raddi, *Asterella angusta* (Steph) Kachroo. are found in low temperature area. However, species like *Plagiochasma* found to complete its life cycle even in xeric habitat. The *Phaeoceros laevis* and *Folioceros udarii* requires low temperature and high moisture to complete their life cycle found in Semadoh corridor. Hence, ecologically the light affects the temperature, then moisture as well as habitat. The angiospermic vegetation lie in the interior and along pathway provide the shade canopy to the forest floor and prevent direct exposure to the sunlight and provide good substrate for bryophyte vegetation. Eldridge and Tozer (1996) also agree the view that temperature and light factors affect the bryophytic vegetation.

D) Biotic factor

In Melghat forest, the rich bryophytic vegetation is restricted to the core forest areas only viz. Gugamal, Semadoh, Chikhaldara region and Belkund-Koha region. The remaining areas found as either tourist places or multiple use areas with intense and continuous human interference. The area under forest is used for collection of fire wood and minor forest products as well as for cultivation of agricultural crops by the tribal residing there, because of which vegetation has been practically decimated.

Herds of grazing animals kept by the tribal graze the vegetation wherever it exists. Bergamini *et al.* (2001) supports the present findings and also suggested that for safeguarding of bryophytic diversity, extensive grazing by cattle is crucial factor, and must be prevented.

E) Soil factor

The nature of soil and its ability to retain water and moisture play an important role in distribution of various species (Table:4.1.1). The lateritic soil in the region at Chikhaldara region is very rich in clay and organic matter. The Brown coloured soil at Semadoh and Belkund region supports the better vegetation at both the sites. It was observed that some plant species like *Plagiochasma intermedium* and *Hyophila involuta* show luxuriant growth while growing on walls in comparison to those growing on moist soil or rocks suggesting that the species might prefer calcium. The distribution of bryophytes seems to be positively related with Nitrogen, Potassium and Phosphorus, (N, P, K) ratio and organic carbon percentage of substratum. Hebrard and Liosel (1994) supported these findings and considered that availability of nutrients and ambient humidity in soil play an important role in the differentiation of terricolous bryophyte communities.

5.2.4 Species and association (Plate: 13)

In Melghat forests, many bryophyte species associates with other cryptogams and small phenarogams. The *Anthoceros erectus* species at Salona Lake and near water bodies found associated with algae *Nostoc* sp. balls or puffs on soil and near water percolations sites where moisture is higher (Plate: 13-G). *Cyathodium cavernarum* and *Cyathodium tuberosum* found very specific at shady and moist regions with dramatic association with pteridophytes like *Selaginella* and *Adiantum* sp. dominantly (Plate: 13, C-D). *Targionia hypophylla* found associated with silver fern *Cheilanthes albumarginatum*, *Tectaria sinnate* and *Selaginella* sp. at Gugamal forest (Plate: 13, A-B). The terricolous *Riccia discolor* also found associated with algal *Nostoc* sp. balls or puffs at Belkund and allied regions (Plate: 13-E). The plants like *Plagiochasma appendiculatum* at water percolation sites near Chikhaldara and Amazari found associated with *Nostoc* sp. puffs (Plate: 13-F). Most of the places were found to be later replaced by fillicales i.e. pteridophytes like ferns (Plate: 13-H). Shaw and Renzaglia (2004) supported these findings and suggested that bryophytes are the

pioneers in process of succession in plants at terricolous habitat and provide the substrate and base until climax of any community.

It is noteworthy that, mosses like *Funaria hygrometrica* found on ash or coal texture of soil at various burnt sites of Semadoh, Gugamal and Ghatang regions. Along with *Bryum coronatum* they cover the whole ground surface with dominant patches of the thalli with lusty green fruiting bodies (Plate: 11, B-C). These fruiting bodies turn pink or dark pink black coloured capsules at the mature stage in late September. The presence of these mosses at burnt sites in Melghat forests supports and confirms the finding by Southorn (1976) that mosses are pioneers in succession at burnt sites in forests.

5.2.5 Phytogeographic analysis

In the study area of Melghat forest, about 20 bryophytic species were recorded for the first time during investigations, out of which 10 belongs to liverworts, 4 belongs to hornworts and 6 belongs to mosses (Table: 4.2.1). In liverworts, the Marchantiales is the most dominant order represented by 10 species, belonging to 3 families and 6 genera (Fig: 4.2.1). Order Anthocerotales is represented by 3 families and 4 genera (Fig: 4.2.2). In mosses, the order bryales is the most dominant with 4 families and 6 genera (Fig: 4.2.3). Hence, the distribution of bryophytes in Melghat forest as compared to other Indian bryogeographical region shows affinities with proper elements (Table 4.2.3). These findings correlated with the findings of Choudhary *et al.*, (2008) showing that the region represents the bryoflora of Central India and especially the Satpura hills.

5.2.6 Fertility, Perennation and Dissemination of bryophytes (Plate: 14)

About 20 species of bryophytes have been collected from Melghat forest and found along with gametophytes and sporophytes. The plants with sporophyte usually complete their life cycle within a limited period of the year. These plants are generally light loving and may lack asexual reproduction. The plants die during the unfavourable climatic conditions and perennate by means of spores. It has been also observed that plants which received long photoperiod show more rapid development of sex organs.

In Melghat forest, vegetative growth period of bryophytes is about four months from June to September and all the fruiting species develop sporophyte by the

end of October. The development of sporophyte depends upon climatic conditions, whether favourable or not and plants are monoecious or dioecious. Person (1943), has supported the views of variation in climatic condition affects the fertility of bryophytic vegetation. Minor or sudden changes in climate highly influence the fertility of mosses. Perennation and dissemination is a remarkable adaptation of bryophytes with their ability to remain live for long periods without water, even under high temperature and then photosynthesis within a second after moistened by rain or dew water.

The Melghat forest remains dry for a considerable part of the year with few exceptions. Most of the bryophytes species appear just after the first showers of rains and complete their life cycle within a short period of one or four months. Most of the species get dried up by completing their life cycle due to high temperature and low moisture availability in the region of Belkund, Semadoh, Ghatang, Raipur and Makhala (Plate: 14, C-D). However, on availability of favourable conditions the plants revive their growth with first showers of rain and multiply through regeneration.

The liverworts thalli completely dried up during summer season where temperature becomes quite high. A few thalloid forms like *Plagiochasma appendiculatum* Lehm. et. Lindenb., *Plagiochasma intermedium* Lindenb. et. Gott, *Plagiochasma rupestre* (Forst.) Steph., *Reboulia hemispherica* (Lim) Radii., *Asterella angusta* (Steph.) Kachroo., fruit abundantly in August to October. However, during dry period or unfavourable condition, they perennate by rolling up their thalli and dark ventral scales cover dorsal surface, thus giving the thallus a peculiar appearance (Plate: 14, E-G). It is also observed that, on favourable conditions, the thallus expands and forms one or more apical shoots while older portions die (Choudhary *et al.*, 2008).

The liverworts *Riccia discolor* Lehm. et. Lindenb and *Riccia gangetica* Ahmad., the thalli becomes shrunken and margin rolled upwards and protects the growing region under xerophytic condition. These plants look like black streaks scattered on soil. But, on suitable conditions of the nature, thalli readily revive and resume their growth. The species *Targionia hypophylla* Linn. commonly multiplies with vegetative growth by forming adventitious shoots on ventral surface which later develops into new plants.

In hornworts like, *Anthoceros erectus* Kash., *Notothylas indica* Kash., *Phaeoceros laevis* (Linn.) Prosk. and *Folioceros udarii* Asthana et Srivastava, the thallus found attached closely to the soil substratum and get rolled down during unfavorable environmental conditions or during end of the season by shading sporophytes. Generally, spores are enclosed in mature sporophytes and their preservation maintained by the thallus. On dry conditions, the sporophytic walls get ruptured through collumella and spores get released into the environment. Under the favourable conditions, they germinate and reappear along with new thalli.

These bryophytic species by means of spores or plant pieces disseminates by wind, rainwater and sometimes animals. The spores get blown away and on favourable condition develop into new colonies. Water is an important factor in distribution of liverworts and hornworts, hence most of them appear near water resources or habitats from where water is flowing or percolating or along shaded areas with trickling water, soil slopes of hillocks and mounds (Choudhary *et al.*, 2008).

In case of mosses, they grow luxuriantly in favourable moist and shady conditions but under xeric conditions the leaves becomes enrolled and scattered on soil or on the rocks or on the tree bark along with stem portion (Plate: 14-H). Such plants like *Hyophila involuta* (Hook) Jaeg., *Funaria hygrometrica* Hedw., *Bryum coronatum* Schwaegr. *Hymenostylium recurvirostre* (Hedw.) Dix. and *Stereophyllum decorum* (Mitt.) Wijk. *et.* Marg. These mosses generally dispersed by the rain and winds along with small animals are responsible for dissemination of spores over larger area. These spores find suitable area and habitats to give rise to new plants. Glime (2006) also supports these findings and suggested human interferences and animals like birds (especially for nests), bats, butterflies etc. also acts as agents of bryophytes dispersal.

5.2.7 Melghat Bryophytes: Hopes and Fears

Bryophyte of Melghat represents a significant ecological indicator of elegant forest ecosystem giving precise idea and impression of nature. These plants are considered today as "remarkable reservoirs" of novel biologically active compounds. The bryophytic spectrum still encompasses an exquisite variety of forms in Melghat forest and their phytochemical investigations thoroughly needed to be investigated with exploration for future.

But there are fears of declining the bryoflora of Melghat due to habitat losses, road constructions, landslides, heavy trampling effect due to unplanned grazing, constant presume of tourists, traffic, nomadic shepherds and their cattle. Hence, along with the motto "Save tigers, Save nature the author like to say

"Save Bryophytes..... Save Nature".

5.3 VAM fungal symbiosis in bryophytes

Fungal symbiosis is one of the key attribute of land plants. Understanding the nature of bryophyte-fungal associations and unraveling the early evolution of fungal symbiosis at the foot of the land plant tree, provides new insight in the Twenty First Century. Although liverworts do not have roots but have rhizoids, many of them are associated with mycorrhiza. The solute exchange between bryophytes and their associated fungi are functionally similar to mycorrhizae of tracheophytes. The liverworts generally considered as the earliest land plants and from the fungal point of view, the symbiotic fungal associations of liverworts considered as the ancestors of true mycorrhizae. The term ancestors is not used in strict phylogenetic sense but considered with individual establishment of plant species with symbiosis in each geological time scale. However, Nebel *et al.*, (2004) provided phylogenetic evidences for affirmation of the liverworts and fungal partnership in nature. Smith and Read (1997) believed that co-evolution of such partners has been essential for the survival by mutualistic and symbiotic way of soil based nutrient supply. Hence, this compatibility of host plants with the fungus was established before more developed advanced root systems.

5.3.1 VAM-fungal symbiosis in liverworts

In the present investigations, it was observed that rhizospheric or mycorrhizospheric soil of collected bryophytes harbor different types of VAM fungal species. About 10 liverwort species were collected from Melghat region out of which 8 species found true mycotrophic in nature (except *Cyathodium* sp.). The liverworts like *Targionia hypophylla*, *Plagiochasma appendiculatum*, *Asterella angusta*, *Reboulia hemisphaerica* and *Riccia gangetica* occurring on terricolous or saxicolous habitat found associated with VAM fungi. The species like *Acaulospora nicolsonii*,

Acaulospora scorbiculata, *Acaulospora rehmi*, *Acaulospora rugosa* and *Acaulospora mellea* (Table: 4.3.1) found common among all the species. However, only three species of *Gigaspora* viz. *Gigaspora albida*, *Gigaspora gigantea* and *Gigaspora rosea* found in most of the liverworts. The *Glomus* species found dominant in all the liverworts and widely distributed among all the locations of Melghat forest. The species like *Glomus aggregatum*, *Glomus albidum*, *Glomus citricola*, *Funneliformis constrictum*, *Rhizophagus diaphanum*, *Claroideoglomus etunicatum*, *Rhizophagus fasciculatum*, *Funneliformis fragilistratum*, *Funneliformis geosporum*, *Glomus glomerulatum* and *Glomus tenerum* found common in almost all liverworts (Fig: 4.3.2). However, the species *Glomus rubiforme* found only in *Reboulia* sp. and *Plagiochasma* sp. In unique findings, the species of *Scutellispora* like *Scutellispora auriglobosa*, *Scutellispora pellucida*, *Scutellispora persica*, *Scutellispora tricalypta* and *Scutellispora weresubi* found only in liverworts rather than hornworts and mosses (Table: 4.3.2). These findings resembles with the observations by Vyas *et al.*, (2007). However, the presence of external colonization in *Cyathodium tuberosum* recorded by Arora (2008) was not found in the present investigation. The occurrence of VAM fungi in species of *Asterella angusta* was earlier recorded by Lingrone and Duckett (1994). In present study it is noteworthy that all the 10 liverworts belongs to order Marchantiales and showed positive interaction with VAM fungi (Russell and Bulman, 2004; Selosse, 2005). In present investigations, the author found that among glomelian fungi, *Glomus* sp. found dominated followed by *Acaulospora* sp., *Scutellispora* sp. and lastly *Gigaspora* sp. Douds and Millner (1999) suggested that this might be due to the local environments that provide conducive conditions for the occurrence of *Glomus* sp. and these fungal species represents as a significant members of the vegetation community.

Selosse (2005) supported the findings that, liverworts imitate mycorrhizas as their fungal associations and nature of most basal extant land plants. Hence, fungal symbioses were common during land conquest and liverworts adaptation to terrestrial life, at the soil atmosphere adaptations. The occurrence of VAM fungal association in Marchantiales and Jungermanniales has been accepted worldwide. Kottke and Nebel (2005) also supported the concept that, the symbiotic fungal associations of liverworts are the possible ancestors of mycorrhizae. He also clarifies that the term an ancestor is not in phylogenetic sense but considering the heritage and congruency between

evolution of liverworts and their specific symbiotic fungi. Nebel *et al.*, (2004) also indicated that, the development of liverworts as the supposedly earliest land plants to the symbiotic association with Glomeromycota. It is interesting to postulate that these fungi were later replaced by the Ascomycetes in many Jungermanniales species and independently by basidiomycetes in the Aneuraceae and in the several families of the Jungermanniales. The associated basidiomycetes belong to the group heterobasidiomycetous groups which are most probably older. Hence, the opinion indicates that the evolution of fungal plant symbiosis started long before the evolution of roots and the formation of true mycorrhizae.

These views confirm the substrate dependence of liverworts and Glomeromycota association with soil inhabiting liverworts.

Pressel *et al.*, (2010) provided new insight to the concept of fungal distribution among liverworts and their patterns of evolution. The anatomical studies in *Haplomitrium* and *Treubia* showed the presence of both extra and intracellular hyphae. The intracellular fungal zone at lower part of the thalli comprises with fine hyphae and fungal lumps. These species showed mucilage presence in the thalli. Generally, all other bryophytes fungal associations showed hyphal entry via the rhizoids but, in *Treubia* the rhizoids remain unaffected but hyphae entered via mucilage-filled spaces between epidermal cells and in *Haplomitrium*, hyphae penetrate directly through epidermal cell walls. The presence of *Glomus* was reported in the two primitive plants like *Treubia* and *Haplomitrium* as endophytes (Lingrone *et al.*, 2007).

Liepina (2012) reaffirmed the occurrence of VAM fungal structures in 29 families of bryophytes in which most of them found symbiotic in association. This is consistent with our idea that mycorrhizal symbiosis occurs among bryophytes.

5.3.2 VAM fungal symbiosis in hornworts

In consideration with VAM fungal infection in liverworts, endotrophic mycelium is well known to occur regularly in certain hornworts like *Anthoceros* and *Phaeoceros* spp. (Schübler, 2000 and Lingrone, 1988). In present investigations, the hornworts under soil analysis showed the occurrence of mycorrhizal spores among the species of *Anthoceros erectus* Kash. *Folioceros udarii* Asthana *et. Srivastava*, *Notothylas indica* Kash and *Phaeoceros laevis* (L) Prosk. Generally, the species of

Acaulospora, *Gigaspora* and *Glomus* found to be associated with hornworts and their soil (Table: 4.3.1). However, the species of *Scutellispora* was not recorded among all the hornworts. This may be due to environmental conditions and sporulations season or soil conditions. This finding resembles with the observations made by Vyas *et al.*, (2008). The species *Anthoceros erectus* found associated with VAM spores like *Acaulospora nicolsonii*, *Acaulospora scorbiculata*, *Gigaspora albida*, *Gigaspora gigantea*, *Gigaspora rosea*, *Glomus aggregatum*, *Glomus albidum*, *Glomus citricola*, *Funneliformis constrictum*, *Rhizophagus diaphanum*, *Claroideoglomus etunicatum*, *Rhizophagus fasciculatum*, *Funneliformis fragilistratum*, *Funneliformis geosporum*, *Glomus glomerulatum* and *Glomus tenerum*. Here the occurrence and density of VAM spores is lesser than liverworts due to thalli occupation on soil surface and substrate availability (Schüßler, 2000). The species *Notothylas indica* Kash. showed mycorrhizal spores in the soil attached to the thalli. These spores are *Acaulospora nicolsonii*, *Acaulospora scorbiculata*, *Glomus aggregatum* and *Rhizophagus fasciculatum*. In this study, the author has found that other species like *Gigaspora* and *Scutellispora* were not found in hornwort *Notothylas indica* Kash. It is noteworthy that, the occurrence of VAM fungal spores found consistently with edaphic factors as well as thalli occupation and establishment in given niche or habitat. Such substrate of soil determines density and diversity of mycorrhizal spores in an ecosystem (Kernaghan, 2005).

The hornwort *Phaeoceros laevis* (Linn) Prosk. found consistently associated with VAM fungal spores in the soils attached to the thalli (Pressel *et al.*, 2010). The spores occurred among the species *Phaeoceros* are *Acaulospora nicolsonii*, *Acaulospora scorbiculata*, *Gigaspora albida*, *Gigaspora gigantea*, *Gigaspora rosea*, *Glomus aggregatum*, *Glomus albidum*, *Glomus citricola*, *Funneliformis constrictum*, *Rhizophagus diaphanum*, *Claroideoglomus etunicatum*, *Rhizophagus diaphanum*, *Rhizophagus fasciculatum*, *Funneliformis fragilistratum*, *Funneliformis geosporum*, *Glomus glomerulatum* and *Glomus tenerum*. This findings common with species *Anthoceros* due to similarity in habitat occurrence and substrate locations in Melghat forests. The occurrence of such association among *Phaeoceros laevis* was recorded first time in India during investigations.

It is interesting that, the species of hornwort *Folioceros udarii* Asthana and Srivastava found associated with the VAM fungal spores like *Acaulospora nicolsonii*,

Acaulospora scorbiculata and *Glomus aggregatum* only. Rests of the other spores were not found associated with this species. This may be due to the habitat specificity of rupicolous habitat and availability of soil substrate. The thallus of *Folioceros* found semitransparent during heavy rains and moisture in muddy soil substrate. Such environmental conditions may affect the density and diversity of mycorrhizal spores. This is the new record from India about the *Folioceros* association with VAM fungal spores and most probably in the world as this species is endemic to India.

5.3.3 VAM fungal symbioses in mosses

In the present investigation, about six species of mosses were recorded from Melghat forests. About four mosses found on terricolous, saxicolous or rupicolous habitat are *Funaria hygrometrica* Hedw., *Bryum coronatum* Schwaegr., *Hyophila involuta* (Hook) Jaeg. and *Hymenostylium recurvirostre* (Hedw.) Dix.. The moss *Funaria hygrometrica* is cosmopolitan in distribution at Melghat forest. This species found associated with the soil occurring VAM fungi like *Acaulospora scorbiculata*, *Gigaspora gigantea*, *Gigaspora rosea*, *Glomus aggregatum*, *Glomus albidum*, *Funneliformis constrictum*, *Rhizophagus diaphanum*, *Claroideoglomus etunicatum*, *Rhizophagus fasciculatum*, *Funneliformis fragilistratum*, *Funneliformis geosporum*, *Glomus glomerulatum* and *Glomus tenerum* (Table: 4.3.1). Parke and Linderman (1980), also reported association of vesicular arbuscular mycorrhizal fungi with moss *Funaria hygrometrica* using pot cultures. Grasso and Scheirer (1981) reported VAM-fungal association in mosses with species *Glomus tenerum* along the leaves and stem of moss.

The another species *Bryum coronatum* also showed presence of VAM-fungal spores as found and resembled with *Funaria hygrometrica*, but lacks *Acaulospora* and *Gigaspora* species. Rabatin (1980), also reported the occurrence of VAM-fungal hyphae among moss tissues like leaves and stem but lacking in rhizoids.

The mosses like *Hyophila involuta* and *Hymenostylium recurvirostre* also showed occurrence of VAM fungal spores like *Glomus aggregatum*, *Glomus citricola*, *Funneliformis constrictum*, *Rhizophagus diaphanum*, *Claroideoglomus etunicatum*, *Rhizophagus fasciculatum*, *Funneliformis fragilistratum*, *Funneliformis geosporum*, *Glomus glomerulatum* and *Glomus tenerum*. These findings supported by the observation of Zhang and Guo (2007). Hence, mosses showed the occurrence of

Acaulospora, *Gigaspora* and *Glomus* species dominance during the course of work. However, *Scutellispora* species was not recorded among mosses (Liepina, 2012). Moreover, no fungal association was recorded in corticolous mosses like *Brachythetium turgidum* Broth. Ex. Dix. and *Stereophyllum decorum* (Mitt) Wijk *et. Marg.* This may be due to epiphytic condition of the clades during evolutionary development. Nebel *et al.*, (2004) suggested that loss of mycorrhization is apparently a derived character and linked to growth as pioneers, on undated places and as epiphytes. Such Epiphytism occurred independently in several groups of bryophytes. Mosses are also known for primary colonizers in early successional habitats and also provide seed beds for higher plants and also acts as bioindicators of air and soil pollution (Poikolainen *et al.*, 2004).

5.3.4 Rhizoidal colonization and spores density (Plate: 25-28)

Most of the bryophytes under study have revealed the presence of characteristic vesicular arbuscular mycorrhizal hyphae and vesicles inside the tissue and appressoria (Table: 4.3.4). Here the bryophyte species like *Plagiochasma appendiculatum* and *Plagiochasma rupestre* have shown consistently high level of mycorrhizas and high rhizoidal colonization of 78%. The spore density was also found high of 478 per 100 gm of soil (Table: 4.3.3). However, in mosses like *Bryum coronatum* and *Funaria hygrometrica* the rhizoidal colonization is quite lesser i.e. 21-22% and spore density is also low. Out of the 20 bryophytes, about 14 species found to be positive for vesicular arbuscular mycorrhizal association. Here the rhizoids found elongated in liverworts and may be related to high nutrient absorption. The species like *Plagiochasma* growing in terricolous habitat showed presence of hyphal loops and round vesicles (Plate: 25,A-B,). The plants like *Targionia* sp., *Asterella* sp., *Reboulia* sp. and *Riccia* sp. also showed more and significant rhizoidal colonization as compared to hornworts and mosses (Plate: 27,A-C). Some mosses like *Brachythetium* sp, *Stereophyllum* sp., *Hyophila* sp. and *Hymenostylium* sp. have not shown any rhizoidal colonization.

It is worth mentioning that smooth walled rhizoids generally infected with the fungal hyphae as compared to tuberculated rhizoids. The entry of hyphae clearly visualized in rhizoids of species like *Plagiochasma*, *Reboulia*, *Asterella*, *Targionia* and *Riccia*. However, the species *Cyathodium* in saxicolous habitat not shown any sign of fungal infection and no spores found in the soil attached to the thallus. This

may be due to habitat and substrate specificity of the species in the region. *Cyathodium cavernarum* also found near water falls, lakes and rock crevices where water is percolating which make it to survive in nutrient poor environment (Arora, 2008).

Rounded elliptical or circular types of vesicles were clearly observed among species like *Targionia* of vesicle size 20.02 μm (Plate: 26-F and Plate: 28-D-I). The species *Plagiochasma appendiculatum* has shown greater size of vesicles of about 40.53 μm in diameter (Plate: 26,A-D, Plate: 28-A). The *Riccia* species showed hyphae in the thallus but less true vesicles inside the thalli. Hence, vesicles were normal feature in Hepaticae showing appressoria and looped hyphae within host tissues (Arora, 2008; Liepina, 2012). Heavy mycorrhization observed in *Plagiochasma appendiculatum* during collection of materials after the month of October with rich and dense fruiting bodies on thallus with broad and large thallus (Plate: 26,A-E). This proves that mycorrhizal association increases the plant fecundity by providing benefits of improved mineral nutrition (Johnson *et al.*, 1997).

The hornworts like *Anthoceros* sp., *Notothylas* sp. and *Phaeoceros* sp. showed globular vesicles inside the tissues of plant body or thallus. They are branched globular but smaller as compared to liverworts with size 13-17 μm (Plate: 26-G and Plate: 27,D-F). No internal colonization was found in *Folioceros udarii* species but hyphal connections seen in few regions. This occurrence of mycorrhizal infections in hornworts supports the views of Schübler (2000). The members of Glomeromycota are easily recognized by non-septate hyphae, arbuscules or vesicles while Basidio- and Ascomycetes are recognized as due to septate hyphae in the present investigation (Nebel *et al.*, 2004). The occurrence of symbiotic fungi in *Phaeoceros laevis* (L) Prosk. in the present findings confirmed with finding of Lingrone (1988). During course of work, a cytological finding confirms the mycorrhizal association in hornworts and exactly matches with glomeromycetes in thalloid liverworts. Hence, the author confirmed that, the endophytes are wide spread in hornworts and liverworts.

Mosses, the largest group of bryophytes generally contain endophytic hyphae of VAM fungi (Rabatin, 1980 Turnau *et al.*, 1999). Mosses accounts phosphorus accumulation in above ground vegetation and mycorrhizal hyphae may be important

avenue of phosphorus movement (Chapin *et al.*, 1987). In the present investigations, mosses like *Funaria hygrometrica* and *Bryum coronatum* showed presence of stained fungus material in the stem and leafy tissues of the plant body. Swollen rhizoids with fungal infections found in rare cases. However, large circular *Glomus* sp. found outside tissue of plant body (Plate: 27,G-H and Plate: 28,B-C). These finding resembles with the findings of Zhang and Guo (2007). No VAM fungal structures found in epiphytic mosses like *Brachythecium* sp. and *Stereophyllum* sp. but commonly occurring rupicolous *Hyophila* sp. and *Hymenostylium* sp. shows fungal hyphal structures in such terricolous moss species (Plate: 27,G-H). No arbuscules were present during all the study work conducted (Table: 4.3.1). Hence, non-septate endotrophic fungus were reported from mosses of Melghat forest. Hence, as per Parke and Linderman (1980), the mosses may be symbiotic in association with VAM fungi in mutualistic way and *Glomus* found as the dominant genus. Furthermore, exploration is needed to trace the mosses and fungal associations using trap cultures.

After screening all the species for probable VAM fungal association, none of the bryophyte was found arbuscular. Appearance or absence of vesicles and arbuscules depends upon the host and the presence of vesicles or absence of arbuscules is not unusual for VA endophytes (Daft and Nicolson, 1974).

5.3.5 Seasons and VAM fungi sporulation

During monsoon season, Melghat forest wears a lusty green carpet and becomes paradise for many plants like bryophytes, pteridophytes and other flora and fauna. Most of the bryophytes species collected during rainy season. However, the best season for VAM fungal sporulation was found in or after the month of August. The climatic conditions like more moist or humus nature favours moderate to high total colonization level and sporulation of VAM fungi. Hence, this reflects the mycotrophic nature of bryophytic community (Abbott and Robson, 1997). Spores of VAM fungi were concentrated mostly in the soil surface layer where spores produced in maximum number. The muddy or clutched soil places found less sporulated as compared to non-muddy or non-saturated free soil. Humus containing soil is found to be rich for VAM fungal sporulation. This finding resembles with the remarkable observations of Muthukumar and Udaiyan (2000) and Alexander (1989).

The species *Plagiochasma appendiculatum* found with high fructifications of sex organs after rainy tissues (Plate: 5-G). This may be due to high level of mycorrhization which favours reproductive growth after luxuriant vegetative growth in the forests. Plants in tropical soils are adapted to nutrient poor environments by increasing their ability to compete for nutrients through association with soil fungi like mycorrhizal fungi or by reducing losses of nutrients by minimizing their biomass turnover (Alexander, 1989). It is notable that, the tropical soil of Melghat forest shows dominance of *Glomus* and *Acaulospora* species of VAM fungi which occurs at the slight acidic or neutral or slight basic nature of soil found among bryophytes. These finding supported by the results obtained by Muthukumar *et al.*, (2003). Hence mycorrhizal colonization in roots occupying a defined volume of soil will depend on a balance between root and fungal activity (Koide, 1993) which is influenced by several factors like soil properties, root phenology, predation, local disturbances, and propagule availability (Brundrett, 1991).

Bryophytes are considered as amphibians of plant kingdom and found in transitional zone from water to land, adapting uniquely on land with VAM fungi in symbiotic association. This also ensures their survival in adverse or extreme condition or difficult places where soil and nutrients are purely lacking. Structural and functional simplicity in bryophytes, especially liverworts make them enable to colonize the VAM fungi in their simple parenchymatous cells and supporting advancement in conduction channel (Arora, 2008).

From present study it is clear that colonization never induces parasitism but it may help in healthy and vigorous growth of the plant by supporting absorption and conduction mechanism. Perhaps the author consider VAM fungi to be a long term strategy that incorporates the flexibility to cope with both present and future environmental conditions.

5.4 Antibiosis in bryophytes

Bryophytes are the oldest, primitive land plants, which have been survived and maintained their place in present flora due to their biologically active compounds. They are also one of the most significant and promising sources of antibiotics and biologically active compounds in nature. These plants possess a typical aromatic smell and anti-feedent or anti-nematic nature. Moreover, all the bryophytic species found never infected by any pathogens besides their occurrence in wet and humid places (Pant and Tiwari, 1990).

Since time immemorial, bryophytes are traditionally used in Chinese, European, North American and Indian medicine to treat illness of cardiovascular system, bronchitis, skin diseases and burns. They also possess anticancer and antimicrobial activity due to their unique chemical constituents (Banerjee and Sen, 1979; Asakawa, 1990; Glime, 2006). In the present investigations seven bryophytes species were screened for determining their antimicrobial sensitivity against selected bacterial and fungal pathogenic species. About four species of liverworts like *Plagiochasma appendiculatum*, *Targionia hypophylla*, *Riccia discolor* and *Reboulia hemisphaerica*, one species of hornwort *Anthoceros erectus* and two species of mosses like *Funaria hygrometrica* and *Hyophila involuta* were used for the experimental purpose.

5.4.1 Antimicrobial sensitivity among liverworts

Preliminary results of screening the extracts of all selected liverworts tested against bacteria and fungi showed that there is a distinct difference between antibiotic activities of the tested species in relation to the solvent used.

Among all the liverworts, the ethanol extracts were recorded as more active or reactive against most of the bacterial and fungal pathogen. The maximum zone of inhibition 13 mm was found in ethanol extracts of species *Plagiochasma* (Fig: 4.4.1-2), *Riccia* (Fig: 4.4.5-6) and *Reboulia* (Fig: 4.4.7-8) against pathogen *E. coli* and *C. albicans*. These findings found similar with the recording of Bodade *et al.*, (2008). However, all the other extracts showed varying levels of activity against all the test organisms. The methanol extracts of all liverworts found significantly reactive against most of the test pathogens as compared to chloroform and acetone extracts. Nevertheless, aqueous extracts of all the liverworts revealed less activity against test

organisms (Banerjee and Sen, 1979). The petroleum ether extracts of all liverworts did not revealed any activity against test bacteria and fungi.

The results obtained in Petroleum ether are in contrast to Khanam *et al.*, (2011) who reported Petroleum ether activity against microorganism in *Marchantia palmata* and Krishnan *et al.*, (2012) who reported that Petroleum ether extract of liverwort *Targionia hypophylla* were active against both gram positive and gram negative bacteria and fungi. Among all the liverworts, the species *Plagiochasma* was most active against all microorganisms followed by *Reboulia* sp., then *Targionia* sp and finally *Riccia* species. The higher degree of activity in *Plagiochasma appendiculatum* supported by Singh *et al.*, (2006) who reported that this plant extract has wound healing activity and used as ethno-medicinal drug by *Gaddi* tribe in *Kangra valley*, Himachal Pradesh to treat skin diseases. Kumar *et al.*, (2000) emphasized the use of *Plagiochasma appendiculatum* for treatment of burns, boils, blisters and skin eruption on the body.

The species *Plagiochasma*, *Reboulia*, *Riccia* and *Targionia* (Fig: 4.4.3-4) were particularly active against bacteria *E. coli* and fungus *C. albicans* and *A. niger*. However, these species were less reactive to the bacteria *S. flexneri* and almost non-reactive to the fungus *R. oryzae*. These findings are in contrast with the findings of Banerjee and Sen (1979) that these liverworts are active against bacteria *P. aeruginosa*. It is striking that all the four liverworts are actively interactive with the fungus *C. albicans* and *A. niger*. Hence, all the liverworts were found more active against fungal pathogens than against the bacterial pathogens. These findings are supported by the studies of Subhisha and Subramanian (2005). However, in some cases like *R. oryzae* no antifungal activity was recorded among all four selected bryophytes (Basile *et al.*, 1998 and Ilhan *et al.*, 2006)

This may be due to the variations in chemical composition of particular species of plants, which can also vary according to the geographical origin and harvesting seasons (Burt, 2004). It also showed that specific antibacterial compounds, effective against the selected bacterial or fungal species, tends to be isolated more effectively from liverworts using ethanol.

5.4.2 Antimicrobial sensitivity in Hornwort

Hornworts are small, evergreen thalloid plants among bryophytes and placed in between liverworts and mosses. The species *Anthoceros erectus* is selected for antimicrobial screening to elicit out its potential medicinal properties. Here only one plant species is selected, as it is very difficult to collect large specimens than others. Considering their vulnerability and habitat destructions other hornworts were not selected under their conservation strategies.

During antimicrobial screening of *Anthoceros* sp. the methanol extract of the plant found more active against gram-positive and gram-negative bacteria as well as fungi (Mewari and Kumar, 2008). However, the aqueous extract and acetone extracts were less reactive as compared to other extracts (Fig: 4.4.9-10). It is remarkable that, the ethanol extracts of *Anthoceros* sp. also showed significant activity against both bacterial and fungal pathogens but lesser as compared to methanol extracts. The more results in antimicrobial activity of both methanol and ethanol extract corresponds to the results obtained by Banerjee and Sen (1979) in hornworts *Anthoceros* sp. and *Notothylas* sp. During extraction process, the methanol, ethanol and chloroform extracts were very dark, grass green in colour and found much saturated than other extracts in *Anthoceros* sp. Both these extracts exhibited higher degree of activity as compared to others may be due to more active and dense chemical compounds (Asakawa, 1988). The bacterial pathogens like *E. coli*, *P. vulgaris*, *S. aureus*, *K. pneumoniae* found more active with maximum zone of inhibition as compared to *S. flexneri*. However, the fungal pathogens like *A. niger* and *C. albicans* found more active in all the extracts tested (Bodade *et al.*, 2008). In the data, the organisms like *E. coli* and *A. niger* showed consistency in their activity as compared to other organisms.

However, no any action or activity was reported by fungus *Rhizopus oryzae* among all the tested extracts of *Anthoceros* sp.

It is also emphasized that among all the extracts, few extracts are more sensitive while others are less and they reciprocates their activity against at least one microorganisms like bacteria and fungi. It can be highlighted that the tested extracts have potential to inhibit bacteria and fungi. The results in this study suggest that the species *Anthoceros erectus* might possess a novel antimicrobial molecule, which has an effect against bacteria as well as fungi. Further research is needed in order to

obtain information about the chemical composition of hornworts species as well as to reveal their mode of action on microbial cells.

5.4.3 Antimicrobial sensitivity among mosses

Mosses are considered as one of the advance group of plants in Bryophytes due to their pioneer conducting tissues and leafy type of gametophytes. They are cosmopolitan in distribution and also depend upon habitats like alpine, arctic, tropical or savannas region. During present investigations two mosses were screened for antimicrobial sensitivity. Moreover, these two species selected due to their mass and enormous availability in Melghat forest.

The moss *Funaria hygrometrica* showed wide range of antimicrobial activity during the screening process (Fig: 4.4.11-12). The methanolic extract of the plant found more active against pathogens followed by ethanol and chloroform. However, the aqueous and acetone extracts were least reactive against the pathogens. These results corresponds to the findings of Banerjee and Sen (1979) and Savaroglu *et al.*, (2011). The gram-positive bacteria like *S. aureus* was found more sensitive in most extracts and gram-negative bacteria *E. coli*, *P. aeruginosa* found consistent effect with most of the extracts (Liang *et al.*, 2006).

The methanolic extract was found more reactive against all microorganisms except *R. oryzae*. The fungus *A. niger* and *C. albicans* found to be active or reactive against most of the extracts except aqueous one. The highest antimicrobial zone of inhibition i.e. 8 mm and 9 mm observed in *P. aeruginosa* and *C. albicans* and this results congruent with the results recorded by Bodade *et al.*, (1998), Pejin *et al.*, (2011) and Sharma *et al.*, (2013). The petroleum ether extracts found almost nonreactive to all the microorganisms during screening with no inhibition effect and contrast to the findings of Cowan (1999). Hence, the moss *Funaria hygrometrica* found as potent source of antimicrobial agent.

The moss *Hyophila involuta* showed (Fig: 4.4.13-14) a wide range of antimicrobial potency. The ethanol extract of this moss was more profound against all the microorganisms, followed by chloroform, methanol and acetone. However, Banerjee and Sen (1979) reported highest antimicrobial activity of *Hyophila involuta* in methanol extract. The aqueous extract exhibited less activity and petroleum ether extracts found almost nullified during the screening. The ethanol extract observed as

most sensitive to bacteria like *E. coli* and *S. typhimurium* (Sabovljevic *et al.*, 2006). Comparatively less reactivity was observed in chloroform, methanol and acetone extract followed by aqueous extracts. The highest microbial zone inhibition of 8 mm was observed in fungus *A. niger* and 7 mm in bacteria *E. coli*. No reactivity was observed in petroleum ether extract and fungus *R. oryzae* among all the plant species. Banerjee and Sen (1979) concluded that, the antibiotic activity detected in various specimens of bryophytes might depend upon its age, season of its collection and ecological niche, which it inhabits.

By comparison of the results obtained, it is confirmed that all the species of liverworts, hornworts and mosses are reactive to various microorganisms. The genus *Plagiochasma* found to be more active antibiotically than hornworts and lastly mosses. It becomes apparent that, the antibiotic activity in these plants varies from species to species (Sanders, 1945). Extracts with organic solvents, particularly ethanol (alcohol) yielded better results than aqueous and other solvents. A comparison of activity in different solvents and careful analysis of antimicrobial spectra reveal the occurrence of a variety of antibiotic substances. It is interesting to note that all the extracts were sensitive to gram-positive *S. aureus* and most of the gram-negative bacteria like, *E. coli*, *P. aeruginosa*, *S. typhimurium*, *K. pneumoniae*, etc. However, all the conventional drugs in use are more active against gram-positive bacterial strains than gram-negative bacterial strains. Hence, this is of considerable interest since conventional antibiotics are generally more active against gram positive than gram-negative bacteria. All the bryophytes species are able to inhibit gram-negative bacteria to a greater extent. These extracts are also active against conventional antibiotic resistant species like *P. aeruginosa* (Castaldo - Cobianchi *et al.*, 1988-89).

Our study show that all the bryophytes possess antifungal activity and *C. albicans* and *A. niger* like fungi are more or less sensitive to all the bryophyte extracts (Ilhan *et al.*, 2006). Hence, the antimicrobial activity may be due to presence of flavonoids, steroids, terpenoids and other polyphenolic compounds (Asakawa, 1988). In an overview of the bioactivity data obtained from the current investigations, it can be highlighted that the tested extracts have potential to inhibit bacteria and fungi. There is further need to investigate chemical characterization of bryophytes species and their mode of action on microbial cells.

5.5 Phytochemical Analysis of Bryophytes

Bryophytes are one of the most significant and promising source of antibiotic and biologically active compound in nature. Historically, a large number of medicinal plants were identified and used by aboriginal people to treat various ailments. Hundreds of medicinal bryophytes have been identified and classified in ethnobotanical literature as potential antimicrobial agent (Banerjee and Sen, 1979). Many bryophytes reported for treatment of ulcers, bronchitis, tympanitis, cystitis as well as skin diseases and burns (Flowers, 1957 and Kang *et al.*, 2007).

In the present investigation most of the bryophytes species have antimicrobial potential and showed characteristic chemical composition. The preliminary phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids among the selected species. Here liverworts found to be more effective than mosses due to their chemical compositions. Bodade *et al.* (2008) suggested that, the antimicrobial tendency of bryophytes can be attributed to active derivatives of Terpenoids (Table: 4.5.1). Krishnan *et al.*, (2012) confirmed that, the bryophytes having alkaloids are pharmacologically active as they have physiological effects on human as well as other animals and serves as therapeutic and anti-malarial drugs. The presence of saponins and glycosides components often referred as a natural detergent because of their foamy nature (Fatoba *et al.*, 2003). Pawar and Arumugam (2011), reported that saponins have anti-carcinogenic properties, antioxidant effects, immune modulation and regulation of cell proliferation. Russell, (2010) reported health benefits like inhibition of growth at cancer cells, cholesterol antibiotic activities of these compounds. He further reported that, cardiac glycosides inhibit the Na/K and ATPase pumps in mammals. The presence of cardiac glycosides reported to be used in treating heart problems. Most of the bryophytes species have relevance in the production of drugs against heart problems and other ailments (Singh *et al.*, 2006). Hence, further research is needed to isolate these secondary metabolites and identify their specific types. Moreover, detectable antibacterial and antifungal compounds are found in most taxa of liverworts.

Flavonoids are extremely common constituents of bryophytes and detected in Marchantiopsida, Anthocerotopsida and Bryopsida by Chopra and Kumar (1988). Among the bryophytes, the liverworts possess appealing cellular bodies which are

characteristic membrane bound cell organelle consisting of ethereal terpenoids and aromatic oils suspended in a carbohydrate or a protein rich matrix. These oil bodies are very important markers for the classification of the Marchantiopsida (Asakawa *et al.*, 2013). This would provide evidence to support present investigations and provide clues for the factors affecting chemical and antimicrobial activities.

5.5.1 Chemical constituents of Bryophytes

Many bryophytes exhibit antimicrobial effects against fungi and bacteria by producing antimicrobial substances are widespread phenomenon. Bryophytes are also known to produce certain chemicals which are naturally toxic to bacteria and fungi (Subhisha and Subramaniam, 2005; Bodade *et al.*, 2008; Krishnan *et al.*, 2012; Cansu *et al.*, 2013).

Bryophytes are the simplest land plants and anatomical barriers are less effective and as a consequence, the synthesis of particular molecules, secondary metabolites with antimicrobial activity are the "Chemical Barriers" having most effective defense system (Harborne, 1988). Basile *et al.* (1998) considered these defense substances belongs to wide range of different chemical classes including flavonoids, alkaloids, terpenoids, glycosides etc. Almost all species of bryophytes are not damaged by insect larvae, fungal pathogens, bacterial attacks, slugs, snails, nematodes and even mammals (Asakawa, 2004). This is caused due to the presence of biological compounds like oligosaccharides, polysaccharides, sugar, alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinone and aromatic and Phenolic substances in bryophytes and are protected against these organisms (Asakawa, 1981). However, the chemical composition of bryophytes differs depending upon species, growth condition and environment or seasons.

5.5.2 GC - MS (Gas-Chromatography and Mass Spectroscopy) Analysis

To trace the possible chemical compounds, Gas Chromatography and Mass Spectroscopic analysis of various bryophytic samples were done. The bryophytes like liverworts, hornworts and mosses showed various types of chemical compounds in their crude methanolic extract.

Caryophyllene is a natural bicyclic sesquiterpene which is a constituent of many essential oil like clove and pepper. This compound is notable for having cyclobutane ring i.e. a rarity in nature (Chopra and Kumar, 1988). Claude, (2013)

found that on insects attacks, these terpenoids will attract nematodes, that will destroy the larvae of these herbivorous insects. This compound was analyzed in *Plagiochasma appendiculatum*, *P. intermedium* and *Anthoceros erectus* and interestingly, the author observed that the thalli of species *Plagiochasma* and *Anthoceros* never infected by any pathogens but provides shelter to many invertebrates, earthworms around the thalli and soil. A typical aromatic smell was always rendered during collection of these plants across Melghat region. Ozturk *et al.*, (2009) reported the antimicrobial activity of such essential oil against *P. aeruginosa* and *S. aureus*. Xian *et al.*, (2006) reported that Caryophyllene from *Marchantia convoluta* with cytotoxic effect of leaf extracts to human liver and lung cancer cells. Hence, these naturally occurring compounds have potential medicinal properties.

3, 7, 11, 15-Tetramethyl-2-hexadecen-01-ol is a diterpene alcohol. It is by-product from the production of chlorophyll and essential material to produce vitamin K1 and vitamin E. It commonly occurs in bryophytes species like *P. appendiculatum*, *A. erectus*, *R. hemisphaerica*, *P. rupestre*, *F. hygrometrica*, *H. involuta* and *S. decorum*. Lalifa (2012), reported antibacterial, anti-fungal and anti inflammatory activity of essential oil like hexadecanoic acid, methyl ester against, *E. coli*, *S. aureus*, *C. albicans* and *P. aeruginosa* extracted from plant *Carduus psychocephalus* L. This finding found congruent with the present investigations of antimicrobial activity.

The bryophytes like *P. appendiculatum*, *A. erectus*, *T. hypophylla*, *F. hygrometrica*, *H. involuta*, and *P. rupestre* showed the presence of chemical compound like n-Hexadecanoic acid or Palmitic acid. This is most common saturated fatty acids either found in plants, animals and even microorganisms. It mainly occurs as its ester in triglycerides (Fats).

Phytol is an acyclic diterpene alcohol that can be used as precursor of synthetic forms of vitamin E and vitamin K. Yoshihiro *et al.*, (2005) showed that phytol is a constituent of chlorophyll and had unique antibacterial activity to inhibit pathogen like *S. aureus*. This activity was reported in present work especially in species *P. appendiculatum* and *A. erectus*. Moreover, Venci and Morton, (1998) reported that *Sumac Flea beetle* use phytol and its metabolites as a shield defense or as chemical deterrents against predation.

The species *Plagiochasma appendiculatum* showed the presence of unique compounds like Hexacosane and Heneicosane more than all other bryophytic species. These compounds are higher alkanes with higher number of carbon atoms. Agnihotri *et al.* (2010) reported the anti-inflammatory properties of these compounds in traditional medicines.

One of the interesting chemical compounds found in bryophytes is Nonacosane. Nonacosane is a straight chain hydrocarbon, occurs naturally and present in several essential oils but can be synthesized artificially too. Naz *et al.* (2013) tested Nonacosane from moss *Funaria parviflora* against bacteria like *S. aureus*, *E. coli*, *S. typhimurium*, *K. pneumoniae* and *S. epidermis* with positive results. These results found congruent with the species *Anthoceros* and *Funaria* in present investigation. Moreover, Nonacosane also found as a major constituent of plant epicuticular waxes along with fatty acids, primary alcohols and aldehydes making of the bryophyte species with shining appearance. It also plays an important role in chemical communication of several insects like female *Anopheles stephensi* mosquito.

Tetratriacontane is a volatile compound and its methanol and chloroform extract in the hornwort species *Anthoceros erectus* have shown antimicrobial activity against gram-positive *S. aureus* and gram-negative bacteria *E. coli*, *S. typhimurium*, *K. pneumoniae* etc. along with fungus *Candida albicans*. These results corresponds to the findings of Karabey *et al.*, (2007).

Stigmasterol is a group of unsaturated plant sterol occurring in the plant fats or oil especially medicinal herbs. This commonly occurs in bryophytes species like *Anthoceros erectus*. Although it is insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Woldeyes *et al.*, (2012) reported the antimicrobial activity of stigmasterol compound against bacteria like *E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhimurium* and found complimentary with the present investigations.

The liverwort *Targionia hypophylla* showed the presence of chemical constituent Longifolene i.e. a tricyclic sesquiterpenoids. It commonly occurs in plants like pines, angiosperms, small amount in fungi and bryophytes. Ücüncü *et al.*, (2010) reported antimicrobial activity of longifolene among various mosses like *Torula*, *Hyphum* and *Pohila* against bacteria *E. coli*, *P. aeruginosa*, *S. aureus* and fungi

Candida albicans. This finding corresponds to the present investigations of antimicrobial activity of *Targionia* species. Hata *et al.* (1993) discovered an “*Acne-Vulgaris*” treatment comprising of longifolene, caryophyllene, centrene and thujopsene as a key ingredients and got U. S. patent no. US-S200429A for such a novel medicine.

The bryophyte species like *Targionia* and *Asterella* possess compound Bicyclo (5.3.0) decane, 2-methylene-5-(1-methylvinyl)-8-methyl. Chen *et al.*, (2013) found this compound as potent composition with potential of anticancer activities among essential oil obtained from *Myrrh* and *Frankincense*.

Patchouli alcohol or patchouli is a terpene compound with typical patchouli scent and used in chemotherapy drugs. Yang *et al.*, (2013) evaluated the antimicrobial activity of patchouli alcohol and pogostone against various bacteria like *E. coli*, *P. aeruginosa*, *S. aureus* and *S. dysenteriae*. The liverworts *Targionia*, *Asterella* and *Plagiochasma* spp. in present study found reactive to these bacterial pathogens with similar promising results.

9-octadecenoic acid and 9, 12 octadecadienoic acid (z-2) methyl esters are commonly called Linoleic acid which is a polyunsaturated fatty acid commonly produced in plants. The bryophytes species like *Targionia*, *Plagiochasma*, *Hyophila* and *Funaria* spp. showed occurrence of these compounds. Park *et al.*, (2013) reported antimicrobial activity of gamma linolenic acid from *Enteromorpha linza* against several bacteria like *Streptococcus mutans* and fungi *Candida albicans*. Likewise, Wei *et al.* (2011) reported antimicrobial, anti-cancerous and antioxidant properties of 9,12 - Octadecadienoic acid against the bacteria like *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Vibrio colerae* in methanol extract of *Peperomia pellucida*.

The moss *Funaria hygrometrica* showed the presence of chemical constituent like Heptadecane, 2, 6, 10, 15-tetramethyl. Heptadecane is an organic compound of alkane hydrocarbon. Interestingly, Ozdemir *et al.*, (2004) reported antimicrobial activity of heptadecane and tetradecane in methanol extract of *Spirulina platensis* against bacteria like *E. coli*, *S. aureus* and fungi *Candida albicans* with promising results. Nonadecane, compound found in species *Funaria* also found effective against bacteria like *S. aureus* and *E. coli* and found as therapeutic agent of pharmaceutical materials reported by Ibrahim *et al.*, (2013).

The liverworts *Plagiochasma intermedium* showed the presence of 1H-Cycloprop[e] Azulene, decahydro-1, 1, 7-trimethyl-4-methylene. The chemical compound like azulene, generally responsible for blue coloration to the liverworts or fungi like mushrooms. It is interestingly found that the *Plagiochasma intermedium* species appears dominantly blue coloured at Gawilgarh fort. Recently, Saraswathy and Lavanya (2013) reported azulene compound in essential oil of *Coleus vettiveroides* and effectively antibacterial against the strains like *K. pneumoniae*, *S. aureus*, *E. coli*, *P. aeruginosa* and in *Proteus mirabilis*. The compound Naphthalene also showed the same properties. Moreover, the compound Cycloisolongifolene, 8-9 dehydro, also found among *P. intermedium*. Kamazeri *et al.*, (2012) reported the antibacterial activity of this compound in extracts of *Curcuma aeruginosa* against bacteria *B. cereus*, *P. aeruginosa* and fungus *Candida albicans*.

Spathulenol is a Gujan-derivative, occurs in various plants like *P. intermedium*. This is a tricyclic sesquiterpene comprising similar chemical structure as azulene. It is a colourless, viscous compound dissolved well in ethanol or water and known earthy, aromatic adour with bitter spicy taste (Saraswathy and Lavanya, 2013).

The compounds like 1, 4-methanoazulene, decahydro-4, 8, 8-trimethyl-9-methylene i.e. longifolene is a sesquiterpenoid and 7-Tetracyclo [6.2.1.0 (3.8 0 (3-9)] undecanol, 4, 4, 11, 11-tetramethyl is aromatic compound and both possesses anti-cancerous activity as per Dr. Duke's online database. It is noteworthy that, the chemical compound gamma-Gurjunenepoxide -(2) i.e. an epoxide did not found with any activity in literature in relation to biological activity and potentials.

Thujopsene is a sesquiterpene occurred in the bryophyte species like *Reboulia hemisphaerica* and *Plagiochasma rupestre* and in many conifers or other plants. Tumen *et al.*, (2013) reported antifungal activity of thujopsene in heartwood extract of *Juniperus* species against wood rot fungi *Glomeophyllum*, *Postia* and *Irpex* species. Moreover, Manter *et al.* (2007) showed antifungal activity of thujopsene against *Phytophthora ramorum* from conifer heartwood. The *Reboulia* species also showed the presence of Longifolenaldehyde a sesquiterpene commonly occurring in bryophytes. Ücüncü *et al.* (2010) reported antimicrobial activity of this compound among Turkish mosses.

In the moss *Hyophila involuta*, an ester compound Dibutyl phthalate was recorded. Dibutyl phthalate (DBP) is also commonly used plasticizer. It is soluble in various organic solvents, e.g. in alcohol, ether and benzene. DBP is also used as an ectoparasiticide. Khatiwora *et al.* (2011) reported the antimicrobial activity of dibutyl phthalate isolated from *Ipomea carnea* and tested against bacteria like *K. pneumoniae*, *P. mirabilis*. Here the extracts of the plant appears to be more susceptible to microorganism *P. aeruginosa*. The activity of the chemical compound Tricyclo [8.6.0.0(2,9)] hexadeca-3-15-diene, trans-2, 9-transoid-9, 10-cis-1, 10 in *Hyophila involuta* moss was not reported till now and can be a novel one if explored in future.

The plant species *Asterella angusta* showed the presence of chemical compound in GC-MS analysis like Humulen. It is also called as α -humulen or α -caryophyllene which is a naturally occurring monocyclic sesquiterpene in number of aromatic plants. Ozturk *et al.*, (2009) reported GC-MS analysis and antimicrobial activity of humulen as essential oil of *Stachys cretica* exhibiting antibacterial and antifungal activity against *P. aeruginosa* and *Candida albicans* respectively. Legault and Pichette (2007) predicted anticancer effect of α -humulen along with Beta and isocaryophyllene.

Another compound analyzed is Germacrenes which is a volatile organic hydrocarbon specifically sesquiterpenoids. Germacrenes are naturally produced in number of plant species with antimicrobial and insecticidal properties but also plays a role in insects pheromones. Mora *et al.* (2013) investigated the potential antimicrobial activities of germacrenes isolated from *Verbesina negrensis* against the bacterial strains like *E. faecalis*, *S. aureus* with crude chloroform extract. The antioxidant activity of 14-methyl-8-hexadecyn-01 was investigated and found in *Punica granatum* extract (Kumar and Bhaskar, 2012).

The corticolous moss *Hymenostylium recurvirostre* showed the presence of unique silicon oxide compounds like Cyclooctasiloxane hexadecamethyl and Cyclononasiloxane hexadecamethyl. These two compounds did not show any biological activity or no antimicrobial property was reported. However, the author suggested that, this is a highly stable silicon oxide compound and even cannot be dissolved in concentrated hydrochloric acid. Hence, it may be stated that, even in acid rains these mosses can withstand against extreme conditions of environment representing as ecological indicators.

The chemical compounds like Heptacosane and Tetracosane are higher alkanes with a higher number of carbon atoms. Formisano *et al.* (2012) isolated Heptacosane compound from essential oils of *Anthemis* plant and tested successfully against *S. aureus*, *E. coli*, *P. aeruginosa* and other seven microorganisms with positive results. Likewise Tetracosane compound was reported by Geethalakshmi and Sarda, (2013) from essential oil isolated from chloroform extracts of *Trianthema decandra* and tested positively against microorganisms like *E. coli*, *P. aeruginosa*, *S. typhimurium* and *Candida albicans*.

Hence, in present investigation, many chemical compounds were found dominantly present among bryophytes species. These compounds distributed variably among different plant species.

Here an attempt has been made by author to establish the co-relation between chemical compound analyzed and their probable antimicrobial sensitivity against microorganisms. Hence, all the bryophytic extract possesses some novel chemical compounds with highly antimicrobial potential is confirmed in the present investigation. It is prominent and notable to say that these bryophytes are "Remarkable Reservoirs of Novel Chemical Compounds" and for further study, characterization of all extracts by elucidation of techniques like NMR, IR and HPLC Analysis will further provide new insight to the drug designing for the betterment of future human race.



CHAPTER SIX

CONCLUSION

6. CONCLUSIONS

The bryophytes represent some of the most species rich lineages of land plants, presenting challenges or opportunities for understanding process of diversification. The most fascinating aspect of their journey in course of evolution is from water towards conquest on the land.

In present investigation, the fascinating world of bryophytes in Melghat forest is explored for the first time. About 20 forest species of bryophytes were explored which belongs to 16 genera and 10 families. The bryoflora of the region showed dominance of the plant species like *P. appendiculatum*, *P. intermedium*, *P. rupestre*, *T. hypophylla*, *C. tuberosum*, *C. cavernarum*, *R. gangetica*, *R. discolor*, *A. erectus*, *F. hygrometrica* and *H. involuta*. However, the species like *R. hemisphaerica*, *A. angusta*, *N. indica*, *P. laevis*, *F. udarii*, *B. coronatum*, *S. decorum* and *H. recurvirostre* were recorded for first time from this region. The distribution of bryophytes responds to topography, elevation, temperature, moisture as well as soil substrate and nutrients. At the higher altitude and maximum precipitation, rich bryophytes diversity observed in Gugamal, Chikhaldara, Semadoh and Belkund region. Most of the species occurs in pH range 6 to 8 i.e. slight acidic, neutral and slight basic nature. However, the distribution found variable among nutrient poor as well as nutrient rich vegetation. Hence, bryophyte species tends to be highly specific for particular microenvironment responding to temperature, light, humidity, precipitation and soil chemistry by making them good ecological indicator species. Considering the role of bryophytes in ecological succession in any ecosystem, much attention is now focused on this group by ecologists and conservation biologists.

Being as a precursor on the land, the bryophytes considered as a most primitive, early land plants on the earth. In present investigation, most of the bryophytes found associated with soil borne VAM fungi. The mycorrhization is a silent process along with the evolutionary aspects among early land plants. The liverworts like *P. appendiculatum*, *R. hemisphaerica*, *A. angusta* and *T. hypophylla* showed the association with such mycorrhizal fungi. About 28 species of genus *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellispora* were found among soil of bryophytes. Moreover, the thallus of these liverworts also showed vigorous presence of endophytic vesicles and ramifying hyphae across the tissues of the plants. Smooth walled rhizoids of the liverworts also showed intra-cellular and extra-cellular

infections of hyphae. The tuberculated hyphae cannot be distinguished for rhizoidal infections as compared to smooth walled one. In *Anthoceros* sp. and *Phaeoceros* sp. heavy VAM fungal infections were seen. In case of mosses not true mycorrhization but, swollen rhizoids, stained plant stem or leaves showed and confirmed the presence of VAM fungal infections. Hence, it is confirmed that bryophytes are in symbiotic association with (VAM) Vesicular Arbuscular Mycorrhizal fungi, since time of their origin.

The phenomenon of antibiosis has been reported to occur in many bryophytes even though they are at a lower level of evolution as compared to the higher plants. Hence, the occurrence of antimicrobial substances in the thalli of several bryophytes is a key attribute of these novel plants to establish as well as to compete on this earth.

During present investigations, about seven bryophytes species were screened for the antimicrobial sensitivity. The species *P. appendiculatum* found most reactive against all the microorganisms. Likewise, the liverworts *T. hypophylla*, *R. discolor*, *R. hemisphaerica*, *A. erectus*, *F. hygrometrica* and *H. involuta* found sensitive to various microorganisms in different extracts. The bacterial organisms like *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* found most reactive against various extracts of bryophytes. Moreover, the fungal pathogens like *Candida albicans* and *Aspergillus niger* found most reactive against all the extracts which were studied. The ethanol extracts of all the bryophytes responded more positively than other extracts. Hence, it is concluded that all the bryophytes extracts reacted to most of the gram-negative bacteria than gram-positive bacteria. It is observed that all the conventional drugs available today reacts more with gram-positive bacterial strains than gram-negative bacteria. These findings will open new avenues and provide insight to the prospects of medicinal world.

The antimicrobial property of bryophytes is well known to the present world due to presence of their medicinally and pharmacologically interesting substances. The presence of alkaloids, flavonoids, tannins, saponins and glycosides confirms the medicinal importance among the bryophytes.

With the advancement of various techniques and modern instruments like (GC) gas chromatography and (MS) Mass spectrometry, isolation as well as structure elucidation of novel compounds can be determined. In present investigations, about

12 bryophyte species samples were analyzed by using GC-MS techniques. The data obtained revealed the presence of chemical constituents like sesquiterpenoids like caryophyllene, longifolene, spathulenol, thujopsene, humulen and germacrene. The terpenes like phytol 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-01 and patchouli alcohol also found common in these species. The hydrocarbons group of alkanes compounds like hexacosane, heneicosane, nonacosane, nonadecane, heptacosane and tetracosane are commonly recorded in analysis. The occurrence of sterol compounds is a common phenomenon in bryophytes like stigmasterol. The unique presence of terpenoids like naphthalene, azulene and cycloprop compounds also represents diverse occurrence of chemical compounds. Hence, the active chemical compounds belong to non-ionized organic acids, alkanes, phenolics, bibenzyls, terpenoids like sesquiterpenoids, flavonoids and related compounds.

The present state of knowledge in this matter reveals that the bryophytes in future may prove to be a rich store-house of hitherto unknown drugs.



CHAPTER SEVEN

REFERENCES

7. REFERENCES

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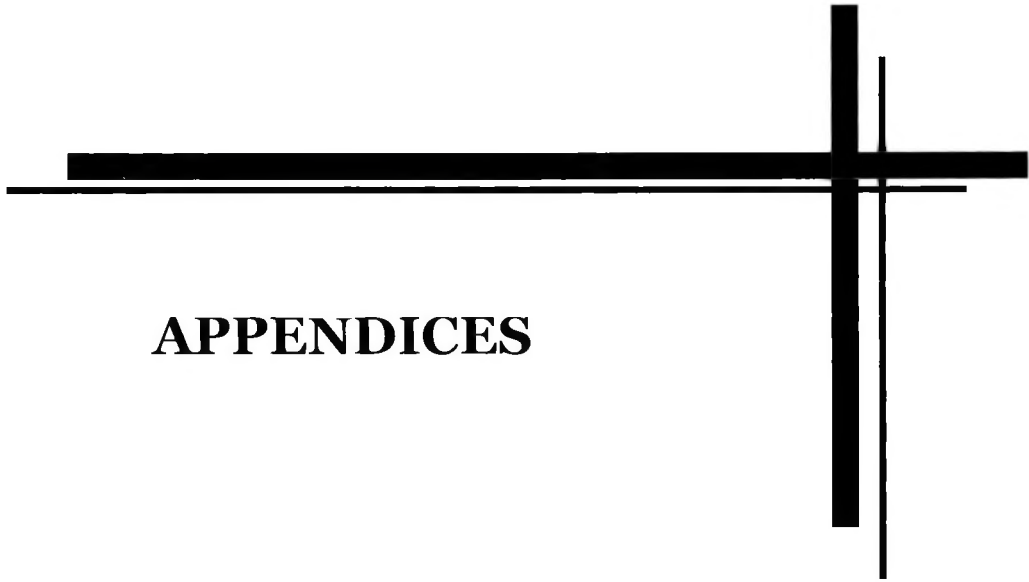
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APPENDICES

ANNEXURE -I

**Permission letter to study and entry in Melghat Tiger Reserve from the
Chief Wildlife Warden, Government of Maharashtra**

**From-XV
Rule 31(1)
Wildlife (Protection) (Maharashtra) Rules, 1975
SANCTUARY/ NATIONAL PARK ENTRANCE PERMIT
(Not Transferable)**

(To be returned to the Issuing Authority within 15 days (Fifteen days) of the expiry of the permit together with an inventory of the research undertaken etc.)

Permit No. 95

Date 13th September, 2011

Subject to the provisions of the Wildlife (Protection) Act, 1972 and Rules made thereunder, and subject to the conditions hereinafter mentioned, permission is hereby granted to Shri Tushar Wankhede, Assistant Professor, Shri Shivaji Science college, Amravati and his team to enter, resides in Chikhaldara, Semadoh, Kolkas Gugamal, Tarubanda, Belkund region region of Melghat Tiger Reserve, Amravati from the date of issue of this permit upto 31/12/2013.

Particulars	Payment Made	
	Fee	Deposit
(1) To conduct survey, collection of data, Photography & collection of Bryophytic plant thallus along with associated soil sample(Approx 100 to 500 gms of soil) for project titled " Study of Bryophytes in Melghat region with reference to soil micro floral association "	(2) To be recovered by Protected Area Manager	(3)

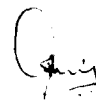
CONDITIONS -

- (1) This permit shall not entitle the holder, to hunt and Trap any animals / birds / butterflies/ plants .
- (2) The permit- holder shall abide by the relevant provisions of the wildlife (Protection) Act. 1972 and the rules made there under.
- (3) The permission is subject to the payment of fees to the concerned officer incharge of the Protected Area as per Rules and in accordance with the Resolution of the Government of Maharashtra as amended from time to time.

Place : Nagpur

Date : 13th Sept, 2011

**O/C approved by PCCF(WL)
M.S. Nagpur**


Chief Wildlife Warden, Maharashtra
&

Principal Chief Conservator of Forest (Wildlife)
Maharashtra State, Nagpur.

SEAL

ANNEXURE –II

Papers presented in the conferences on various parameters

Sr. No.	Title of the Paper Presented and Author	Title of Conference	Organized by
1.	<i>Soil Diversity of Bryophytes from Melghat region in Amravati District. (M.S.)</i> (*Wankhede T.B. & Manik S.R.)	National Conference on “Emerging Trends in Biodiversity & Environment” Best Poster Award Prize	Department of Botany, Institute of Science, Aurnagabad. (M.S.) 26 February, 2010
2.	<i>Ecological Influences on Bryophytic Diversity of Melghat Forest, Maharashtra.</i> (*Wankhede T.B. & Manik S.R.)	XXXIII Conference of IBS and International Symposium on the New Horizons of Botany.	Department of Botany, Shivaji University, Kolhapur. (M.S.) India 10-12 November, 2010
3.	<i>Diversity of soil microfloral association with Bryophytes of Melghat Forest, Maharashtra</i> (*Wankhede T.B. & Manik S.R.)	International Conference on Microorganisms in Environmental Management and Biotechnology	Department of Biotechnology & Bioinformatics Centre. Barakatulla University, Bhopal, (M.P.) India 01-03 July, 2011
4.	<i>In Vitro Screening of Selected Bryophyte Species of Melghat Forest For Antibacterial Activity</i> (*Wankhede T.B. & Manik S.R.)	52 nd Annual Conference of AMI & International Conference on Microbial Biotechnology for Sustainable Development.	Department of Microbiology, Punjab University, Chandigarh. Punjab. 3-6 November, 2011
5.	<i>Antifungal Activity of Liverworts from the Melghat Forest</i> (*Wankhede T.B. & Manik S.R.)	National Conference on Mycodiversity with its Sustainable Exploration and Biotechnological applications & 38 th Annual Meeting of Mycological Society of India.	Department of Botany, Shri Shivaji Science College, Amravati (M.S.), India 6-7 February, 2012
6.	<i>An assessment of antibacterial activity of extracts from some Bryophytes.</i> (*Wankhede T.B. & Manik S.R.)	53 rd AMI Meeting & International Conference On Microbial World : Recent Innovation And Future World	Department of Biotechnology, KIIT University, Bhubaneshwar. Odisha 22-25 November, 2012
7.	Fungal diversity in the soil sample of the bryophyte <i>Plagiochasma appendiculatum</i> Lehm. Et Lind. (*Wankhede T.B. & Manik S.R.)	39 th Annual meeting (National) of Mycological Society of Indi Current perspectives of fungi in Health care and Environment a	Department of Botany, Bangalore University, Bangalore. (K.N.) 13-14 March, 2013
8.	Chemical Composition of the Liverwort <i>Plagiochasma appendiculatum</i> Lehm. et Lindenb. (*Wankhede T.B. & Manik S.R.)	Current Advances in Biotechnology and Annual National Meeting of Society for Biotechnologists (India)	Department of Biotechnology, S.G.B. Amravati University, Amravati. (M.S.) India 26-27 November , 2013
9.	GC-MS Analysis of the Liverwort <i>Reboulia hemisphaerica</i> (L) Ralii (*Wankhede T.B. & Manik S.R.)	XXIII National Conference of IAAT and Seminar on “Recent Advances in Plant Taxonomy Research”	Department of Botany, P.G.T.D. R.S.T.M. Nagpur University, Nagpur (M.S.) 27-29 December, 2013

