ASSESSMENT OF ARBUSCULAR MYCORRHIZAL SYMBIOSIS IN SOME LIVERWORTS OF JAMMU AND KASHMIR WITH INSIGHTS INTO THEIR ANTIOXIDANT PROPERTIES

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in
BOTANY

By

Mamta Verma
Registration Number: 41900701

Supervised by

Dr. Anupam Kumar (15332)
Department of Biotechnology
(Associate Prof.)
Lovely Professional University,
Punjab

Co-Supervised by

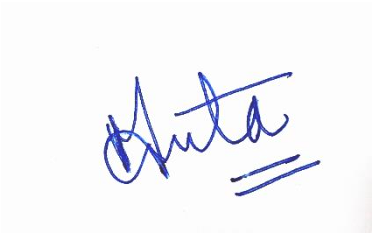
Dr. Sandeep Kotwal
Department of Botany (Astt. Prof.)
Govt. PG college, Doda, (J&K)
affiliated to University of Jammu,
Jammu

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2024

DECLARATION

I, **Mamta Verma**, hereby declare that the presented work in the thesis entitled **"Assessment of Arbuscular Mycorrhizal Symbiosis in some Liverworts of Jammu and Kashmir with insights into their antioxidant properties"** in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Anupam Kumar**, Associate Professor, in the **Department of Biotechnology**, **School of Bioengineering and Biosciences** of Lovely Professional University, Punjab, India and **Dr. Sandeep Kotwal**, Assistant Professor in the **Department of Botany**, Govt. PG college Doda, affiliated to University of Jammu, Jammu. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



(Signature of Scholar)
Name of the scholar**: Mamta Verma**
Registration No:41900701
Department/school: Department of Botany, School of Bioengineering and Biosciences
Lovely Professional University,
Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Assessment of Arbuscular Mycorrhizal Symbiosis in some Liverworts of Jammu and Kashmir with insights into their antioxidant properties"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Botany**, **School of Bioengineering and Biosciences**, is a research work carried out by Mamta Verma, 41900701 and is a bonafide record of her original work carried out under my supervision and that no part of this thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Dr. Anupam Kumar (15332)
Department of Biotechnology
(Associate Prof.) Lovely Professional University, Punjab

(Signature of Co-Supervisor)

Dr. Sandeep Kotwal
Department of Botany (Asst.Prof.)
Govt. PG college, Doda affiliated to University of Jammu, Jammu

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to everyone who has supported and guided me throughout my Ph.D. journey.

First and foremost, I am immensely grateful to my supervisor, Dr. Anupam Kumar and my co-supervisor, Dr. Sandeep Kotwal, for their invaluable guidance, constant support and encouragement. Their insights and expertise have been crucial in shaping this research.

I am thankful to Prof. Joydeep Dutta, Head of Department of Botany, LPU for providing insightful comments and constructive criticism at different stages of my research. I would also like to extend my appreciation to research committee members Prof. D.K. Pandey and Prof. Ashish Bajaj for their constructive feedback and helpful suggestions that significantly enhanced the quality of this thesis.

I would like to extend my deepest thanks to Professor Anima Langer (Retd.), Department of Botany, University of Jammu for generously sharing her knowledge of species identification. Her expertise and dedication have been instrumental in the success of my research. Her mentorship has enhanced my understanding and skills in this field. I am also grateful to the HOD and faculty members (Prof. Yash pal Sharma, Prof. Namrata Sharma, Prof. Veenu Kaul, Prof. Susheel Verma) of Department of Botany, University of Jammu for providing the necessary laboratory facilities and resources. The supportive environment and state-of-the-art facilities were essential for the successful completion of my research.

I am also immensely grateful to my parents for their unconditional love and support. Their sacrifices, encouragement and belief in my abilities have been the foundation of my academic and personal achievements. I am profoundly thankful to my husband, Sushil Kumar Verma, for his unwavering support and patience. His encouragement and

understanding have been my anchor through the highs and lows of this academic endeavour. To my adorable children, Sabal and Mivaan, thank you for your endless love and for being a constant source of joy and inspiration. Your patience and understanding during my long hours of work have been remarkable.

Thanks to my friends and colleagues at Department of Botany, University of Jammu for their camaraderie and for creating a stimulating and enjoyable work environment. I am especially grateful to my friend Sat Pal Pakhroo (Forest Conservator, Chenab Circle, Jammu) for helping me in the plants collection. I am also grateful to Kiran Verma, Dr. Som Nath, Dr. Aarti, Priya Verma, Anita Devi, Dr. Samita, Nisha Goswami, Shaveta Jandial, Dr. Usha, Dr. Bheekham Pal, Dr. Madhavi Prashar, Dr. Faisal Mushtaq for their assistance and moral support during the challenging times and last but not the least a special word of thanks to my younger brother Sukh Dev Verma who was always there for my help through thick and thin during this entire journey.

Thank you all for making this achievement possible

ABSTRACT

Liverworts, a type of non- vascular plant, have been used by various cultures for their medicinal, ecological and cultural significance. Although there is enormous amount of research being carried out on the AM fungi as well as antioxidant capacities of higher plants like angiosperms and gymnosperms, but little attention is paid towards these miniature bryophytes because of their small size. There is very less literature so far available which suggests correlation between AM fungal symbiosis and antioxidants in liverworts. Although some work of this type has been undertaken in other countries, but very little is known about the diversity of AM fungal association of Indian bryophytes and their antioxidants relationship.

The territory of Jammu and Kashmir has a very rich bryo-diversity (420 species) and is very less explored for this type of work. Hence, there is a huge potential in utilizing these ethno-botanically important amphibians of plant kingdom to increase agricultural yield and in healthcare as ecosystem friendly antibiotics and antioxidants. Detailed studies on the AM fungi and antioxidant potential of various groups of bryophytes are expected to throw light on several questions of academic, economic and evolutionary interest. Keeping this in mind the study was undertaken.

The rhizoids and anatomical sections of thallus were cleared and stained using KOH and trypan blue staining method. AM symbiosis was observed in 7 out of 21 liverworts species studied. AM fungal structures such as vesicles and arbuscules and AM fungus spores were observed. The associated AM fungus was multiplied by Trap culture in *Sorghum species*. AM fungal species was identified as *Glomus aureum*. AM colonization was observed to be influenced by various factors such as habitat, temperature, pH and soil water content. Ascorbic Acid content (As A), Proline content, Glycine-Betaine content (GB), Glutathione content (GSH), Glycine-Betaine (GB), Total Phenol content (TPC), Superoxide Dismutase

(SOD), Catalase (CAT), Guaiacol Peroxidase (GPOX), Ascorbate Peroxidase activity (APOX) and Glutathione Reductase (GR) were also calculated. The antioxidant potential was observed to be positively correlated with the AM colonization. The bryophytes that have a robust defense mechanism to grow under various habitats, hold a rich reservoir of unique phytochemicals, are not studied well for antioxidants profile. AM fungus on the other hand enhances the uptake capabilities of plants, particularly for immobile ions and for water, nitrogen and phosphorus through their hyphae extending into the soil thereby improving plant nutrition The positive correlation between AM colonization and antioxidant potential suggests that the presence of AM fungi might contribute to the liverworts' ability to withstand oxidative stress, potentially enhancing their overall health and survival in varying habitats.

CONTENTS

S.No.	Chapter	Page No.
1.	**INTRODUCTION**	1-5
2.	**REVIEW OF LITERATURE**	6-23
2.1	Mycorrhiza	
2.1a	Types of mycorrhizae	
2.1b	Host range of mycorrhizal fungi	
2.1c	Benefits of AMF associations	
2.1d	Glomalin	
2.2	Antioxidants in Bryophytes	
2.2a	Antioxidants activities in mosses	
2.2b	Polyphenol Activities	
2.2c	Diversity of Liverworts and antioxidant compounds	
2.3	Ethnomedicinal values of bryophytes	
3.	**MATERIALS AND METHODS**	24-40
3.1	Arbuscular Mycorrhiza	
3.1a	Cleaning and staining of rhizoids	
3.1b	Structural details of mycorrhizae	
3.1c	Quantification of mycorrhiza	
3.1d	Identification of AM fungi associated with liverworts	
3.2	Antioxidant activities	
3.2a	Preparation of plant extract	
3.2b	Protein concentration	
3.2c	Carbohydrate estimation	

3.2d	Enzyme activities	
3.2e	Non-enzymatic antioxidants	
4.	**RESULTS AND DISCUSSION**	41-105
4.1	AM in studied liverworts	
4.2	Identity of endosymbiont	
4.3	Mycorrhizal colonization	
4.4	Factors influencing the growth of AM	
4.4a	Habitat	
4.4b	Temperature	
4.4c	pH	
4.4d	Soil water content	
4.5	Arbuscular mycorrhiza and plant growth	
4.6	Antioxidants in studied liverworts	
4.6a	Protein content	
4.6b	Soluble sugar content	
4.6c	Enzyme activities	
4.6d	Non-enzymatic antioxidants	
4.6e	Oxidative stress markers	
4.6f	Photosynthesis	
4.7	Relationship between mycorrhizal colonization and antioxidants	
5.	**SUMMARY AND CONCLUSION**	106-110
6.	**BIBLIOGRAPHY**	111-140
7.	**ANNEXUERS**	141-146

LIST OF FIGURES

Figure No.	Title	Page No.
1	Map of India showing UT of Jammu & Kashmir, explored districts and various collection sites of Jammu & Kashmir.	27
2	Diverse habitats of liverworts.	28
3.	Rhizoids of *Asterella multiflora* stained in trypan blue showing fungal hyphae and vesicles.	43
4.	V.S. of the thalli of *A. multiflora* showing AM fungal association inside the storage zone of thallus along with arbuscules.	44
5.	Rhizoids of *Asterella wallichiana stained* with trypan blue showing fungal hyphae and vesicles.	46
6.	V.S. of thalli of *A. wallichiana* showing AM fungal invasion inside the storage zone of thallus along with arbuscules.	47
7.	Rhizoids of *Plagiochasma appendiculatum* showing AM fungal associations	49
8.	V.S. of the thalli of *P. appendiculatum* showing fungal invasion inside the storage zone of thallus along with arbuscules.	50
9.	Rhizoids of *Reboulia hemispherica* stained with trypan blue showing arbuscular mycorrhiza.	54
10.	Thallus of *Reboulia hemispherica* showing mycorrhizal invasion in midrib region of the plant.	55
11.	Rhizoids of *M. paleacea* showing AM fungus growth pattern.	57
12.	V.S. of the thalli of *M. paleacea* showing fungal invasion in the storage zone filled with arbuscules.	58
13.	Rhizoids of *Marchantia papillata* stained with trypan blue showing oval vesicle inside rhizoid.	60
14.	V.S. of the thalli of *M. papillata* showing fungal invasion and cells of the storage zone filled with arbuscules.	61
15.	Trap culture of liverworts and Isolated Sporocarp of *Glomus aureum*.	63

LIST OF TABLES

Table No.	Title	Page No.
1.	List of sites of collection along with their altitude.	25
2.	Methods used to perform different experiments.	26
3.	List of taxa undertaken for study.	39
4.	Characteristic features of mycorrhizal hyphae associated with *Asterella multiflora* growing under different habitats.	42
5.	Characteristic features of mycorrhizal hyphae associated with *Asterella wallichiana* growing under different habitats.	45
6.	Characteristic features of mycorrhizal hyphae associated with *Plagiochasma appendiculatum* growing under different habitats	48
7.	Characteristic features of mycorrhizal hyphae associated with *Reboulia hemispherica* growing under different habitats.	53
8.	Characteristic features of mycorrhizal hyphae associated with *Marchanta paleacea* growing under different habitats.	56
9.	Characteristic features of mycorrhizal hyphae associated with *M. papillata* growing under different habitats.	59
10.	Relationship between Min/Max. temperature and Percent Mycorrhizal Colonization (PMC)	67
11.	Table showing pH range of collection sites of various liverworts.	74
12.	Data regarding the Moisture Content (MC) of the soils and the Percent Mycorrhizal Colonization (PMC) in different areas	80
13	Data of Biochemical estimations of liverworts.	85

ABBREVATION USED

AM	Arbuscular Mycorrhiza
A.M	*Asterella multiflora*
A.W	*Asterella wallichiana*
P. A	*Plagiochasma appendiculatum*
R.H	*Reboulia hemispherica*
M.PP	*Marchantia papillate*
M.PL	*Marchantia paleacea*
J	Jammu
U	Udhampur
K	Kathua
R	Reasi
J1	Akhnoor
J2	Bantalab
J3	B. C. Road
J4	Botanical Garden, University of Jammu
J5	Dablehar
J6	Domel
J7	Gadigarh
J8	Jhajjarkotli
J9	Jourian
J10	Mamka
J11	Maha-Maya
J12	Miran-sahib
J13	Nagbani
J14	Nagrota
J15	Palace road
J16	Sidhra
J17	Talab-Tillo
U1	Devika
U2	Katra
U3	Mansar Lake
U4	Ramnagar
U5	Udhampur
K1	Basohli
K2	Mahanpur
K3	Marta nagrota
R1	Bhamla
R2	Kalidhar
R3	Sunderwani

CAT	Catalase
GPOX	Guaiacol peroxidase
SOD	Superoxide dismutase
APOX	Ascorbate peroxidase
GR	Glutathione Reductase
AsA	Ascorbic acid
GSH	Glutathione
Pr	Proline content
GB	Glycine- betaine
MDA	Malanoaldehyde
TPC	Total Phenol Content
Chl a	Chlorophyll a
Chlb	Cholorophyll b
CAR	Caratenoids
PMC	Percent Mycorrhizal colonization
MC	Moisture Content
BSA	Bovine Serum Albumin
FC	Folin-Ciocateau
g	Gram
mg	Milligram
UA	Unit Activity
GAE	Gallic Acid Equivalent
F.W.	Fresh Weight
H_2O_2	Hydrogen Peroxide
EDTA	Ethylenediaminetetraacetate
NADP	Nictinamide Adeninenucleotide
DDT	Dithiotheritol
DCM	Dichloromethane
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
+	Present
-	Absent
Temp	Temperature
Min.	Minimum
Max.	Maximum

CHAPTER-1

INTRODUCTION

Bryophytes are characterized by their diminutive size and the absence of roots and true vascular systems commonly found in higher plants. They are a distinctive and diverse group within the larger category of embryophytes that stand out due to their remarkable species diversity, second to angiosperms (Christenhusz & Byng, 2016). In the life cycle of bryophytes, gametophyte stage is dominant, which leads an independent existence. Concurrently, the sporophyte is reliant, either partially or entirely, on the gametophyte for essential resources such as water and nutrients. This dual-phase life cycle, where the dominant stage is the gametophyte, distinguishes bryophytes from higher plants where the sporophyte is the more conspicuous phase. Bryophyte gametophytes exhibit structural diversity, with some possessing a thalloid structure, resembling a flat and undifferentiated structure, while others differentiate into structures resembling roots (rhizoids), stems (cauloids) and leaves (phylloids). This structural organization reflects an intriguing evolutionary adaptation, serving as a transitional link between green algae and vascular plants. The transitional nature of bryophytes suggests that they likely emerged soon after the initial colonization of land by plants. This proposition aligns with the idea that bryophytes played a pivotal role in the early establishment and adaptation of plant life on terrestrial environments (Bowman *et al*., 2016). Through their unique life cycle and structural features, bryophytes contribute to our understanding of the evolutionary trajectory of land plants and their crucial role in shaping terrestrial ecosystems. Traditionally, bryophytes were often ignored in forest ecosystem studies or simply grouped with litter and soil components (Glime, 2024).

Bryophytes encompass three major groups: hepatics (liverworts), hornworts and mosses. These groups contribute significantly to ecosystems by performing various essential roles, such as water retention, acting as ecological indicators, enhancing soil quality and participating in nutrient cycling (Zhu *et al*., 2022; Rousk *et al*., 2017; Glime, 2020). Consequently, bryophytes hold ecological importance for their involvement in soil stabilization, nutrient cycling and support for biodiversity. One crucial function of bryophytes is their capacity to retain water, playing a pivotal role in maintaining moisture levels in their immediate environment. This feature is particularly beneficial in ecosystems with varying water availability, as bryophytes help regulate water retention and release, influencing the overall water balance (Xiao & Bowker, 2020).

Bryophytes also serve as valuable ecological indicators, reflecting the environmental conditions of their habitats. Their presence or absence can provide insights into factors such as moisture levels, nutrient availability and overall ecosystem health. This makes them useful tools for monitoring and assessing environmental changes and disturbances. In addition, bryophytes contribute to soil quality improvement. Through their growth and decay processes, they enhance soil structure and fertility. The rhizoids of bryophytes play a role in binding soil particles, preventing erosion and aiding in soil stabilization, particularly in disturbed or vulnerable environments. Furthermore, bryophytes participate actively in nutrient cycling. Their decomposition releases organic matter into the soil, contributing to nutrient availability for other organisms. This nutrient cycling is integral to the overall functioning of ecosystems, supporting plant growth and sustaining biodiversity. In summary, the ecological significance of bryophytes is multifaceted, encompassing water regulation, ecological monitoring, soil stabilization and nutrient cycling. By performing these vital roles, bryophytes contribute to the overall health and resilience of terrestrial ecosystems. Researchers have found that rhizoid-sphere communities in bryophytes, which contribute to nutrient cycling and soil health, change under drought conditions (Berdaguer et al., 2024). Furthermore, their simplicity and evolutionary significance make them valuable model organisms for scientific research (Yadav *et al*., 2023).

Mycorrhizae encompass a variety of mutualistic associations between soil fungi and plant roots. While bryophytes lack true roots, they possess rhizoids and many of them form associations with mycorrhizal fungi (Rimington *et al*., 2018). Arbuscular mycorrhizae are prevalent endomycorrhizal associations in most land plant species, involving fungi from the phylum Glomeromycota (Schubler *et al.,* 2000). These associations are characterized by the development of intracellular structures such as arbuscules and vesicles, serving as key sites for nutrient and carbon exchange between the plant and fungus (Smith & Read, 2010; Fitter *et al.,* 2000). Arbuscules, formed through the joint development of plant and fungal structures, play a central role in nutrient and carbon transfer. Other intraradical structures like vesicles, coils and intracellular hyphae growing in root cortical tissue are interconnected with an extraradical mycelium (Smith & Read, 2010; Allen, 2007). This extraradical mycelium serves multiple functions, including spore formation and nutrient uptake (Friese & Allen, 1991). Bryophytes, despite lacking true roots, engage in

mycorrhizal associations, particularly with arbuscular mycorrhizae. This interaction highlights the adaptability of mycorrhizal relationships across a range of plant forms, including those with rhizoids instead of true roots. The intricate structures formed during these associations contribute to efficient nutrient and carbon exchange, emphasizing the importance of mycorrhizae in nutrient cycling and plant health.

During the early land plants evolution, bryophytes played a crucial role as early colonizers in challenging environments characterized by desiccation, nutrient scarcity and heightened ultraviolet radiation (Krings *et al*., 2007). Their ability to thrive in such inhospitable conditions marks them as pioneers in ecological succession. A great amount of ecological success has been achieved by bryophytes in diverse habitats, from aquatic to desert and from arctic to tropical. Bryophytes, as the initial group to transition to a terrestrial habitat, underwent significant morpho-anatomical, physiological and reproductive modifications to adapt to the altered environmental conditions.

An important adaptation appears to be the establishment of arbuscular mycorrhiza (AM) symbiosis with fungi, a feature observed prior to the evolution of true mycorrhiza (Redecker *et al*., 2000). The limited or underdeveloped root systems in early bryophytes, coupled with the scarcity of essential nutrients such as phosphorus in primitive soils, drove the evolution of AM symbiosis as a crucial mechanism for plant colonization of land (Cairney, 2000; Antoine *et al*., 2021; Veresoglou *et al*., 2022).

The coevolution of roots with arbuscular mycorrhizae (AM) in vascular plants underscores the profound significance of this symbiotic relationship (Brundrett, 2002). The development of arbuscular mycorrhizal associations in early plants is recognized as a unique occurrence, signifying a monophyletic innovation that played a crucial role in enabling vascular plants to swiftly colonize continents (Delaux, 2017). The extensive prevalence of arbuscular mycorrhizal (AM) associations among terrestrial plants in various ecological niches, excluding aquatic environments, has been extensively documented (Pressel *et al*., 2021). This symbiotic interaction between arbuscular mycorrhizal fungi and liverworts plays a pivotal role in the colonization and establishment of plants in terrestrial ecosystems.

Antioxidants are the compounds that neutralize the free radicals produced during the metabolic processes. Reactive oxygen species (ROS) possess the potential to induce oxidative stress at elevated concentrations, contributing to various degenerative diseases, ageing, apoptosis and food rancidity (EL-Beltagi & Mohamed, 2013). Plants serve as a primary source of antioxidants, derived from phytochemicals. Notably, natural antioxidants from plants exhibit a more favorable impact on the human body compared to synthetic counterparts, as plant constituents align with living physiology, making them well-suited for human consumption. The recognition of the importance of biologically active compounds from plant origins has grown substantially in recent years, given their capacity to combat diseases arising from oxidative stress. The exploration and utilization of higher plants, particularly angiosperms, as sources of antioxidants have become common place due to their potential health benefits. Nevertheless, bryophytes, which possess strong defence mechanisms enabling survival in highly diverse habitats and harbour a wealth of distinct phytochemicals, remain insufficiently explored in terms of their antioxidant properties. AM fungus, on the other hand, extends the uptake capabilities of plants, particularly for immobile ions and for water, nitrogen and phosphorus through their hyphae extending into the soil thereby improving plant nutrition. They have, therefore, come to be used as biofertilizers to increase the productivity of economically important crops and trees. Hence, there is a huge potential in utilizing these ethno-botanically important plants to increase agricultural yield and as eco-friendly antibiotics and antioxidants in modern healthcare.

Arbuscular mycorrhizal associations in Angiosperms, Gymnosperms and Pteridophytes have long been known. In Bryophytes, it has been studied in many countries by various research workers (Brundrett, 2002; Duckett & Read, 1995; Duckett *et al*., 2006; Bidartondo *et al*., 2003; Carafa *et al*., 2003; Ligrone *et al*., 2007 & Pressel *et al*., 2021). In the context of India, the understanding of the diversity of Arbuscular Mycorrhizal fungal associations with bryophytes remains significantly limited. Similarly, in recent years, the antioxidant capacities of pteridophytes and angiosperms were evaluated (Souri *et al*.,2008; Begara-Morales *et al*., 2018; Souri *et al*., 2020). Currently, there is a scarcity of data available concerning bryophytes, with only limited information at our disposal.

The territory of Jammu & Kashmir (North-Western Himalayas) has a very rich bryodiversity (420 species). It is reported to house nearly 120 liverworts and hornworts (Tanwir, 2005; Tanwir & Langer, 2006) belonging to large number of families, of which more than 100 species have been collected from Jammu region alone. Out of these, twelve species are reported to form Arbuscular Mycorrhizal associations (Verma & Langer, 2011; Sharma, 2017). Only two species from District Reasi, Jammu & Kashmir have been studied so far for antioxidants (Sharma *et al.,* 2015). Hence, there is a large scope for research on these amphibians of plant kingdom. Therefore, this study was conducted to study AM and antioxidant activities in liverworts. Detailed studies on the AM fungi and antioxidant potential of various groups of bryophytes are expected to throw light on several questions of academic, economic and evolutionary interest.

REASEARCH OBJECTIVES:

Due to (i) limited information on AM fungal symbiosis and antioxidant properties of these plants from J&K and (ii) the richness in hepatic diversity of the area, this research work has been undertaken with the following objectives:

1. To screen and sample the diverse hepatic taxa for AM fungal symbiosis.
2. To isolate and study the cultural characteristics of AM fungi associated with the collected hepatics.
3. To study the effect of various abiotic factors on symbiosis and antioxidant potential of collected hepatic taxa.
4. To analyze the correlation between the AM fungal symbiosis and antioxidant potential.

CHAPTER-2

REVIEW OF LITERATURE

2.1) Mycorrhiza

The term "Mycorrhiza," which means 'Fungus Root,' was coined by Albert Bernhard Frank, a German botanist, in 1885. Frank proposed that mutualistic plant and fungus partnerships were vital for the nutritional sustenance of both organisms. Cooke (1977) provided a definition of fungal symbiosis, encompassing all associations where fungi encounter a living host to obtain metabolites or nutrients in various ways. In contrast, Harley (1984) specifically defined mycorrhizae as partnerships between fungal hyphae and higher plant organs dedicated to absorbing substances from the soil. On a broader scale, Hawksworth *et al*. (1988) presented a comprehensive definition of mycorrhiza, albeit less precise, as it did not exclude pathogenic associations. This broader definition encompassed various interactions involving fungal hyphae and plant roots. The historical progression of these definitions reflects evolving perspectives on the multifaceted relationships between fungi and plants, emphasizing their crucial roles in nutrient exchange and ecological balance. Smith noted that most mycorrhizal associations are found in roots, which have adapted to accommodate fungi. Building on this, Smith (1997) supplemented the information by stating that mycorrhizal associations also take place in the subterranean stems of specific plant species. Read *et al.* (2000) reported the occurrence of Glomalean fungi in bryophyte thalli also. Brundrett (2002) broadened the definition of mycorrhiza as “a symbiotic association essential for one or both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate contacting organ) of a living plant, that is primarily responsible for nutrient transfer”.

2.1a) Types of mycorrhizae:

In 1885, Frank made a distinction between two types of mycorrhizae:

i. Ectotrophic mycorrhizae, characterized by a distinct external sheath of fungal tissue that encloses the root.

ii. Endotrophic mycorrhizae, which lack a sheath but exhibit both inter- and intracellular penetration of the host by the fungus.

Subsequently, in 1969, Peyronel *et al*. categorized mycorrhizal associations into endomycorrhiza, ectomycorrhiza and ectoendomycorrhiza, depending on the fungi's specific location within the roots. Various other authors, such as Lewis (1987), Read (1991) and Harley *et al.* (1984), have also contributed to the classification of mycorrhizae.

Brundrett (2002) recognized seven types of mycorrhizae based on a combination of morphological, morphogenetic and physiological characteristics. These are (i) Vesicular arbuscular mycorrhiza (AM) (ii) Ectomycorrhiza (iii) Arbutioid (iv) Ectendomycorrhiza, (v) Monotropoid mycorrhiza (vi) Ericoid mycorrhiza and (vii) Orchidoid mycorrhiza. The same author in the year 2004 further classified each type into various categories based on the regulation of fungus by host plants.

2.1b) Host range of mycorrhizal fungi: AM fungi are common occurrences in bryophytes, pteridophytes, gymnosperms and angiosperms throughout the world.

Angiosperms: A researcher collected data for 6,507 angiosperm species, out of which 67% had AM, 15% had other association types and 18% were nonmycorrhizal (Trappe, 2005). Ragupathy *et al.* (1990) found the formation of AM in 33 out of the 70 tropical hydrophytes. Bagyaraj, 1991 reported the occurrence of VA mycorrhizae in a large number of important diverse economical important crops and tropical legumes. Smith & Smith 2011 compiled data on mycorrhizae in angiosperms. Their research revealed that among forty-one families of angiosperms, exclusively the "Paris type" of Arbuscular mycorrhiza was documented, while in thirty families, only the "Arum type" was observed. Additionally, twenty-one families exhibited instances of both classes, showcasing intermediate forms between them. This diversity in mycorrhizal associations highlights the intricate relationships between plants and fungi across various angiosperm families.

Arbuscular mycorrhizal (AM) association was observed in 71% of vascular plants (Fernández *et al*., 2019). They cannot survive without host plants (Kloppholz *et al*., 2011; Sugiura *et al*., 2020). Many plants are categorized as "nonhost" plants. Some important families of angiosperms such as Caryophyllaceae and Brassicaceae predominantly include these non-host plants (Wang and Qiu, 2006).

As indicated by Smith & Read (2010) and Bidartondo *et al*. (2011), early terrestrial plants that established themselves on land probably depended on arbuscular mycorrhizal (AM) associations to meet their nutritional requirements. The endurance of AM symbiosis has been notable throughout evolutionary timelines, despite occasional diversifications into various mycorrhizal patterns (Lokhandwala & Hoeksema, 2019; Feijen *et al*., 2018). This resilience stands in contrast to the dynamics observed in other types of mycorrhizal associations.

According to Smith & Read (2010) & Bidartondo *et al*. (2011) early terrestrial plants that colonized the land most likely relied on arbuscular mycorrhizal (AM) associations to fulfill their nutritional needs. The AM symbiosis exhibits a remarkable endurance across evolutionary time, despite irregular diversifications towards various mycorrhizal association patterns (Lokhandwala & Hoeksema, 2019; Feijen *et al*., 2018) this is in contrast to other mycorrhizal associations. Mycorrhizae have also been observed in various medicinal plants, suggesting potential for enhanced medicinal plant cultivation (Chanda *et al.*, 2024).

Gymnosperms: Newman & Redell (1987) compiled data available for the gymnosperms and found that ninety eight percent of gymnosperms form AM associations. Stockey *et al*. (2001) observed mycorrhizal association in the extinct conifer *Metasequoia milleri*.

Muthukumar and Udaiyan (2002) discovered arbuscular mycorrhizal (AM) associations in Cyadaceae and Zamiaceae. In 2006, Wang and Qiu conducted a comprehensive survey involving 3,617 species from 263 land plant families to assess their mycorrhizal status. Their findings revealed that all gymnosperm families host mycorrhizal associations. Moreover, certain gymnosperms, including cycads (Muthukumar and Udaiyan, 2002) and ancient conifers were identified as capable of developing arbuscular mycorrhiza (Strullu-Derrien *et al.*, 2018).

Evidence from extinct Voltairean conifers, specifically *Notophytum* from the middle Triassic approximately 220 million years ago, showed the presence of arbuscular mycorrhiza. Harper *et al.* (2015) found that this symbiotic fungal relationship in early transitional conifers was significant. This underlines the relevance of the association.

Furthermore, during the Jurassic Period, it was discovered that certain current gymnosperms belonging to the Araucariaceae family, such as the ones that were investigated by Padamsee *et al.* (2016), were capable of harboring arbuscular mycorrhizal fungi. Reports of AMF colonization extend to other gymnosperm families, including Pinaceae (Cazares & Trappe, 1993; Smith *et al*., 2010), Cupressaceae (Aalipour *et al.*, 2020; Bush, 2008) and Podocarpaceae (Russell *et al*., 2002). Observations of AMF vesicles have been documented in the seedling roots of Abies (Cazares & Trappe, 1993; Smith *et al*., 2010), Pinus (Smith *et al.*, 2010; Alicia Franco *et al.*, 2021) and Pseudotsuga (Cazares & Trappe, 1993) within the Pinaceae family.

Pteridophytes

Sedebeck recorded a fungal invasion in 1875 in the thallus of *Equisetum*bya *Pythium* species. Muthukumar & Udaiyan (2000) examined seventy-one species of pteridophytes which belong to 30 families and observed AM in as many as sixty of these. An account of arbuscular fungi that are connected with pteridophytes of Dujiangyan, which is located in southwest China, was provided by Zhang *et al*. (2004). *Glomus, Acaulospora, Archaeospora, Entrophospora* and *Gigaspora* are the five fungal genera that were separate from one another by the authors. In 2005, Turnau and colleagues revealed the presence of mycorrhizal symbiosis in the chlorophyllous gametophytes and sporophytes of a fern known as *Pellaea viridis.*

Duckett & Ligrone (2005) conducted research on the parenchymatous cells of the rhizomes and underground gametophytes of *Tmesipteris* and *Psilotum*. He documented glomeromycotan fungi that was aseptate and fungus was intracellular that were similar to the 'Paris type' of AM that is present in higher plants.

Mycorrhizal arbuscular structures have been discovered in both roots and gametophytes of pteridophytes that aid in growth. They obtain water and minerals from their surroundings (Muthukumar & Prabha, 2013; Muthukumar *et al*., 2014). The importance of symbiosis in leptosporangiate ferns is lower when compared to that of lineages that diverged shortly afterward. Furthermore, epiphytic species are not likely to be mycorrhizal fungal hosts due to the environment in which they grow (Gemma *et al.*, 1992; Lehnert *et al.*, 2017).

Regardless, colonization may occur. In ferns and lycophytes, the percentages are close to 60% and 80%, respectively (Muthukumar & Prabha, 2013; Muthuraja *et al*., 2014). Roots of many other pteridophytes had also known to form AMF colonization (Lara-Perez *et al*., 2015; Krzyzanski *et al*., 2022). Recent findings have expanded understanding of mycorrhizal associations beyond seed plants, examining relationships in pteridophytes (Kumari *et al*., 2024).

Bryophytes

The presence of mycelium in bryophyte tissues has been documented for many years. Nemec (1899) noted the common occurrence of mycelium in the leafy members of Jungermanniales. Reports on fungi colonizing liverworts date back to the 1800s (Stahl, 1949; Boullard, 1988; Rimington, 2015), with the colonization primarily confined to the rhizoids and vegetative thallus. Boullard (1988) introduced the term 'mycothallus' to describe such associations.

This phenomenon has long been recognized, as Nemec (1899) reported mycelium presence in the leafy members of Jungermanniales. Stahl (1900) attempted to correlate invasion incidence in hepaticae with the nutrition theory in mycotrophic plants. Beaverie (1902) observed *Fusarium* mycelium in the thallus of *Conocephalum conicum,* while Golenkin (1901) provided records for the Marchatiaceae, describing endotrophic invasion in various liverwort species. Cavers (1904) challenged the idea, citing observations on *Conocephalum* and *Monoclea*. Gallaud (1905) specialized the endophyte in liverworts, noting its distinct characteristics and intracellular distribution in *Pellia*. He believed fungal invasion was widespread in hepatics, limited to the gametophytic generation and rare near the sporogonium.

Humphrey (1906) observed tuberous swellings on *Fossombronia longiseta* stems, finding mycelium present in each case. Ridler (1923) supported Gallaud's observations on *Pellia,* describing arbuscules composed of "fine and branching threads" filling thallus cells with the mycelial network. Magrou (1927) confirmed the immune status of strongly growing fertile fronds of *Pellia* to invasion up to the period of dispersal. Chaudhari and Rajaram

(1925) noted fungal invasion in vigorous thalli of *Marchantia nepalensis*, typically limited to tissue beneath air canals.

Parke & Linderman (1980) reported vesicular arbuscular mycorrhiza in the moss *Funaria hygrometrica* growing on the soil surface of *Asparagus- Glomus epigaeus* pot cultures. Ecological characteristics of the interaction between the AM fungus *Glomus tenuis* and the moss *Pogonatum* were documented by Rabatin (1980). He cultured moss along with fungal inoculum and observed that cleared and stained moss had characteristic fine hyphae and *Glomus tenuis* spores along leaves and stems. Ligrone (1988) documented the frequent occurrence of fungal hyphae in the parenchyma of the gametophyte of *Phaeoceros laevis*, along with the formation of intra-cellular arbuscule-like structure. Similarly, a report of Ligrone & Lope in year 1989 suggested arbuscular mycorrhizal like associations in *Conocephalum conicum*. In their study, Read *et al.* (2000) extensively documented the colonization of zygomycetous fungi in various existing lower land plants. These fungi demonstrated the formation of structures similar to those typically observed in vesicular-arbuscular mycorrhiza of gymnosperms and angiosperms. Notably, the authors made a groundbreaking observation by reporting the newfound capability of glomalean fungi to colonize a thalloid liverwort (*Pellia epiphylla*), resulting in the development of arbuscules and vesicles. This discovery expanded our understanding of mycorrhizal associations in a broader range of lower land plant species.

Schubler (2000) reported the formation of an arbuscular mycorrhiza like symbiosis between the hornwort *Anthoceros punctatus* and *Glomus claroideum*. Kottke *et al.* (2003) observed similar symbiotic associations of hepatics with heterobasidiomycetes. As a result of their investigations, they discovered that Jungermanniales are connected to sebacinoid mycobionts, whereas *Aneura pinguis* established connections with *Tulasnella* species. Birch trees, *Cryptothallus mirabilis* and *Tulasnella* species were all involved in an intriguing interaction that was documented by Bidartondo *et al.* (2003). According to the results of their investigation, the interaction with *Tulasnella* is an essential component for the development of this liverwort. Experiments involving labeling gave further evidence that the $^{14}CO_2$ that was supplied to birch seedlings was transported to *Cryptothallus* via *Tulasnella.* The authors Nebel *et al.* (2004) brought to light a considerable similarity

between the evolution of liverworts and the evolution of symbiotic fungus. For the purpose of shedding light on the evolutionary connection of these organisms, they developed a theory that suggested that the symbiotic fungal associations of liverworts could potentially be the forefathers of mycorrhizae.

They observed that the Jungermanniales have sebacinoidmycobionts while *Aneura pinguis* is associated with *Tulasnella* species. Bidartondo *et al.* (2003) reported an interesting relation between *Cryptothallus mirabilis, Tulasnella* species and birch trees. They showed that interaction with *Tulasnella* is necessary for growth of this liverwort and by using labeling experiments they showed that $^{14}CO_2$ provided to birch seedling is transferred to *Cryptothallus* by *Tulasnella*. Nebel *et al.* (2004) indicated a strong congruency between the evolution of liverworts and symbiotic fungi and hypothesized that 'The symbiotic fungal associations of liverworts are the possible ancestor of mycorrhizae'. Pocock & Duckett (1984) indicated the probable absence of symbiotic association in mosses. According to Russell and Bulman (2005), the arbuscular-mycorrhizal fungus of the genus *Glomus* and the liverwort *Marchantia foliacea* have a unique symbiotic relationship. Tanwir (2005) reported mycorrhizal association in five liverwort species (*Marchantia kashyapii, Asterella blumeana, A. angusta, A. mussuriensis* & *Conocephalum conicum*) growing in district Poonch of J&K (Northwest Himalaya). Duckett *et al*. (2006) discovered a highly distinct glomeromycotean connection with the *Treubia* (Treubiaceae) primitive antipodean liverwort. The authors epitomized very early stages in the evolution of glomeromycotean symbiosis. Upson *et al.* (2007) gave description of association between the mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort *Cephaloziella varians*. Ligrone *et al*. (2007) studied Glomeromycotean association in liverworts and they observed that fungal endophytes found in *Conocephalum, Haplomitrium, Pellia and Fossombronia*, were affiliated with *Glomus* group A. In contrast, the endophyte identified in *Monoclea* was associated with *Acaulospora*. This distinction in fungal associations provides valuable insights into the diverse relationships between liverworts and specific fungal groups, contributing to our understanding of the intricate symbiotic interactions within plant-fungal partnerships. They also provided analyses of these connections at the molecular, cellular and taxonomic levels. Schistochilaceae, a family of leafy liverworts, was examined by Pressel *et al*. (2008) and they found novel ascomycetous endophytic

associations in the members of this family. Verma & Langer (2011) found AM association in eleven species of liverworts from Jammu, Northwest Himalayas. Liepinia (2012) observed that four out of twenty-one hepatics studied form fungal symbiosis. Desiro *et al.* (2013) used electron microscopy and molecular phylogenetics to study fungal symbiosis in bryophytes. They found that the association with Glomeromycota was more frequent. Similar glomeromycotean associations have also been noted in *Mannia fragrans* from West Bengal (De., 2017) and *Marchantia nepalenis* from J&K (Verma & Langer, 2014). In both instances, smooth-walled rhizoids exhibit greater fungal hyphal colonization than tuberculated ones, through which they advance into the midrib's gametophyte parenchyma and spread from cell to cell through the wall of two neighboring cells via plasmodesmata. Liverworts belonging to Marchantiopsida and Pelliidae exhibit a diverse array of fungal colonization patterns and structures, reminiscent of those found in AM fungi associated with angiosperms (Read *et al*., 2000).

In angiosperms Arum and Paris types are particular kinds found within the larger category of arbuscular mycorrhizal (AM) associations, which demonstrate the symbiotic connections between plant roots and fungi belonging to the Glomeromycota phylum. Outlined below are the distinctions between these types:

The Arum-type mycorrhiza involves fungal hyphae penetrating the root cells of the host plant to create complex, highly branched structures called arbuscules. In general, arbuscules are found only in the cortical cells of the root, allowing for nutrient exchange between the plant and the fungus. In Paris-type mycorrhiza, fungal hyphae penetrate the intercellular spaces of the root and create coils or dense networks of hyphae. In this type, fungal colonization is mainly extracellular, with hyphae surrounding the outer part of root cells without developing arbuscules inside them.

Essentially, the main difference is found in the location and structure of fungal colonization within the root cells. Arum-type mycorrhiza displays arbuscules developing within the cells, whereas Paris-type mycorrhiza involves colonization outside the cells with the development of coils or hyphal networks. Both types have important functions in enabling nutrient exchange between the plant and the fungus (Smith & Smith, 2011). Liverworts, excluding Haplomitriopsida, lack gametophytic intercellular gaps.

Consequently, the AMF colonization in liverworts is classified as Paris-type (Ligrone, 2007). However, Rimington *et al.* (2018) findings showed that, after discounting intercellular hyphae, all of the structures formed by Glomeromycota in liverworts belong to both types. Kobae *et al.* (2019) isolated AM fungi from the thalli of *Marchantia paleacea*. Rimington, *et al.* (2020) concluded that 78% of hornworts and lycophytes (up to 100%) harbour fungal symbiosis but members of Mucoromycotina and Glomeromycotina are involved only in AM symbiosis in bryophytes. Studies have specifically observed mycorrhizal associations in the liverwort species *Asterella multiflora*, adding insight into mycorrhizal diversity in bryophytes (Verma *et al.*, 2024).

2.1c) Benefits of AMF associations

Uptake and transfer of nutrients

Extraradical mycelium can access more soil than plant roots alone can, which increases AMF's ability to collect water and nutrients (Brito *et al*., 2014). The immediate benefit of phosphorus uptake and transport stands out the most, but it is also crucial to absorb other immobile minerals like zinc as well as mobile elements like nitrogen and sulphur. The plant roots and the soil environment aid in the growth of the fungus's mycelium, which connects the roots into a three-dimensional network. In nutrient-poor environments, it functions as an effective system for nutrient uptake particularly phosphorus. Additionally, the mycelium helps to generate the water-resistant aggregates required for adequate soil tilth (Kazadi, 2020). Mycorrhizal symbiosis plays significant roles in ecological restoration and agriculture by promoting plant health and growth (Martin & van der Heijden, 2024). Fungal associations are key to enhancing plant growth by improving nutrient uptake, a beneficial interaction especially in nutrient-poor soils (Kumar *et al*., 2024). AMF raises the number and quality of horticulture plants' production, increases their disease resistance and increases the availability of nutrients and water to them (Chahal *et al*., 2022). The reduction of abiotic challenges like salt and metal ion toxicity and biotic pressures like soil-borne illnesses are further ecological advantages of AMF colonization plants. Arbuscular mycorrhizal symbioses are beneficial for a variety of field crops, in large part, because they

create a dense hyphal network in the soil, boost plant uptake and improve nutrient exploitation (Catska *et al*.,1997; Johnson *et al*., 2016).

Soil structure

One important component of AMF that improves soil functioning and has an impact on the advantages is the establishment and maintenance of soil structure. In addition to improving host plant nutrition, the extra-radical mycelium of AMF significantly affects the stability and agglomeration of soil particles (Wright & Upadhyaya, 1998). Abetter soil structure results from the association of the soil AM fungus for storing carbon and other organic matter that is preserved by aggregates. The struggle with saprotrophic bacteria and fungus slows down decomposition. Mycelial exudates contribute carbon to the subsoil and AMF also produces very stable organic matter components by combining minerals with them (Sosa-Hernández,2019).

Prevents Soil erosion and nutrient leeching

AMF effectively combats nutrient leaching and soil erosion in both natural and cultivated soils. In order to connect and link particles of soil without compressing the soil, the highly ramified AMF mycelium creates a three-dimensional matrix (Chahal *et al*., 2020). This stabilises soil aggregates. Along with promoting plant growth and the establishment of root systems, the hyphal web of AMF shields the soil from wind and water erosion and supports water retention and nutrient supply (Samuel & Veeramani, 2021).

Use of AM fungus in heavy metal contamination phytoremediation

Leyval *et al.* (1997) found that mycorrhizal fungi are used as phytoremediation agent in polluted soils. They suggested that cell walls of AM fungi have several sites for binding metals which save the host plants from the toxicity of metals. Entry *et al.* (1999) reported the accumulation of Cs^{137} and Sr^{90} from contaminated soil of grass species (*Paspalum notatum, Sorghum hatepense* and *Panicum virgatum*) inoculated with *Glomus mossae.* Joner & Leyval (2000) conducted a study indicating that the development of a more efficient mycorrhizal symbiosis is possible when heavy metal-tolerant arbuscular mycorrhizal (AM) fungi are present in soil contaminated with metals. The research

suggested that mycorrhiza might play a vital role in safeguarding plant roots from the adverse effects of heavy metals. Nevertheless, the effectiveness of this protective mechanism varies among different isolates of mycorrhizal fungi and in the presence of different heavy metals (Khan, 2005; Wang *et al*., 2023). Davies *et al.* (2001) found that mycorrhizal fungi enhance the accumulation and tolerance of chromium in sunflowers. Sunflowers inoculated with *Glomus intraradices* accumulate more chromium as compared to those which are not inoculated. AMF are thought to improve heavy metal tolerance. The ability of plants to tolerate heavy metal exposure is significantly influenced by various plant and fungal species, as well as the specific heavy metal present in the root environment (Khaliq *et al*., 2022). Arbuscular mycorrhizal fungi (AMF) play a crucial role in regulating the allocation of heavy metals within plants, preventing their transportation from roots to shoots (Wu *et al.,* 2016). Research has shown that maize plants exposed to AMF retained heavy metals such as cadmium (Cd), lead (Pb) and zinc (Zn) in their roots (Zhan *et al*., 2018). Despite high levels of heavy metals in their tissues, plants influenced by AMF exhibited minor stress symptoms, suggesting a potential reduction in toxic effects due to enhanced phosphorus (P) nutrition and improved growth (Khalid *et al*., 2023). AMF has been identified as a factor capable of hindering the absorption of heavy metals in certain plant species. Through the activation of the antioxidant defence system and restriction of the uptake of metals such as Cadmium (Cd) and Lead (Pb), the AM fungus associated with *Cnadulla officinalis* demonstrated the ability to mitigate the adverse impacts of heavy metal exposure (Hristozkova *et al*., 2017).

2.1d) Glomalin

Glomalin is produced by AMF hyphae, a protein that sequesters toxic metal ions. It can bio-stabilize metal-polluted soils. It was discovered by an American research service soil scientist who named it after fungal order Glomales (Wright *et al*., 1998). It is a complex glycoprotein produced by Arbuscular Mycorrhizal Fungi in their hyphal walls (Wright *et al*., 1998; Deepika, 2021). With the senescence of hyphae, glomalin is stored within the soil where it accumulates until it becomes as much as 5% of the soil carbon (Rillig *et al*., 2003) and Nitrogen (Lovelock & Robinson, 2002). After the discovery of this glycoprotein,

a number of studies have been carried out by different workers to understand its role in natural ecosystems (Wright & Upadhy,1998; Rillig *et al*., 2003& Deepika, 2021).

Wright & Upadhyay (1998) studied undisturbed, disturbed and soils under elevated CO_2 for understanding the relationship between aggregate stability and protein glomalin in temperate regions. One year later, Wright & Upadhyay (1998) used strips of horticultural films to trap extra-radical hyphae emanating from roots of Sorghum colonised by *Gigaspora rosea, Glomus intraradices* and *G. caledoniam* and found these films to be effective traps for hyphae and protein glomalin. Lovelock *et al*. (2004) that more fertile soils had less glomalin and vice versa. Rosier *et al*. (2006) assessed the use of the Bradford method and ELISA test in the quantification of glomalin and found that soil organic matter may influence the accuracy of these methods. They discouraged the use of these methods under conditions where significant extraneous protein additions occur (*i.e.*, manure, sewage etc.).

Burrows (2014) conducted a study investigating the impact of plant diversity on the infectivity of AM fungi and the synthesis of glomalin. The results, based on three years of data collection, revealed that monocultures exhibited the lowest levels of glomalin production. Notably, glomalin production was found to be more closely associated with plant diversity. The study identified a positive correlation between glomalin levels and spore density. In a separate investigation in 2015, Sathyarahini and colleagues explored the relationship between total of glomalin content and soil particles in various fruit crops. Their findings demonstrated that soil aggregates measuring three to four millimetres exhibited significantly higher levels of total glomalin compared to two-millimetre aggregates. This observation underscores the role of glomalin in the overall process of soil aggregation. Deepika (2015) provided additional insights into the nature and function of glomalin. She argued that seasonal variations, soil edaphic conditions, land use systems and diverse plant species collectively influence the status of AM fungi and the production of glomalin. Another notable conclusion she drew was that glomalin has the capacity to sequester heavy metals.

According to some authors, Glomalin is extremely recalcitrant (resistant to being destroyed) and challenging to dissolve in water. These characteristics make glomalin (or

its components) particularly stable substances that guard the surface of the soil aggregate (Vlcek & Pohanka, 2020).

In short, we can say that arbuscular mycorrhizal associations are symbiotic relationships between plants and certain fungi that provide a range of benefits to the host plants. Here are some key factors that could explain increased growth in AM associated plants:

1. **Nutrient Uptake:** It is well known that arbuscular mycorrhizal fungi have the ability to improve the efficiency with which host plants take in nutrients, particularly for critical elements such as phosphorus and nitrogen. The fungi have a bigger surface area compared to the roots of the plant, which enables them to investigate a greater volume of soil and gain access to nutrients that the plant might not have access to otherwise. According to Smith and Read (2010) and Wurzburger *et al*. (2017), plants that have mycorrhizal connections can experience higher growth because of improvement in their ability to absorb many important nutrients specially phosphorus.

2. **Improved Capacity to Take in Water:** Mycorrhizal fungi have the ability to enhance a plant's capacity to take in water from the soil. This is especially helpful in situations where there is a limited supply of water, such as when there is a drought or dry circumstances. According to Kapoor and Mukerji (1987) and Keffe and Sylvia (1993), it is possible for improved water uptake to assist in the maintenance of turgor pressure within plant cells and to boost overall growth.

3. **Stress Tolerance:** Mycorrhizal connections have the potential to increase a plant's resilience to a variety of environmental challenges, including infections, salt and heavy metals. Plants are also able to devote a greater amount of their energy and resources to growth when they are better protected from the environmental conditions that cause them stress (Begum, 2019).

4. **Enhancement of Photosynthesis:** Mycorrhizal fungi have the ability to affect the efficiency with which host plants produce photosynthetic compounds. The ability of these fungi to indirectly boost photosynthesis, which ultimately results in higher

biomass production, can be attributed to their ability to aid with nutrient uptake and stress tolerance (Chandrasekaran *et al.*, 2019).

5. **Resource Allocation**: Plants that have mycorrhizal associations may devote a greater portion of their resources (for example, carbohydrates) to growth rather than investing extensively in root development for the purpose of incorporating nutrients and water into their bodies. It is possible that this change in resource allocation will result in larger buildings that are located above ground (Chen *et al*., 2018; Beyschlag, 2024).

2.2) Antioxidants in Bryophytes

The study of antioxidants in plants has gained significant attention due to their potential health benefits and ecological importance. Bryophytes, including liverworts, hornworts and mosses, represent an intriguing group of non-vascular plants that have been traditionally overlooked in antioxidant research. However, recent studies have shed light on the remarkable antioxidant properties observed in various bryophyte species. This review synthesizes the current understanding of the antioxidant activity within bryophytes, highlighting their significance in the broader context of plant biology and human health.

Bryophytes generate a variety of secondary metabolites that give these plants powerful antioxidative defences to withstand biotic and abiotic stressors (Xie *et al.*, 2009; Gahtori & Chaturvedi, 2019; Mathew *et al*., 2023). These plants have evolved active chemical and molecular defences to make up for the lack of any unique morphological and anatomical defence mechanisms. Cell organelles and membranes are shielded from oxidative damage by antioxidant defences. ROS interact with crucial cell components including proteins and lipids under unfavourable circumstances, disrupting the structure of the cell and ultimately resulting in cell damage (Asakawa *et al.*, 2013a). Cells are shielded from oxidative stress caused by internal as well as external adverse environments by antioxidant enzymes. Bryophytes have potential to inhibit the release of superoxide anion radicals as well as calmodulin,5-lipoxygenase, cyclooxygenase and hyaluronidase. They also had antimicrobial, antitumor, cytotoxic, cardiotonic, allergy-causing, irritancy and tumour-

effecting, insect anti-feedant, molluscicidal and tumour-effecting properties (Yayintas & Irkin, 2018; Cianciullo *et al.*, 2021).

2.2a) Antioxidants activities in mosses:

Some of the mosses from Turkey like *Oxytegus tenuirostris*, *Eurhynchium striatum* and *Rhyncho stegiummurale* showed antioxidant properties (Yayintas *et al*., 2017). Similarly, *Eurhynchium striatulum* and *Homalotheciums ericeum* had very good free radical scavenging capacities (Erturk *et al*., 2015). *Saninonia uncinata,* an alpine moss, that have secondary metabolites which impart immunity against the environmental stresses like UV, high temperatures and drought (Bhattarai *et al.,* 2008). The same author in the year 2009 examined the antioxidant potential of the compounds isolated from them showed a two to seven-fold increase from their extracts (Bhatterai *et al*., 2009; Mishra *et al*., 2014). According to Pin-Der-Duh X *et al.* (1999) the reducing power of plant extracts, which is dependent on the availability of reductones, is directly related to the antioxidant activity of plants. By giving a hydrogen atom, which causes the chain of free radicals to break, the reductones operate as antioxidants (Gordan, 1990). The secondary metabolites in mosses include terpenoids, bibenzyls and flavonoids. They are abundant in fats, acetophenols and other compounds. Their antibacterial efficacy is influenced by the unique chemical makeup, compound structure, functional groups and any potential additive or destabilizing interactions between compounds (Asakawa *et al*., 2013b). A significant amount of terpenoids have been reported from the bryophyte, *Thuidium tamariscellum.* These terpenoids were reported to impart antioxidant activities in plants (Mohandas & kumaraswwamy, 2018). The terrestrial moss *Pseudoscleropodium purum,* which can be utilized as a biomarker for detecting pollution, was also found to contain glutathione, which was found to be an important antioxidant chemical (Varela *et al*., 2018). Several other mosses, in addition to the species mentioned above, have substantial antioxidant activity (Vats & Alam, 2013; Oyedapo *et al.*, 2015). The moss *Sphagnum magellanicum* was shown to contain a number of phenolic compounds, including caffeic, vanillic, chlorogenic, gallic,3-4 hydrozybenzoic, p-coumaric and salicylic acid, according to reverse-phase HPLC (Alam, 2021). Aromatic and terpenoids compounds isolated from mosses had biological properties that demonstrated antibacterial and antifungal activity (Cianciullo *et*

al., 2021; Manoj & Muruganan, 2012)

2.2b) Polyphenol Activities:

Numerous lifestyle disorders can be cured because of the amazing antioxidant properties of polyphenolics (Kasote *et al*., 2015). There may be one or more aromatic rings containing hydroxyl groups in polyphenolic compounds. In general, side chain conjugation with aromatic rings and the quantity of free hydroxyls has a direct impact on the phenolics' ability to act as antioxidants (Gordon, 1990). Studies on *Thuidium tamariscellum's* phytochemistry revealed that the moss had a large number of terpenoids. According to reports, the plant's significant terpenoids content is primarily responsible for its high antioxidant properties (Mohandas & Kumaraswamy, 2018). Additionally, studies showed that liverworts typically have greater total flavonoid concentrations than mosses. The values of these substances were often greater in acrocarpous mosses than in pleurocarpous mosses. Compared to bryophytes grown in full sunlight, lower light levels had higher total flavonoid concentrations. The highest total flavonoid concentrations were found in epiphytic bryophytes, while the lowest concentrations were found in aquatic bryophytes. Low-latitude species have higher flavonoid content than high-latitude species (Zhan *et al*., 2018). Additionally, studies showed that liverworts' antioxidant levels were comparable to those of vascular plants. Some species have indeed been investigated for their resistance to high nitrogen concentrations (Koranda, 2007), drought and water stress (Wasley, 2006). A few mosses crude extracts (Basile *et al*., 1999; Dulger *et al*., 2002; Yayintas *et al.*, 2017) include hypnogenols, dihydroflavonols, biflavonoids, hydroxy flavonoids and polycyclic aromatic hydrocarbons. Plants produce flavonoids in response to microbial infection. Their ability to interact with soluble proteins outside of cells as well as with bacterial cell walls is likely what causes this behavior (Basile *et al.*, 1999).

2.3) Diversity of Liverworts and Antioxidant Compounds:

Bryophytes exhibit a rich diversity of antioxidant compounds, including phenolic compounds, flavonoids, ascorbic acid, carotenoids and enzymes such as catalase and superoxide dismutase. These compounds are vital in scavenging ROS and mitigating oxidative stress, suggesting a sophisticated antioxidant defence system within these plants.

There have been reports of strong antioxidant activity in a number of bryophytes. Few of these bryophytes had highly effective antioxidant enzyme systems, while some others had a variety of phenolic and flavonoid chemicals that can scavenge free radicals. One of the studies of liverwort *Marchantia polymorpha* identified a peroxidase ezyme that was distinct from any peroxidase previously identified in vascular plants (Hirata, 2002). Similar to this, a research of antioxidant enzymes in the liverwort *M. polymorpha* and the moss *Brachythecium velutinum* revealed the importance of the ascorbate peroxidase in the elimination of hydrogen peroxide (Paciolla, 2003). A different study found that *Plagiochasma appendiculatum* extract significantly increased superoxide dismutase and catalse activity while reducing lipid peroxidation (Singh, 2006)**.** The extracts of liverworts like *Plagiochasma appendiculatum, Marchantia polymorpha* and *M. linearis* had also been reported to have antioxidants activities (Singh *et al*., 2006, Krishnan & Murugan, 2013). Similarly, methanolic extract of *Plagiochila beddomei Steph.* showed an enormous number of biological activities (Manoj *et al*., 2012). Two bryophytes *Plagiochasma appendiculatum* and *Pellia endiviifolia* from Reasi district of J&K are also reported to have antioxidant activities (Sharma *et al*., 2015). Superoxide radical scavenging activity has also been reported from the mosses. The ethyl acetate extract of *M. polymorpha* has somewhat stronger antioxidant activity than the ethanolic extract, according to the results of the DPPH and ABTS assays used to screen for antioxidant properties. Other investigations established that *M. polymorpha* contained phenols, tannins, flavonoids, saponins and glycosides. These investigations also showed antagonism between colon cancer cell lines and flavonoids isolated from *M. linearis* cell suspension cultures (Krishnan & Murugan, 2013a; 2013b). Despite being a widespread component of all continents' vegetation, bryophytes are still largely under-utilized in conventional medicine. So, we can say that these desiccation-tolerant plants are an interesting source of several antioxidants that could be employed for new medication discovery since they store a variety of rich biochemical compounds. These antioxidants are abundant in liverworts and mosses, which may one day provide a source for molecules with important medical and cosmetic applications (Aslanba, 2017; Dixit, 2021). Bryophytes' antioxidant activity, like that of other plants, is regulated by a number of variables, including tissue type, altitude and seasons (Thakur & Kapila, 2017).

2.3) Ethnomedicinal values of bryophytes

Bryophytes have been used traditionally by the local tribes of many countries including India, Europe, North America and China for the treatment of tonsillitis, disease of cardiovascular system, bronchitis, skin disease and burns (Xie, 2009; Gahtori & Chaturvedi, 2019). The majority of bryophytes are known for their antimicrobial, anticancerous, cytotoxic, cardiotoxic and antioxidant activities (Dey & Mukherjee, 2015; Negi *et al.*, 2013; Dziwak *et al*., 2022; Dey & Gosh, 2022). The therapeutic properties of bryophytes may be attributed to the presence of antioxidants (Irshad & Chaudari, 2002; Sindhi *et al*., 2013). In India, about 22 bryophytes including one from the Andaman and Nicobar Islands, have been reported to be used in ethnomedicines (Dagar & Dagar, 1999). Liverworts are among the bryophytes and are utilized therapeutically over the world, particularly in Indian and Chinese medical systems for the treatment of hepatitis and various skin problems (Chassagne *et al*., 2019; Saroya, 2011; Atwood, 2024). In the system of alternative medicine, many bryophytes are utilized as medications.

CHAPTER-3

MATERIALS AND METHODS

Plant collection

Liverwort taxa i.e. *Pellia endiviifolia, Asterella multiflora, A. wallichiana, Mannia foreaui, M. indica, Plagiochasma appendiculatum, P. articulatum, Reboulia hemispherica, Athalamia pusilla, Wiesnerella denudata, Marchantia paleacea, M. papillata, M. polymorpha, M. subintegra, Preissia quadrata, Riccia billardieri, R. cruciata, R. crystallina, R. discolor, R. melanospora* and *Targionia hypophylla* included in the dissertation were collected from various areas in districts Jammu, Kathua, Reasi and Udhampur of Jammu region of UT of J & K (Fig.1). For the sake of convenience short form J, K, R and U were assigned to Jammu, Kathua, Reasi and Udhampur respectively. Jammu is situated on the banks of Tawi river, located at 32.73°N 74.87°E and has an average elevation of 327 m (1,073 ft). It has a humid subtropical climate with summer reaching 44ºC and winter falling below 4ºC, receives normal annual rainfall of 1246 mm with 26%-89% humidity. Liverwort taxa were collected from diverse habitats such as epilithic (on rock, brick wall, cemented wall, stone wall), non-epilithic (on humus rich soil and garden soil), sub-aquatic (stream bank); either under shady conditions or exposed to sunlight (Fig.2). These samples were identified on the basis of morphological criteria established by Rawat *et al*., 2015 and photographed using Nikon Eclipse 400 camera and studied for AM association. The plants were carefully removed in thin patches from the substrate, accompanied by the adhering rhizosphere soil. Subsequently, the rhizoids were separated from the attached soil and gently rinsed under tap water. The rhizoids were separated from thallus.

3.1) Arbuscular mycorrhiza

3.1a) Cleaning and staining of rhizoids & scales

To examine the occurrence of arbuscular mycorrhizal (AM) formation and the internal structure of the endophyte within rhizoids, a series of procedures were carried out:

Rhizoids and scales underwent boiling in a 0.01% KOH solution for 2-3 hours. Following this, they were allowed to reach room temperature for at least 1 hour and then thoroughly rinsed 4-5 times to eliminate any residual KOH. The cleaned rhizoids and scales were then stained with a trypan blue solution, composed of phenol, lactic acid, glycerine, distilled

water in the ratio of 1:1:1:1 and a small amount of trypan blue dye. Stained rhizoids and scales were mounted in glycerin and examined under a microscope to identify the presence of arbuscular mycorrhizal (AM) fungal hyphae, vesicles and arbuscules. These observations were recorded using a NIKON ECLIPSE camera.

3.1b) Structural details of Arbuscular mycorrhizal fungi: The AM fungus was studied from the vertical sections (V.S) of thallus. The sections were cut with hands by sandwiching thallus in between two halves of potato blocks. In 0.001% KOH these vertical sections of thallus were placed for few seconds, then stained in trypan blue for two minutes and then mounted in glycerin. Slides were then waxed and photographed.

Sporophytic tissue was also studied using the same protocol.

3.1c) Quantification of Mycorrhiza: The colonization of AM was determined by:

i) the presence of arbuscules, inter/intracellular hyphae and vesicles within rhizoids after cleaning and staining of rhizoids and

ii) colonization of fungus inside the thallus.

The degree of AM colonization or Percent Mycorrhizal Colonization (PMC) was determined by method given by Nicolson (1967) as follows:

$$PMC = \frac{\text{No. of rhizoids colonized}}{\text{Total no. of rhizoids scanned}} \times 100$$

It is broadly classified into five groups as follows (Kormanic, 1982).

Class 1: up to 5% Class 4: 51-75%

Class 2: 6-25% Class 5: 76-100%

Class 3: 26-50%

For pH analysis, soil samples were dissolved in distilled water in the ratio of 2:1 and stirred well. After half an hour, soil solutions were filtered in another beaker and pH was recorded using pen type pH meter of Hanna make.

Table 1: List of sites of collection along with their altitude and coordinates.

Areas	Site of collection	Site. No.	Altitude (m)	Coordinates
Jammu	Akhnoor	J1	301	32.896957,74.735489
	Bantalab	J2	400	32.767942,75.032325
	B. C. Road	J3	312	32.742079,74.859514
	Botanical Garden, University of Jammu	J4	340	32.719376,74.861272
	Dablehar	J5	305	32.539274,74.732035
	Domel	J6	650	32.875188,74.957428
	Gadigarh	J7	300	32.675132,74.852326
	Jhajjarkotli	J8	540	32.883546,74.943542
	Jourian	J9	320	32.836490,74.579444
	Mamka	J10	310	32.575621,74.759774
	Maha-Maya	J11	460-500	32.730612,74.890623
	Miran-sahib	J12	310	32.644421,74.818523
	Nagbani	J13	287	32.756011,74.749121
	Nagrota	J14	340-400	32.798345,74.915432
	Palace road	J15	340	32.766221,74.874727
	Sidhra	J16	400-440	32.758082,74.892160
	Talab-Tillo	J17	336	32.723934,74.837027
Udhampur	Devika	U1	650	32.924449,75.129469
	Katra	U2	830	32.995650,74.943074
	Mansar Lake	U3	666	32.696612,75.144321
	Ramnagar	U4	830	32.804446,75.315356
	Udhampur	U5	680	32.916410,75.143439
Kathua	Basohli	K1	515	32.500599,75.818538
	Mahanpur	K2	460	32.537096,75.641812
	Marta -Nagrota	K3	400	32.562951,75.647652
Reasi	Bhamla	R1	620	33.046438,74.474899
	Kalidhar	R2	720	33.044712,74.476051
	Sunderwani	R3	633	33.045976,74.491973

Table 2: List of taxa undertaken for study.

S.No.	Order	Family	Taxon	Site No.
1	Metzgeriales	Pelliaceae	*Pellia endiviifolia*	J13, J14
2	Marchantiales	Aytoniaceae	*Asterella multiflora*	J1, J5, J10, J12, J13, J14, J15, R1
			A. wallichiana	J11, J12, J13, J14, R1
			Mannia foreaui	J4, U1, U4
			M. indica	J6, J16
			Plagiochasma appendiculatum	J3, J5, J7, J8, J11, J12, J13, J14, U4, U5, R2
			Plagiochasma intermedium	J17, J13, U4,
			Reboulia hemispherica	J12, J13, J14, J5, J8, J15, J16, J17, R3, U1, U2, U5
		Cleveaceae	*Athalamia pusilla*	R3
		Conocephalaceae	*Wiesnerella denudata*	R2
		Marchantiaceae	*Marchantia paleacea*	J4, J8, J9, J11, J12, J13, J14, K1, K2, K3, R1, R2, R3
			M. papillata	J2, J6, J9, J11, J12, J13, J14, K1, K3, R2, R3
			M. polymorpha	R1, J14
			M. subintegra	U1, U5
			Preissia quadrata	R3, R4
		Ricciaceae	*Riccia billardieri*	J4
			R. cruciate	J4
			R.crystallina	J4
			R.discolor	J4
			R.melanospora	J4
		Targioniaceae	*Targionia hypophylla*	R2

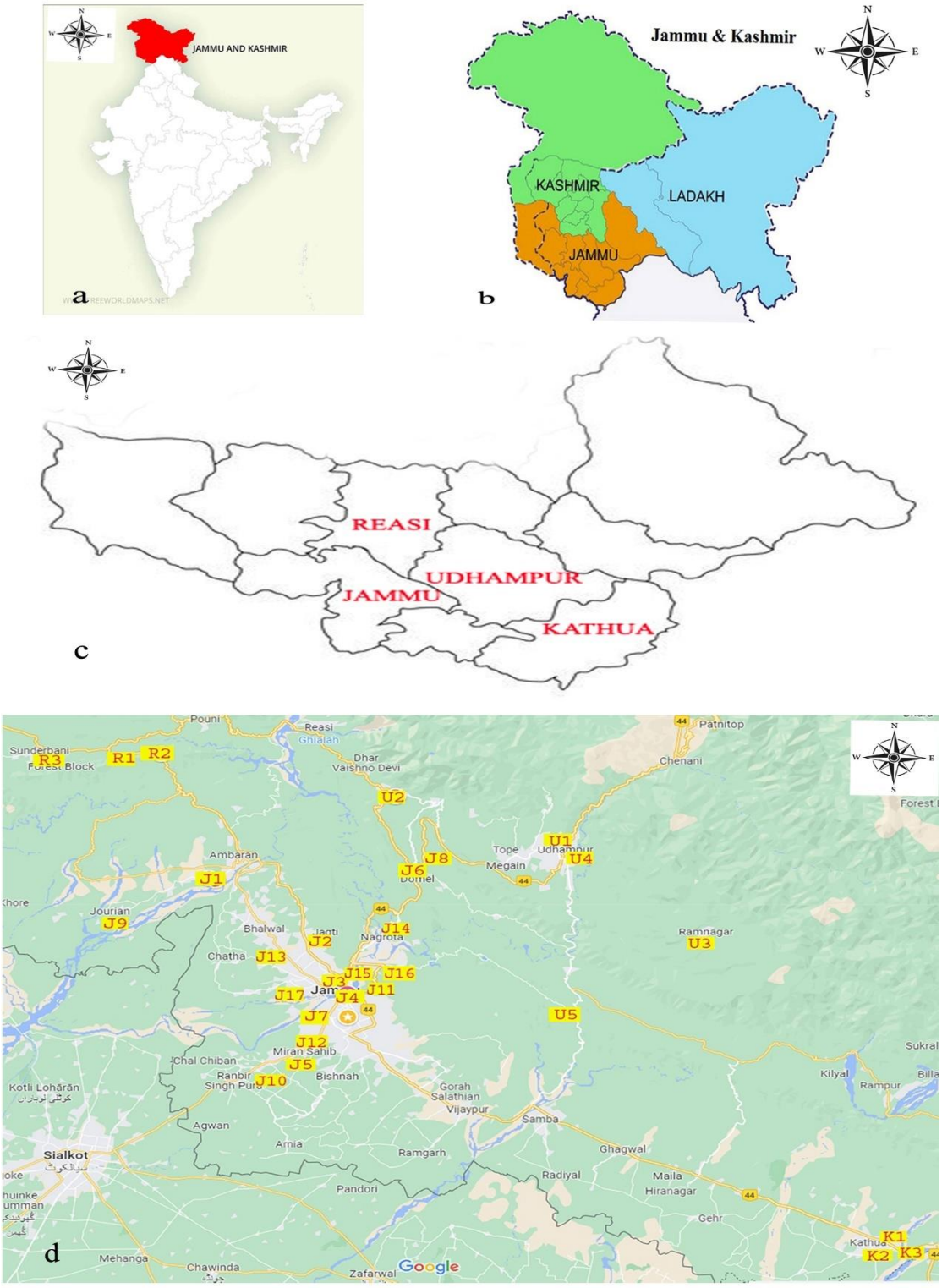


Fig. 1a-1d: Map of India (Fig.1a) showing UT of Jammu & Kashmir (Fig.1b), explored districts (Fig.1c) and various collection sites (Fig.1d) of Jammu & Kashmir.

Fig.2a-2h: Diverse habitats of liverworts epilithic on rock (Fig. 2a), brick wall (Fig. 2b), cemented wall (Fig. 2c), stone wall (Fig. 2d); stream bank (Fig. 2e & f); non-epilithic on humus rich soil (Fig. 2g) and garden soil (Fig. 2h),

Temperature and altitude of collection sites were recorded with the help of thermometer and altimeter.

For determining moisture content, 5 gm of fresh soil samples were weighed and dried in an oven at 80°C for 24 hrs and then weighed again. The soil moisture content is measured by using formula (Sharma, 2017).

$$\% \text{ moisture content (MC)} = \frac{\text{weight of moist soil (M)} - \text{weight of dry soil (D)}}{\text{weight of dry soil (D)}}$$

Identification of AM fungi associated with liverworts

Multiplication of AM fungi

Rhizosphere soil attached to the patch of liverworts was collected. The patch was chopped into small fragments and mixed thoroughly with the associated soil using an axe. Garden soil and sand in the ratio of 1:4 was taken and autoclaved at 15 psi pressure for 1hour for three consecutive days. Then the chopped blend was mixed with coarse sand (autoclaved) in the ratio of 1:1. This mix then was put in small plastic pot (15-cm size). Cultures were grown in a greenhouse for at least four months. Spores were collected from these cultures.

Wet sieving was used to isolate the AMF spores (Oehl *et al*., 2003a). Twenty-five grams of dried rhizospheric soil was put through a series of sieves with mesh sizes of 1000, 500, 125 and 32 µm as part of the technique. Using a dissecting microscope with a magnification of up to 90 times, spores were counted. About 40 to 70 percent of the spores were mounted in a mixture of Melzer's reagent (Brundrett *et al.,* 1994).

The identification manuals of Schenck & Perez, 1990 and INVAM Website were used as a basis for multiplication and identification as much as possible. The liverwort samples along with the spores were submitted to the herbarium of Botany, University of Jammu (Acc. No. 18018 to 18100).

3.2) ANTIOXIDANT ACTIVITIES

3.2a) Preparation of Plant Extract

By utilising a pre-cooled mortar and pestle situated, 0.5 grams of thallus tissue were combined with 3 millilitres of 0.1 M potassium phosphate buffer. Twenty minutes later,

the mixture was centrifuged at 13,000 rpm. The estimation of protein content and antioxidant enzyme activity was performed utilising the supernatant.

Protein concentration was determined using the Lowry method (Lowry *et al*., 1951) using a BSA stock solution (1mg/ml).

Reagent A: mixture of 50 ml of 2% sodium carbonate and 50 ml of 0.1 N NaOH solution (0.4 grams in 100 ml of distilled water).

Reagent B: 10 ml of 1.56% copper sulphate solution combined with 10 ml of 2.37% sodium potassium tartrate solution.

Reagent C is prepared by combining 2 ml of reagent (B) with 100 ml of reagent solution (A) FC (Folin – Ciocalteau). Diluted threefold with distilled water.

Procedure

Bovine serum albumin (BSA) reference standard was created by dissolving 15 mg of BSA in 100 ml of distilled water. Various concentrations of BSA solutions were created through successive dilutions of the original standard solution. 0.1ml of both the sample and standards (0.1, 0.2, 0.3, 0.4 and 0.5) were pipetted into individual test tubes and then diluted to 1ml with pure water. 2 millilitres of alkaline copper sulphate reagent (Reagent C) were added. Thoroughly blended the solutions. The solution was left at room temperature for 10 minutes. Next, 0.2 millilitres of Folin Ciocalteau reagent solution were added to each tube and incubated for 30 minutes. Calibrate the spectrophotometer with a blank sample and measure the absorbance at 660 nm. Create a standard calibration curve by plotting absorbance versus protein concentration. Measure the absorbance of the unknown sample and calculate its concentration by referencing the reference curve.
Protein concentration calculation: Protein concentration was determined by utilising the standard calibration curve in Excel through the formula $Y = mx + c$.

Estimation of carbohydrates: The carbohydrate content was assessed using the method described by Yem (1954).

Anthrone reagent: Combine 200 mg of anthrone reagent with 100 cc of concentrated sulfuric acid.

Glucose solution of standard concentration:

a) Prepare a standard stock solution by weighing 100 mg of Glucose and transferring it into a 100 ml volumetric flask containing distilled water (resulting in a solution of 100 mg of Glucose in 100 ml of Distilled water).

b) In a volumetric flask, begin the process of preparing a working standard by diluting 10 milliliters of the stock standard solution with one hundred milliliters of distilled water.

Procedures

In each of the five individual test tubes, dispense between 0.2 and 1 milliliter of the working standard solution. Fill each test tube with water until the total volume reaches one milliliter. The contents of a test tube should be combined with four milliliters of anthrone reagent, then the test tube should be covered with a lid and placed in a water bath for ten minutes. After the test tube has been allowed to reach room temperature, a photoelectric colorimeter should be used to determine the optical density at 620 nm. In order to make a blank solution, simply combine one milliliter of distilled water with four milliliters of anthrone reagent at the same time.

Plot the glucose concentration (10 to 100mg) on the x-axis and the absorbance at 620 nm on the y-axis to create a calibration curve on graph paper. Calculate the sugar concentration in the sample using the calibration curve.

Carbohydrate quantity calculation

The carbohydrate quantity was determined by comparing it to the standard calibration curve in Excel using the equation: The equation is in the form of $y = mx + b$.

Assessment of antioxidant enzyme activity Extraction of Enzymes

Crushing was performed on 0.5 grams of fresh thalloid tissue using a mortar and pestle that had been pre-chilled, together with 5 milliliters of ice-cold 0.1M potassium phosphate buffer. Over the course of ten minutes, the homogenate was centrifuged at a speed of

13,000 revolutions per minute and the supernatant that was obtained was used to determine the levels of activity of several enzymes.

3.3d) Enzyme Activities

Catalase enzyme activity (CAT) (EC 1.11.1.6)

The approach described by Aebi (1983) was followed in order to determine the total amount of catalase activity.

Prepare a potassium phosphate buffer solution with a concentration of 0.1M and then add hydrogen peroxide (H_2O_2) in accordance with these instructions. The reaction mixture consisted of 0.1 ml of enzyme extract, 0.2 ml of hydrogen peroxide and 2 ml of potassium phosphate buffer with a concentration of 0.1M. The test cuvette was used to measure the change in absorbance at a wavelength of 240 nm.

$$\text{Unit activity (units/min/gm tissue)} = \frac{\text{Change in the absorbance/min x total volume}}{\text{Extinction coefficient x Vol. of sample taken}}$$

Extinction Coefficient = 6.93×10^{-3} ml^{-1} cm^{-1}

$$\text{Specific activity (UA/mg)} = \frac{\text{Unit activity}}{\text{Protein content}}$$

Guaiacol peroxidase activity (GPOX) (EC1.11.1.7)

GPOX activity was determined using the method established by Putter in 1974.

Procedure

The reaction consists of 2.5 ml of 0.1M phosphate buffer, 0.50µl of guaiacol solution, 0.1 ml of enzyme extract and 0.50µl of H_2O_2 solution in a test cuvette. The spectrophotometric measurement was used to determine the rate of production of the oxidised guaiacol product at 436 nm.

$$\text{Unit activity (units/min/g tissue)} = \frac{\text{Change in the absorbance per minute x total volume}}{\text{Extinction coefficient x Volume of sample taken}}$$

The extinction coefficient is 25 Mm-1cm-1.

Specific activity is calculated by dividing the unit activity by the protein content.

Superoxide dismutase activity (SOD)(EC1.15.1.1)

SOD activity was determined following the methodology outlined by Kono (1978).

Procedure

A total of 1.8 milliliters of sodium carbonate buffer, 750 microliters of Nitrobluetetrazole (NBT-96 µM) and 150 microliters of triton-X-100 (0.6%) are included in the reaction mixture by volume. The process of reaction commenced with the introduction of 150 microliters of hydroxylamine hydrochloride, which was then followed by an incubation period of two minutes. After that, one hundred microliters of extract were added. 540 nm was the wavelength at which the NBT reduction was measured after the content was transferred to a cuvette.

$$\text{Unit activity (x)} = \frac{\text{Change in absorbance per minute (Blank)} - \text{Change in absorbance per minute (test)}}{\text{Change in absorbance per minute (blank)}} \times 100$$

Specific activity is calculated by dividing the unit activity by the protein content.

0.1ml of enzyme extract results in X% inhibition. Thus, 50% inhibition is achieved by adding 50 x 70 = y µl of the sample

Ascorbate Peroxidase activity (APOX) (1.11.1.11).

APOX activity was assessed following the method described by Nakano & Asada (1981).

Chemicals :0.1M Potassium phosphate buffer 100µL of Ethylenediaminetetraacetic acid (EDTA), 1.2ml of Ascorbic acid and H_2O_2

Procedure

The reaction mixture was composed of potassium phosphate buffer, ethylenediaminetetraacetic acid (EDTA) 100µL, ascorbic acid 1.2 ml, H_2O_2,100 µl and 0.2 ml enzyme extract, resulting in a final volume of 3 ml. The reduction in absorbance at 290 nm during a period of 1 minute was measured.

APOX activity was quantified as:

$$\text{Unit activity (units/min/g tissue)} = \frac{\text{Change in the absorbance per minute x total volume}}{\text{Extinction coefficient x Volume of sample taken}}$$

The extinction coefficient is 2.8 mM-1.

Specific activity (UA/mg) = Unit activity/ Protein content

Glutathione reductase activity (GR) (1.6.4.2)

Glutathione reductase activity was determined using the method described by Kranner *et al.* (1998).

Chemical substances :100mM Potassium phosphate buffer, 2mM EDTA and 0.2mM NADP Glutathione in its oxidised form, GSSG, at a concentration of 0.5mM.

Process

The reaction mixture comprised 0.1 ml of potassium phosphate buffer, 50µl of EDTA, 50µl of nicotinamide adenine dinucleotide phosphate, 50µl of Glutathione and 50µl of enzyme extract.

$$\text{Unit activity (units/min/g tissue)} = \frac{\text{Change in the absorbance per minute x total volume}}{\text{Extinction coefficient x Volume of sample taken}}$$

The extinction coefficient is 6.2mM-1.

The specific activity (UA/mg) is equal to the unit activity divided by the protein content.

3.3e) Non-enzymatic antioxidants

Ascorbic acid (AsA)

Ascorbic content was evaluated according to Cakmak & Marschner, (1992) method.

Reagents

Methanol, 5% metaphosphoric acid, Phosphate buffer (150 mM, pH-7.0), Ethylenediaminotetraacetate, (5mM) DDT (dithiothreitol), N- ethylamine, Trichloroacetic acid, Orthophosphoric acid, Ethyle alcohol, Ferric chloride, 2, 2 bipyridine.

Procedure

A total of ten millilitres of methanol was used to homogenize about one gram of newly harvested thalloid tissue. A mixture of 0.5 millilitres of the extract and 2.5 millilitres of a solution containing 5% metaphosphoric acid was then subjected to centrifugation at a speed of 10000 revolutions per minute for a period of ten minutes. It was composed of 0.3 millilitres of the supernatant that had been centrifuged, 0.7 millilitres of phosphate buffer with 150 millimolar concentration of EDTA and 0.1 millilitres of 10 millimolar DDT. The excess DDT was removed by adding 0.1 millilitres of N-ethylmaleimide to the mixture. Finally, add 0.5 millilitres of trichloroacetic acid, 0.5 millilitres of 2,2-bipyridine, 0.5 millilitres of ethyl alcohol and 0.2 millilitres of ferric chloride. At a wavelength of 525 nm, the absorbance of the mixture was measured after it had been incubated at 45 degrees Celsius for a period of half an hour. The concentrations of ascorbic acid, which ranged from 0 to 100µg/ml, were employed as a positive control in order to determine the amount of ascorbic acid present in the tissue sample.

Computations

Ascorbic acid concentration was determined by fitting the data to a standard calibration curve in Excel using the equation $Y = mx + b$.

Glutathione (GSH)

The glutathione levels were determined using the Sedlak & Lindsay (1968) technique.

Procedure

0.5 grams of fresh weight thalloid tissue was homogenised in 5 millilitres of tris-buffer (0.2M). The homogenate was centrifuged at 13,000 rpm at 10°C for 10 minutes. A reaction mixture was prepared by combining 0.1 ml of supernatant with distilled water, DTNB (0.001M) and methanol in a test cuvette. The reduction in absorbance was then measured at 412 nm. The GSH concentration was measured using reduced glutathione as a standard within a concentration range of 0-10mg/ml.

Proline concentration The proline concentration was assessed following the method described by Bates *et al.* (1973).

Reagents: Dissolve 1.25g of ninhydrin in 30mL of glacial acetic acid and 20mL of orthophosphoric acid by heating and stirring. 3% solution of sulphosalicyclic acid in water, glacial acetic acid, toluene and proline.

Process 0.5 grams of thalloid tissue should be homogenized in 10 milliliters of an aqueous solution of sulphosalicylic acid at a concentration of three percent to start the procedure. A cleared solution can then be obtained by filtering the combination through Whatman No. 2 filter paper after the mixture has been filtered. It is necessary to put two milliliters of this filtered solution into a tube and then to add two milliliters of glacial acetic acid and two milliliters of acid ninhydrin. It is recommended that the combination that was produced be placed in a bath of boiling water and allowed to undergo heating for a period of one hour. Incorporate 4 ml of toluene into the reaction mixture and stir vigorously for 20-30 seconds. Allow the layers to separate, then heat the toluene layer to room temperature. Determine the intensity of the red colour produced at a wavelength of 520 nm. Measure the proline content in the sample by conducting a series of tests with pure proline standards and establishing a standard curve. Determine the quantity of proline in the test sample by utilising the data acquired from the standard curve.

Glycine-betaine (GB): Glycine betaine content was estimated according to method given by Greive & Grattan, 1983.

Reagents: Hydrochloric acid, potassium iodide, iodine and 1,2-dichloro methane

Procedure: 0.5 grams of thalloid sample was mixed thoroughly in 5 millilitres of ethanol. Filtered extract was combined with 1ml of 2N HCL after filtration. Approximately 0.5 ml of the mixture was placed in a test tube and 0.1 ml of the samples were chilled on an ice bath for 90 minutes. Then, 10 ml of DCM was added and vigorously shaken. An upper layer was eliminated and the bottom organic layer was analysed spectrophotometrically at 365 nm using a UV/VIS specord M-40 spectrophotometer. The betaine concentration was determined by analysing a standard curve with betaine as the reference standard.

Computation

Glycine betaine was quantified against a standard calibration curve in Excel using the equation $Y = mx + b$.

Total Phenol content (TPC)

Phenol content was quantified using the Ragaazzi & Veronese (1973) technique.

Reagent: Sodium carbonates 2.5% and FC (Folin ciocalteau) reagents

Procedure: The homogenization process was carried out in 10 milliliters of distilled water using 0.5 grams of fresh weight thalloid tissue. Let the mixture remain undisturbed for half an hour in the dark after combining 1.5 milliliters of the extract with three milliliters of FC reagents. Add three milliliters of sodium carbonate solution after that. In order to determine the absorbance of a blue solution at a wavelength of 680 nm, a spectrophotometer was utilized.

Estimation of MDA content for oxidative damage assessment. MDA content was determined using the Heath & Packer (1968) technique.

Reagents: Thiobarbituric acid (TBA, 0.5%), Trichloroacetic acid (TCA, 0.1%)

Process: Malondialdehyde (MDA) 0.5 grams of thalloid tissue was homogenised in 5 millilitres of 0.1% trichloroacetic acid (TCA) to homogenise the tissue. Centrifuge the homogenised samples at 13,000 rpm for 10 minutes, then transfer the supernatant to a fresh

tube. 3 millilitres of a solution containing TCA (20%) and TBA (0.5%) was added to the supernatant and thoroughly mixed. The solution is heated to 95°C for 15 min. and then cooled. Thiobarbituric acid (TBA) interacts with malondialdehyde (MDA) to produce a red compound in an acidic buffer. To assess the MDA content, the absorbance of the resultant red compound is measured at 532 nm and 699 nm wavelengths using a spectrophotometer.

Calculations

Lipid peroxidation was assessed by measuring the quantity of MDA produced.

$$\text{MDA (µmol/g F.W.)} = \frac{\text{Absorbance x entire volume (millilitres)}}{\text{Extinction coefficient x volume of sample (millilitres) x weight of the tissue}} \text{ X } 1000$$

Extinction coefficient = 155^{-1} cm ^{1}

Photosynthetic pigments

0.5 grams of thallus were homogenised with 5 millilitres of 95% ethanol before and after experiencing terminal water deficit to create a slurry. The samples underwent centrifugation at 13000 revolutions per minute for 10 minutes. The operation was conducted in dim light to reduce photo-oxidation. The measurements were conducted using a spectrophotometer at wavelengths of 664 nm for Chl a, 648 nm for Chl b and 470 nm for carotenoids, following the equation by Lichtenthaler (1987) for estimation.

Chlorophyll a = 13.36A664-5.19A648

Chlorophyll b = 27.36A648-8.12A664

Total carotenoids = 1000A470-2.13Chla-97.63chlb/209

Table 3: Methods used to perform different experiments.

	Experiment	Method used	Instrument Used
AM associations	Staining and clearing of rhizoids	Verma & Langer, 2014	Nikon Eclipse Japan 500 microscope, Thermofisher Precision Water bath and Fisherscientific Vertical Autoclave
	Degree of colonization	Nicolson,1967	
	Multiplication of AM fungi	Brundrett *et al*., 1994	
	Identification of AM fungi	Schenck & Perez,1990	
Biochemical constituents	Protein	Lowry *et al.,* 1951	Thermofisher UV-Vis Bench Top Spectrophotometer and Reagents (Merk).
	Carbohydrate	Yem &Willis, 1954	
	Catalase (CAT)	Aebi, 1984	
	Guaiacol peroxidase (GPOX)	Putter,1974	
	Superoxide dismutase (SOD)	Kono, 1978	
	Ascorbate peroxidase (APOX)	Nakano & Asada,1981	
	Glutathione Reductase (GR)	Kranner *et al*.,1998	
	Ascorbic acid (AsA content)	Cakmak & Marschners, 1992	
	Glutathione (GSH content)	Sedlak & lindsay, 1968	
	Proline content	Bates *et al.,*1973	
	Glycine- betaine (GB)	Grieve & Gratan,1983	
	Lipid peroxidation-MDA formation	Heath & Packer, 1968	
	Total Phenol Content	Ragaazzi & Veronese, 1973	
	Photosynthetic Pigment	Lichtenthaler,1987	

CHAPTER-4

RESULTS AND DISCUSSION

Twenty-one liverwort species from two orders (Metzgeriales & Marchantiales) and thirteen genera were examined for the AM fungi in rhizoids and thallus during the current investigation. Additionally, observations of the fungal partner's vesicles and arbuscules at different phases of development were also made. Only six of the twenty-one species of liverworts that were studied showed AM associations. The antioxidant potential of these six species were subsequently studied both enzymatically and non-enzymatically. Following is a description of the observations made for each taxon.

Asterella multiflora was examined for arbuscular mycorrhizal associations in 16 populations from different sites in the Jammu area, which included samples from sandy soil, moist soil, stone walls, cement walls and banks of canals (Table 4).

Both tuberculated and smooth-walled rhizoids were colonized by fungi in all studied samples (Figs. 3a and 3b), but the frequency of mycorrhizal colonization was substantially higher in the smooth walled type of rhizoids. Aseptate fungal hyphae were also seen (Fig. 3c). Typically, fungi created one (Fig. 3b) or three (Fig. 3c) parallel hyphal strands that crossed the rhizoids. Rhizoids typically contained Y- (Fig. 3d) and H-connections (Fig. 3e). Many rhizoids had branching hyphae at their distal end (Fig. 3f). Rarely, several rhizoids had darkly stained cylindrical and oval, softly pigmented vesicles (Figs. 3g and 3h). Mycorrhizal colonization was calculated between 59-74%.

The presence of mycelium in the thallus tissue was examined in six populations. The central mid rib region of the storage zone was occupied by fungus in all of the studied samples (Figs. 4a and 4b). The remainder of the storage zone, the photosynthetic tissue and the wings were uninvaded (Fig. 4a). Only a few centre cells in the midrib area of the plant were tightly packed with fungal threads (Fig. 4c), whereas cells surrounding the central cells had hyphae dispersed throughout them (Fig. 4d). In certain cells, arbuscules were also observed (Fig. 4e).

Asterella wallichiana: In Jammu district, sixteen populations of *A. wallichiana* were examined for mycorrhizal connections throughout the year in a variety of habitats,

including the bank of a canal, soil-capped stone and cemented walls in shady or open conditions (Table 5).

Both smooth walled as well as tuberculated rhizoids were found to have fungal hyphae. The majority of the rhizoids (Figures. 5a–5f) included hyphae that were similar to those in *A. multiflora*. Hyphae also created H- connections (Fig 5d) and Y-connections (Fig. 5e) in rhizoids with smooth walls. Vesicles were also seen in rhizoids with smooth walls (Fig. 5f). Mycorrhizal colonization was 54.9-73%. The presence of mycelium in the thallus tissue of five populations of the fungus *A. wallichiana* was also examined. Fungus was only observed in a few cells in the upper midrib region of the plant's thallus (Fig. 6a). Some cells in the invaded area had no hyphae whereas some were completely filled with hyphae, which could be seen moving from one cell to another (Fig. 6b). Arbuscules were occasionally developed in some cells (Fig. 6c).

***Plagiochasma appendiculatum*:** Thalli of 19 populations from diverse locations in Jammu, Udhampur and Reasi Districts were collected month wise throughout the year and analyzed for arbuscular mycorrhizal relationships (Table 6). Mycorrhizal colonization was 52.2-70.4%.

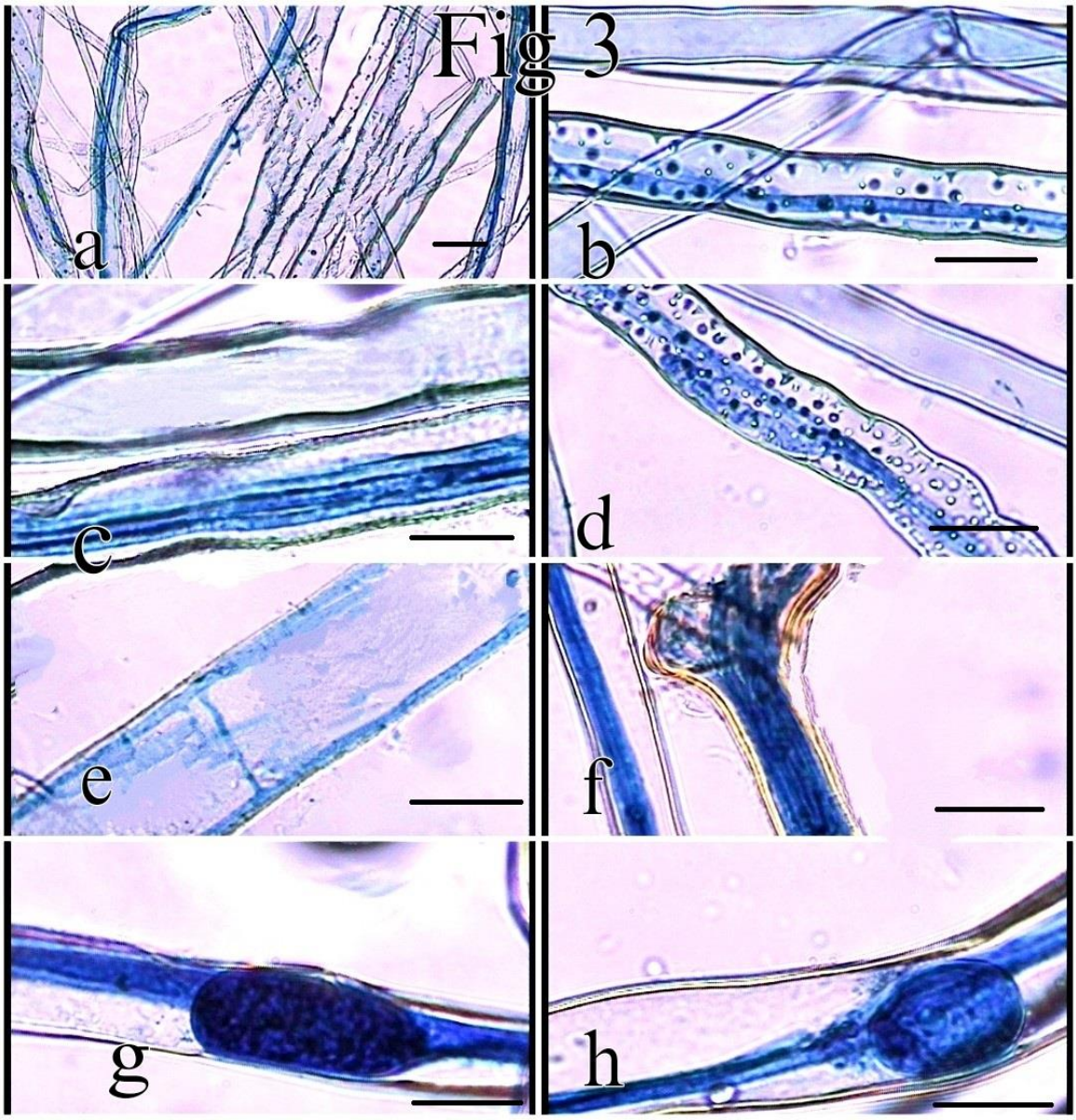
In every sample, mycorrhizal connections were observed. Both tuberculated (Fig. 7b) and smooth walled (Fig. 7a) rhizoids were colonized by the fungi in invaded plants. On a contrast, tuberculated rhizoids were found to be less colonized than smooth walled rhizoids. In general, fungus hyphae were aseptate (Fig. 7a). A single (Fig. 7b), two (Fig. 7c), or more than two (Fig. 7d) hyphal strands were formed as the fungus expanded. The fungus formed showed Y-connections and H-connections (Fig.7e). Oval shaped vesicles were also observed in many rhizoids (Fig.7f).

The few layers of the storage zone were colonized by fungi in all of the samples analyzed (Fig. 8a). Hyphae could be seen moving from one cell to another (Fig.8b). In certain cells, arbuscules were also seen (Fig. 8c). The top storage zone and photosynthetic tissue were clear of invasion (Fig. 8a).

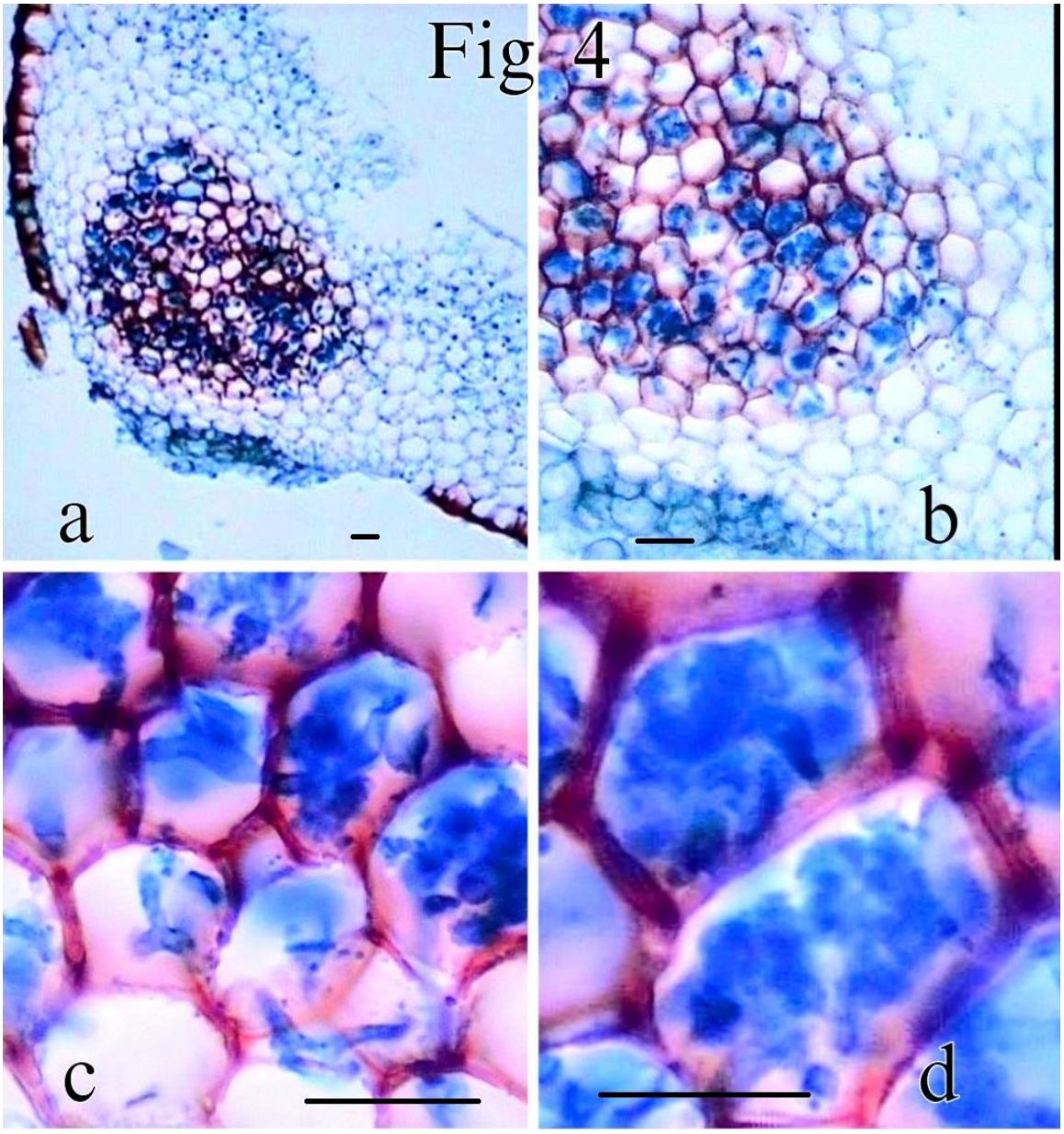
Table 4: Characteristic features of mycorrhizal hyphae associated with *Asterella multiflora* **growing under different habitats**.

S.No.	Site No.	Habitat; Sun exposure	Fungal characterstics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal spores	AM associations	Population 1	Population 2	Population 3	Average PMC
1	J1	Moist rock; shady.	+	+	-	-	+	58.1	68.1	54.6	60.3
2	J5	Soil capped stone; exposed to sunlight.	+	+	-	-	+	60.3	60.6	56.5	59.1
3	J12	Cemented wall near water; exposed to sunlight.	+	-	+	-	+	70.8	60.8	60.7	64.1
4	J12	Cemented wall near water; exposed to sunlight.	+	+	+	+	+	75.2	55.6	64.9	65.2
5	J10	Soil capped stone; exposed to sunlight.	+	+	-	+	+	73.9	64.8	74.5	71.0
6	J13	On brick wall, exposed to sunlight.	+	+	+	-	+	74.2	64.2	61.3	66.5
7	J5	On brick wall, exposed to sunlight.	+	-	-	+	+	75	75.6	59.1	69.9
8	J13	On brick wall, exposed to sunlight.	+	+	+	-	+	74.9	74.9	55.5	68.4
9	J12	On the bank of canal; moist soil; shady.	+	+	-	-	+	74.5	74.6	70.8	73.3
10	J10	Soil capped stone; exposed to sunlight.	+	+	+	-	+	71.7	76	75.2	74.3
11	J15	On rock, exposed to sunlight.	+	-	+	+	+	69.1	69.1	73.9	70.7
12	J5	Soil capped stone; exposed to sunlight.	+	+	+	-	+	60.5	60.5	74.2	65.0
13	U1	Moist rock; shady.	+	-	+	+	+	69.1	69.1	73.9	70.7
14	J10	Soil capped stone; exposed to sunlight.	+	+	-	-	+	60.3	60.6	56.5	59.1
15	R1	On clay soil; shady.	+	+	-	-	+	74.5	74.6	70.8	73.3

Abbreviations used: +: Present -: Absent



Figs. 3a-3h: Rhizoids of *Asterella multiflora*. Figs. 3a &b: Smooth walled (Fig. 3a) and tuberculated rhizoids (Fig. 3b) showing AM (10X & 40X resp.). Fig. 3c: Three hyphal strands running across the rhizoid (40X). Figs. 3d and 3e: Y- (Fig. 3d) and H-connections (Fig. 3e) formed inside rhizoids(40X). Fig. 3f: Fungal hyphae at the distal portion of the rhizoid (40X). Fig. 3g &h: Rhizoids possessing cylindrical (Fig. 3g) and oval (Fig. 3h) vesicles (100X). Bar:20µm

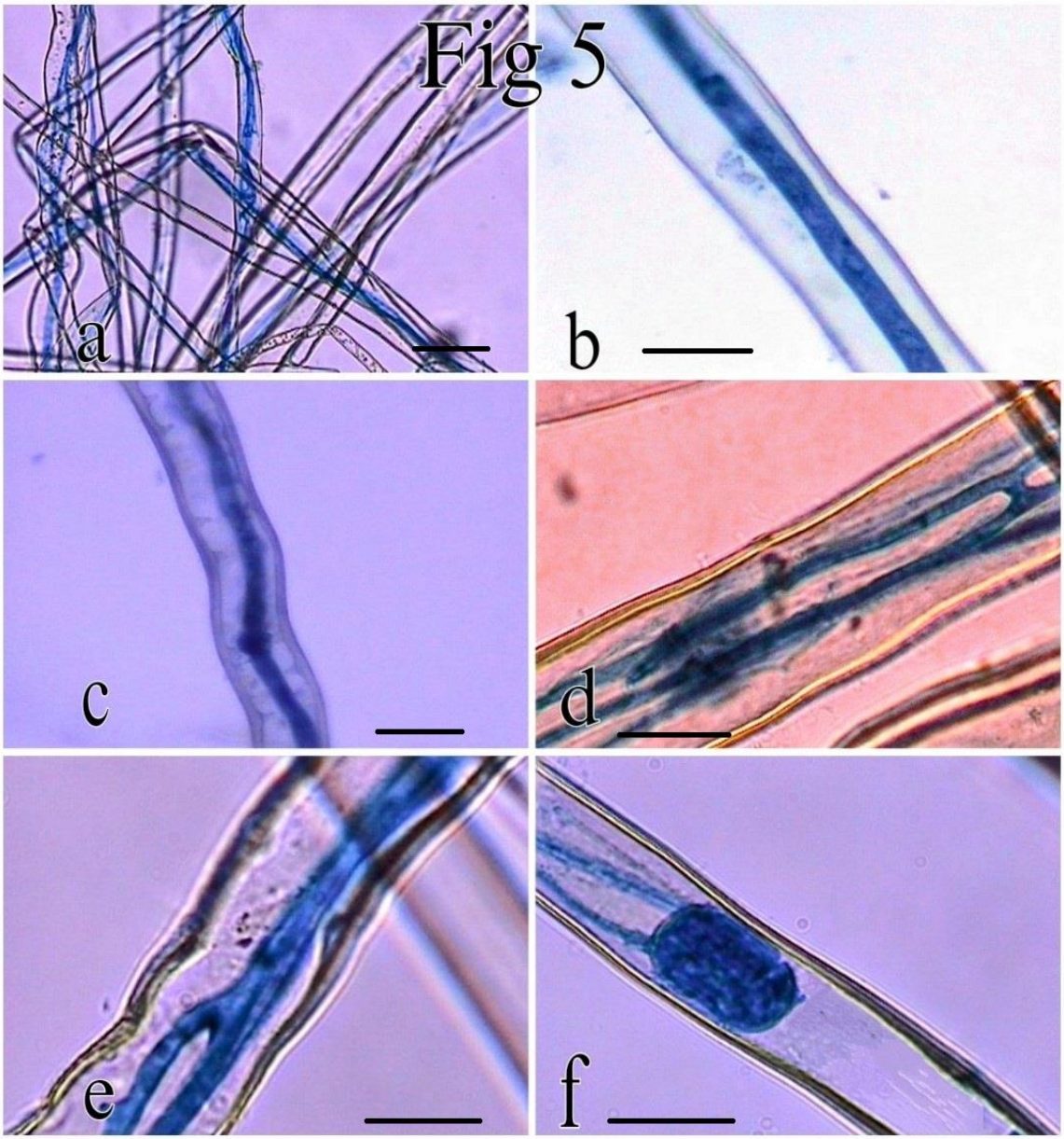


Figs. 4a-4e: V.S. of the thalli of *A. multiflora.* Note fungal invasion in the midrib region in Figs. 4a and 4b (10X & 40X). Figs.4b &4c: Magnified views of midrib region showing central, compactly filled cells and cells having intermingled hyphae (40X). Fig.4d: A magnified view of cells of midrib region showing arbuscules (100X). Bar:20µm

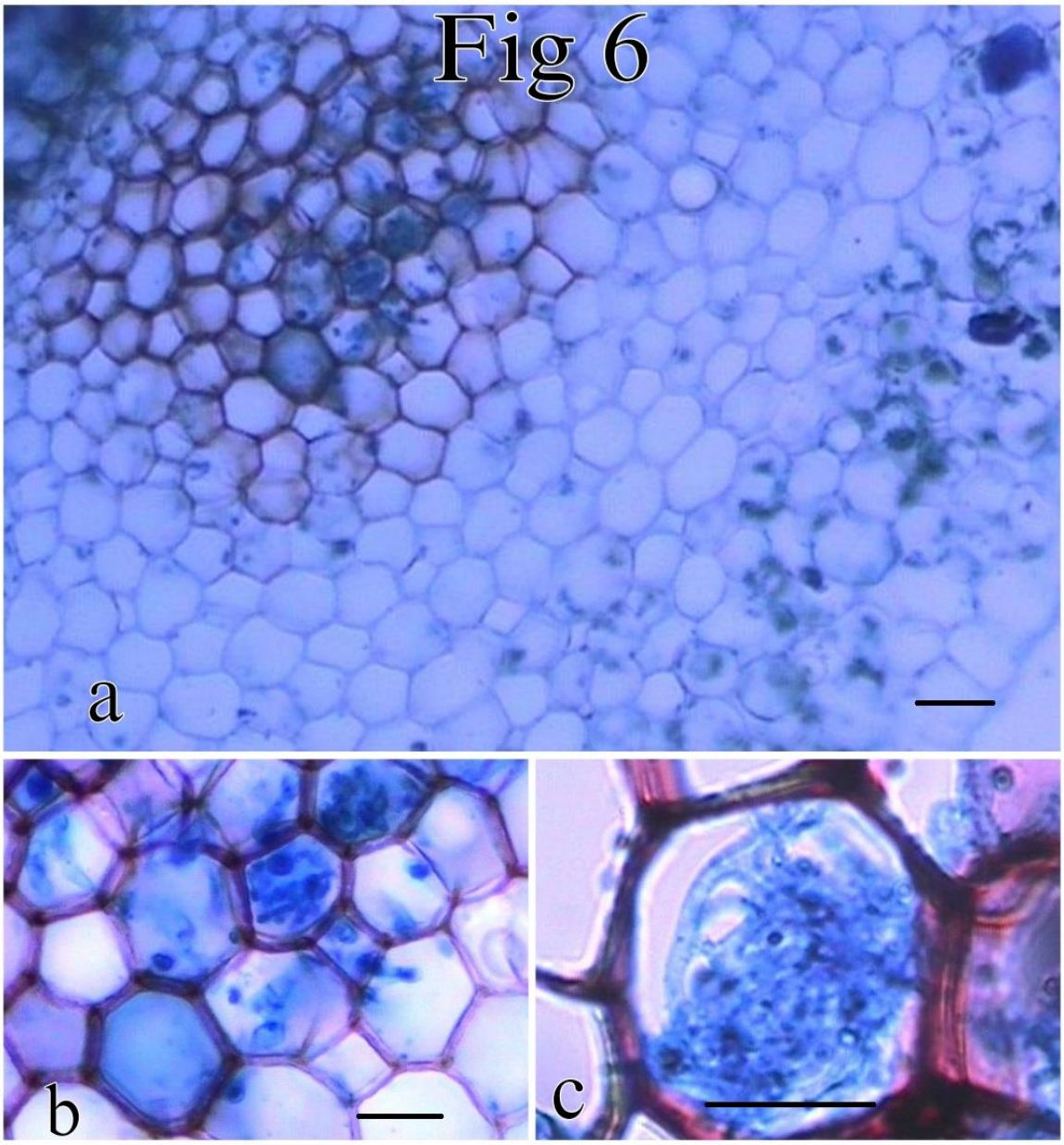
Table 5: Characteristic features of mycorrhizal hyphae associated with *Asterella wallichiana* **growing under different habitats**.

S.No.	Site No.	Habitat; Sun exposure	Fungal characteristics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal spores	AM associations	Population 1	Population 2	Population 3	Average PMC
1.	J12	On the bank of canal; shady	+	+	+	-	+	66.3	76.5	69.3	70.7
2.	J12	On cemented wall; shady	+	-	+	-	+	76.2	66.2	76.2	72.8
3.	J14	On stone wall, exposed to sunlight	+	-	+	-	+	76.5	66.5	76.5	73.1
4.	J12	On the bank of canal; shady	+	+	+	+	+	48.3	53.2	63.2	54.9
5.	J11	On cemented wall; shady	+	+	+	-	+	53.2	63.72	53.2	56.7
6.	J12	On the bank of canal; shady	+	+	-	-	+	48.3	48.2	46.1	47.5
7.	J14	On stone wall, exposed to sunlight	+	+	-	-	+	60.2	73.2	67.2	66.8
8.	J17	On the bank of canal; shady	+	+	+	-	+	72.9	43.9	47.9	54.9
9.	J12	On stone wall, exposed to sunlight	+	+	+	-	+	70.8	72.8	47.8	63.8
10.	J13	On the bank of canal; shady	+	-	+	-	+	67	56.3	76.3	66.5
11.	J12	On the bankof canal; shady	+	+	+	-	+	65.7	68.7	77	70.4
12.	J17	On moist soil; shady	+	-	+	-	+	56.5	48.6	76.5	60.5
13.	J14	On stone wall, exposed to sunlight	+	-	+	-	+	76.2	66.2	76.2	72.8
14.	J14	On sandy soil, exposed to sunlight	+	+	+	-	+	53.2	63.72	53.2	56.7
15.	J11	On cemented wall, exposed to sunlight	+	+	+	-	+	76.2	66.2	76.2	72.8
16.	J12	On rock; shady	+	+	+	-	+	76.5	66.5	76.5	73.1

Abbreviations used: +: Present -: Absent



Figs. 5a-5f: Rhizoids of *Asterella wallichiana stained* with trypan blue. Figs. 5a-5c: Smooth walled (Fig. 5b) and tuberculated rhizoids (Fig. 5c) showing aseptate hyphae (10X & 40X). Note three (Fig. 5d) hyphal strands (40X). Also note the H- connection in Fig. 5d. Fig. 9e: Y- connection formed inside a rhizoid (40X). Fig. 5f: Oval vesicle inside the smooth walled rhizoid (40X). Bar:20µm

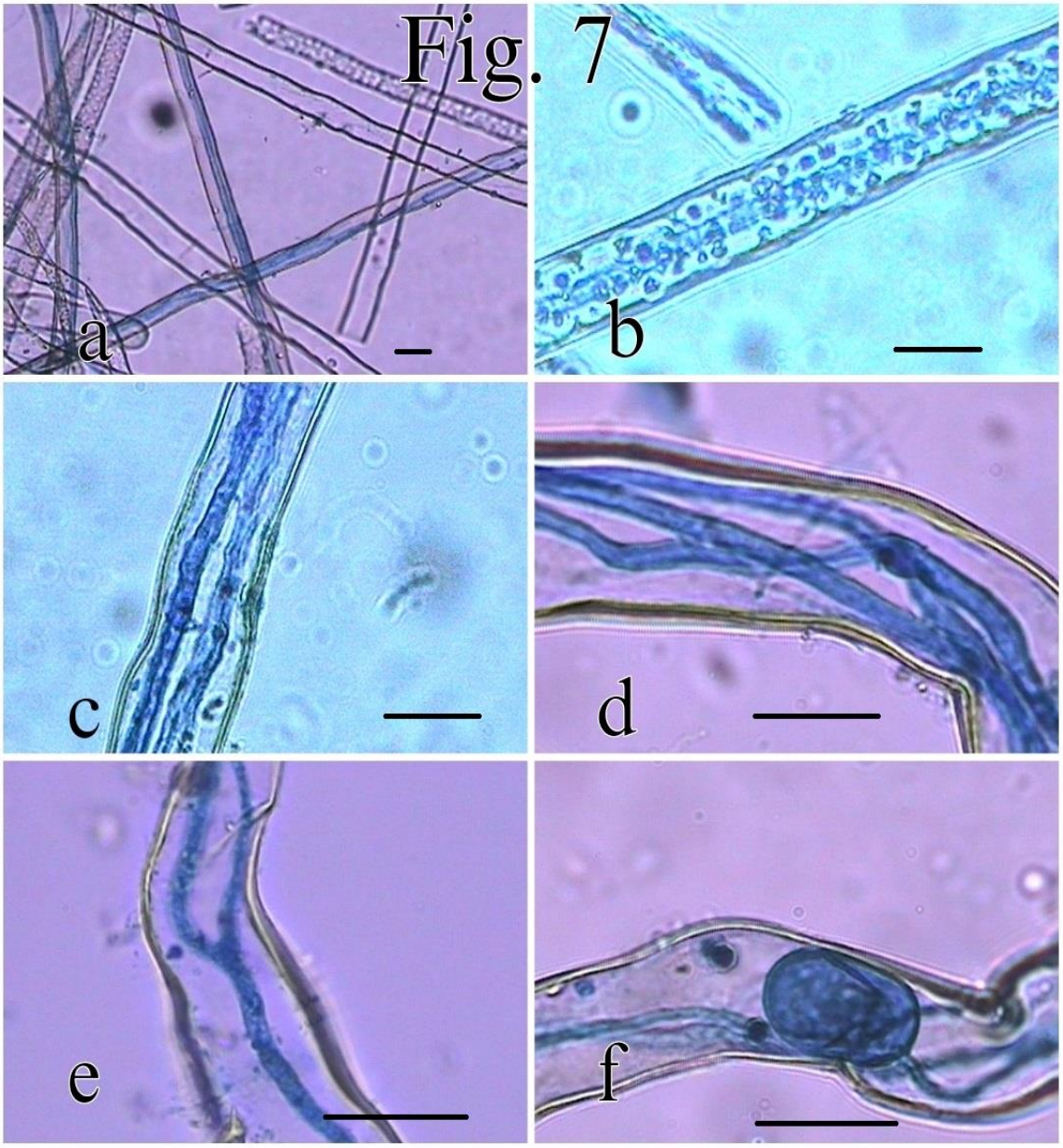


Figs. 6a-6c: V.S. of thalli of *A. wallichiana* showing fungal invasion. Note fungus occupied upper middle storage zone in Fig. 6a (40X). Fig. 6b: Magnified view of cells of the storage zone showing fungal invasion (40X). Fig. 6c: A magnified view of a cell showing an arbuscule (100X). Bar:20µm

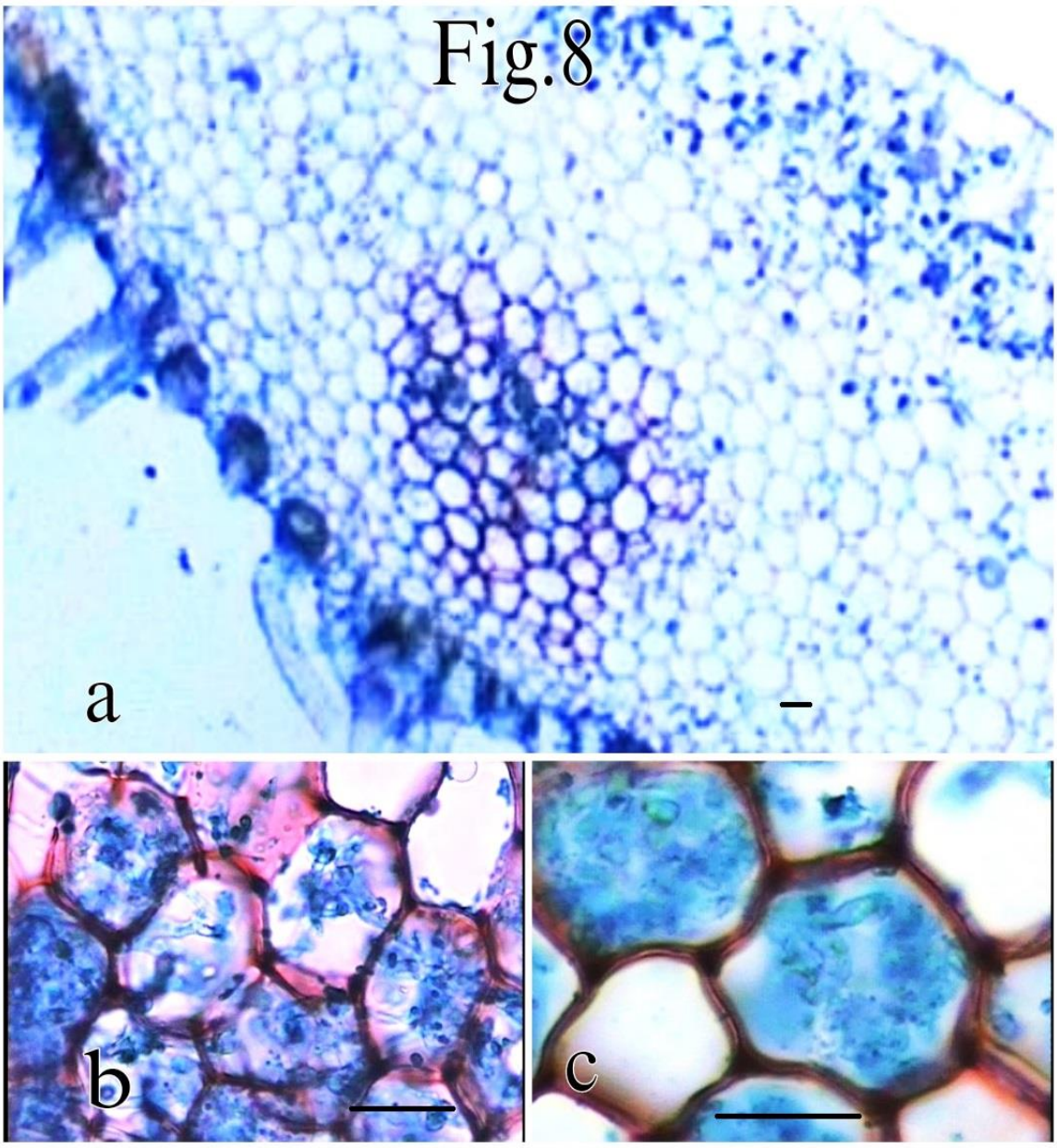
Table 6: Characteristic features of mycorrhizal hyphae associated with *Plagiochasma appendiculatum* growing under different habitats

S.No.	Site No.	Habitat; Sun exposure	Fungal characteristics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal	AM associations	Population 1	Population 2	Population 3	Average PMC
1	J14	On the stone exposed to sunlight.	+	+	-	+	+	58.1	66.2	64.9	63.0
2	J5	On brick wall, exposed to sunlight.	+	+	-	-	+	60.3	66.5	43.5	56.7
3	J7	On the soil surface; shady.	+	+	+	-	+	70.8	59.1	76.5	68.8
4	J5	On cemented wall, exposed to sunlight.	+	+	-	-	+	75	68.7	53.2	65.6
5	J12	On the bank of canal; shady.	+	+	-	-	+	74.9	74.6	43.7	64.4
6	J11	On the bank of canal, exposed to sunlight.	+	+	+	-	+	74.2	66.1	48.2	62.8
7	J8	On cemented wall; shady.	+	+	-	-	+	75	63.1	73.2	70.4
8	J14	On the rock; shady.	+	+	-	-	+	64.9	47.8	43.9	52.2
9	J5	On brick wall, exposed to sunlight.	+	+	-	-	+	44.5	34.5	80.8	53.2
10	J8	On cemented wall; shady.	+	+	+	-	+	71	65.2	65.1	67.1
11	J14	On the stone exposed to sunlight.	+	+	+	-	+	69.1	65.3	67.7	67.7
12	J6	On the brick wall; shady.	+	-	+	-	+	60.5	62.5	74.6	65.8
13	J5	On cemented wall, exposed to sunlight.	+	-	+	-	+	74.2	66.1	48.2	62.8
14	J12	On the bank stream; shady.	+	-	+	-	+	74.9	74.6	43.7	64.4
15	J11	On the bank stream; shady.	+	+	+	+	+	74.9	74.6	43.7	64.4
16	J8	On cemented wall; shady.	+	+	+	-	+	71	65.2	65.1	67.1
17	U4	On the stone, exposed to sunlight.	+	+	+	-	+	69.1	65.3	67.7	67.7
18	J3	On cemented wall; shady.	+	+	+	-	+	71	65.2	65.1	68.1
19	J8	On stone, near stream; shady.	+	+	+	-	+	69.1	65.3	67.7	67.3

Abbreviations used: +: Present -: Absent



Figs. 7a-7f: Rhizoids of *Plagiochasma appendiculatum* showing AM fungal associations. Figs. 7a and 7b: Smooth walled (Fig. 7a) and tuberculated rhizoid (Fig. 7b) showing fungal hyphae (10X & 40X resp.). Figs. 7b-7d: Rhizoids showing one (Fig. 7b), two (Fig. 7c) and three (Fig. 7d) hyphal strands running inside rhizoids. Figs. 7e: Y- connection. Fig.7f: Oval vesicle inside rhizoid. Bar:20µm



Figs. 8a-8e: V.S. of the thalli of *P. appendiculatum* showing fungal invasion.Fig.8a: Note invasion in the storage zone (10X). Fig. 8b: Fungus invading through cell to cell in the storage zone of the the thallus (40X). Fig. 8c: Magnified view of cells having arbuscules(100X). Bar:20µm

***Reboulia hemisphaerica**:* Arbuscular mycorrhizal associations were examined in 18 samples of *R. hemispherica* that were gathered from diverse locations in the Jammu, Kathua, Reasi and Udhampur districts. These samples were taken while they were growing in a variety of habitats, including near a canal's bank, on rocks, stones, brick walls and in forest litter that was either shaded or exposed to direct sunlight (Table 7).

Both smooth walled (Fig. 9a-d) and tuberculated (Fig. 9e) rhizoids had fungal colonization. In the majority of cases, smooth walled rhizoids were far more likely to experience mycorrhizal colonization than tuberculated rhizoids. Additionally, several rhizoids have more than one hyphal thread (Fig. 9d). In other instances, Y-connections and H-connections were also seen (Fig. 9e and 9f). Mycorrhizal colonization was observed between 63.9-75.5%.

The midrib region of every thallus under investigation has been colonized by fungus. The storage zone and remaining portions of the photosynthetic tissue were free of invasion. Rhizoids allowed fungus to enter the thallus (Fig. 10a), where it colonized the midrib region of the thallus (Fig. 10b). Arbuscules were also observed in the cells of storage zone (Fig.10c).

Marchantia paleacea: Arbuscular mycorrhizal associations were checked in 16 samples of *Marchantia paleacea* thalli, which had been collected on a monthly basis throughout the year from various locations in the Jammu, Kathua and Udhampur districts. These samples were collected as they were growing in a number of habitats, including the bank of a canal, moist soil, sandy soil, stone wall, brick wall, cemented wall and rock, either in the shade or open to the sun (Table 8). Mycorrhizal colonization was observed between 69.7-79.4%.

All of the examined samples were found to contain mycorrhizal associations. An aseptate fungus (Fig. 11a) was frequently discovered in association with both smooth-walled (Fig. 11a) and tuberculated (Fig. 11c) rhizoids in all invaded samples. However, compared to tuberculated rhizoids, smooth walled rhizoids had a far higher incidence of mycorrhizal colonization. The rhizoids often had one, two, or three hyphal strands running through them. The growth patterns of the fungus included coils (Fig. 11d) could also be detected.

At the terminal location of the hyphae, darkly pigmented vesicles were produced (Fig. 11e). These vesicles varied in size and shape, being spherical (Fig. 11e) to cylindrical (Fig. 11f).

Fungal endophyte in thallus was also looked for in all the populations where fungus invades rhizoids. Every plant that was looked at, V. S. examining the storage zone's middle and upper part of the thallus revealed AM fungus in the cells (Fig. 12a). The upper 1-2 layers of the storage zone and the photosynthetic tissue were unaffected by the invasion. Even in the storage zone's lower 5–12 layers, no signs of invasion were discovered (Fig. 12a). In several storage zone cells, hyphae and arbuscules were entirely filled (Fig. 12c). A growing arbuscule had a primary hypha that resembled a trunk and generated fine branches.

***Marchantia papillata*:** 18 populations of this species were studied. Arbuscular mycorrhizal invasions in *M. papillata* obtained from several sites in the Jammu and Udhampur Districts were investigated. These samples were taken when they were growing in a variety of environments, including a bank canal, a cemented wall, a rock, in the shade, or in the sun (Table 9). Additionally, a few samples from the Kathua district were also examined.

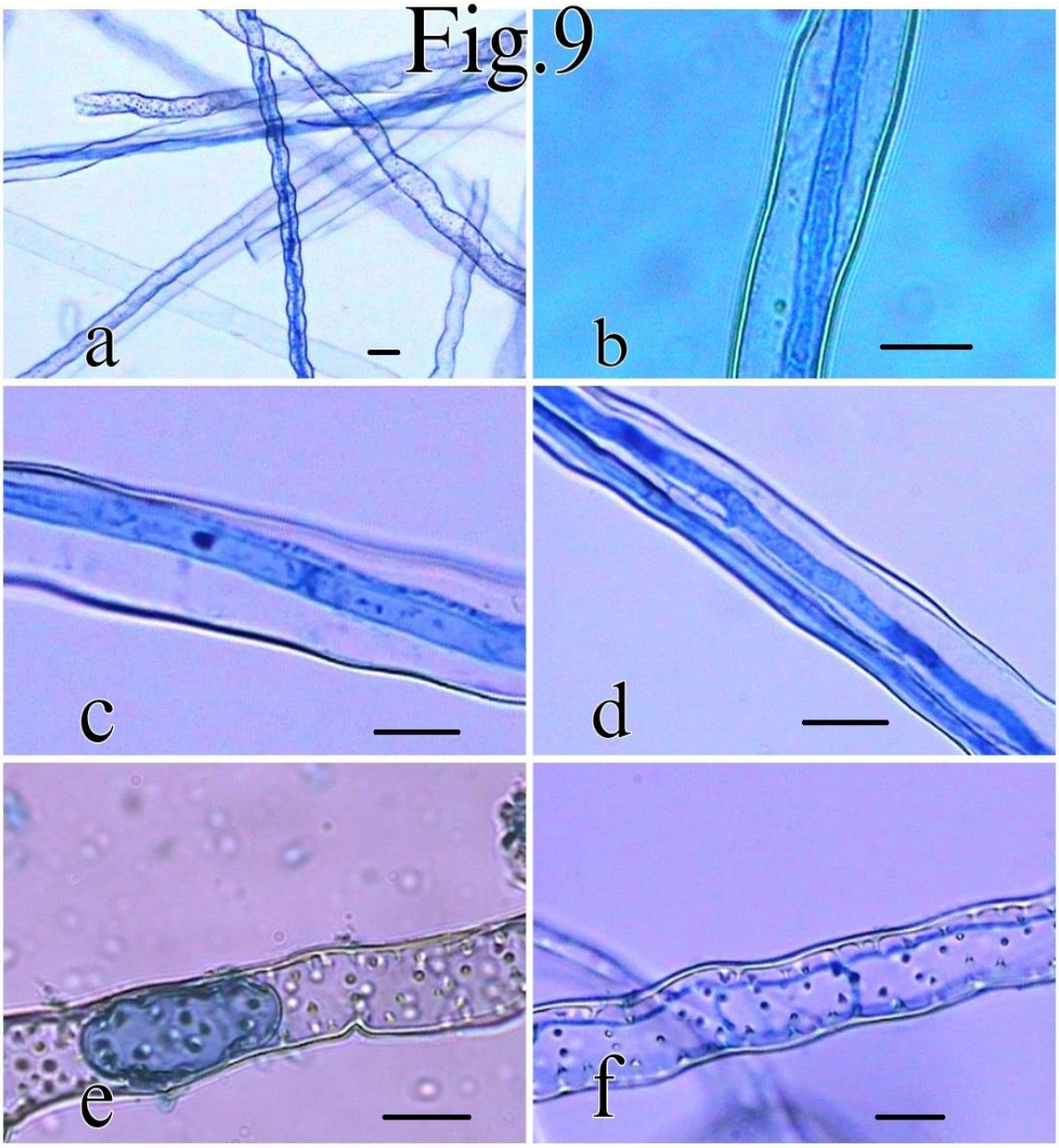
Both tuberculated (Fig. 13b) and smooth-walled (Fig. 13a) rhizoids were colonized by fungus in samples that had been invaded. However, compared to tuberculated rhizoids, smooth walled rhizoids had a higher frequency of mycorrhizal colonization. The mycelium was aseptate. By developing one (Fig. 13b), two, or, in exceptional cases, more than two (Fig. 13c) hyphal strands, fungi developed inside rhizoids. Hyphal strands created Y- and H- connections (Figs. 13d and 13e). Oval shaped vesicles were also observed in several rhizoids (Fig.13f). Mycorrhizal colonization was observed between 68.6-79.4%.

All the samples of *M. papillata* were examined to see if mycelium was present in the thallus. Every plant under investigation, with one exception, had fungus attached to cells in a zone between the midrib and the photosynthetic area. Like the photosynthetic tissue, the thallus' wings were not invaded (Fig. 14a). The majority of the invaded area's cells were entirely covered in fungus hyphae (Fig. 14a & b). Arbuscules filled certain cells (Figs. 14c).

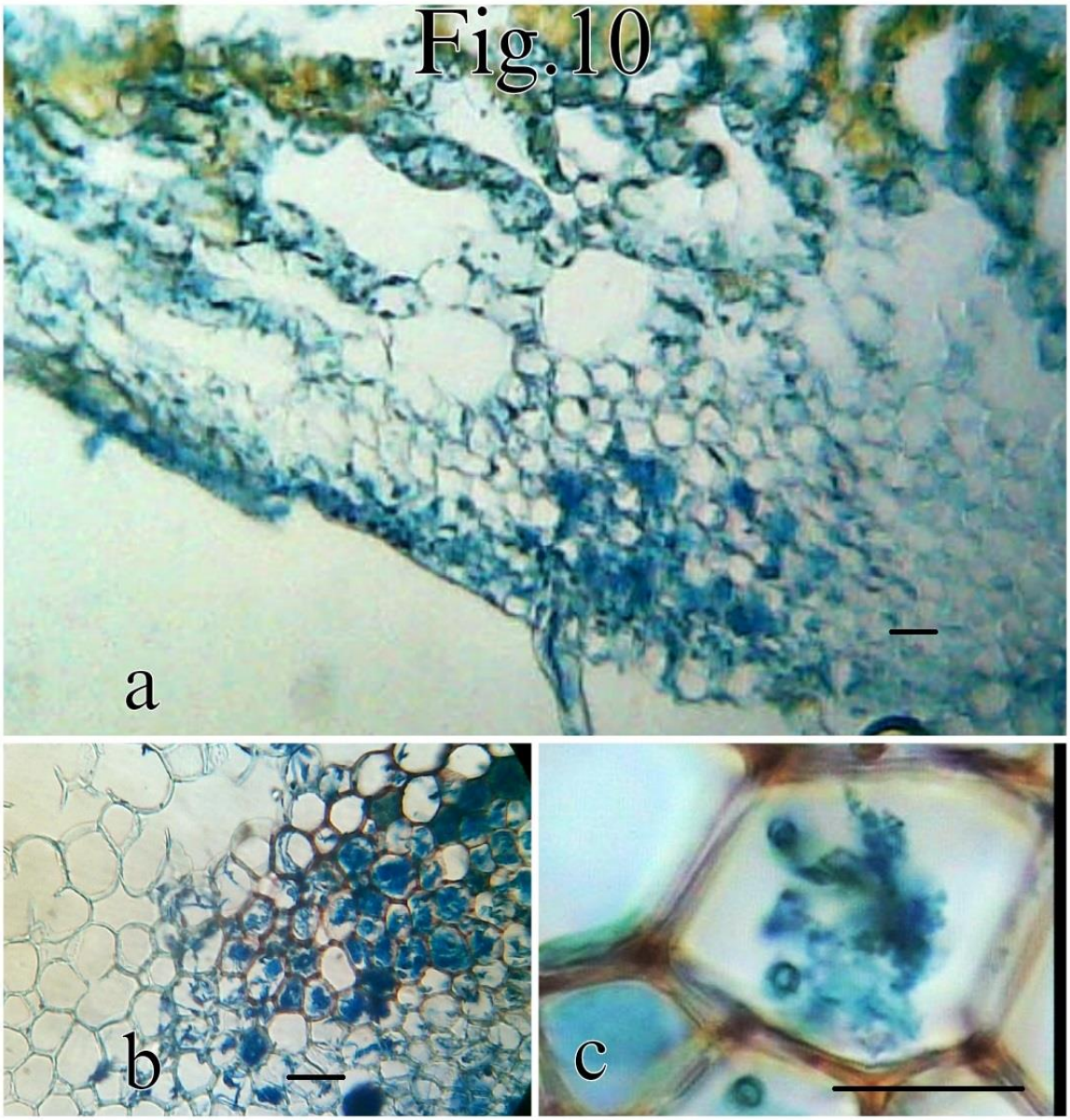
Table 7: Characteristic features of mycorrhizal hyphae associated with *Reboulia hemispherica* **growing under different habitats**.

S.No.	Site No.	Habitat; Sun exposure	Fungal characteristics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal spores	AM associations	Population 1	Population 2	Population 3	Average PMC
1	J14	On the bank of the canal; shady.	+	+	+	-	+	75.2	67.1	55.2	65.8
2	J17	On rock, exposed to sunlight.	+	-	+	-	+	46.3	59.6	55.1	53.6
3	J14	On stone; shady.	+	+	+	-	+	76.9	42.8	59.9	59.8
4	J13	On brick wall, exposed to sunlight.	+	+	+	-	+	77.9	55.6	65.2	66.2
5	J8	On cemented wall; shady.	+	+	+	-	+	76	65.5	75.5	72.3
6	J16	Under the forest litter; shady.	+	+	+	-	+	66.2	64.2	61.3	63.9
7	J15	Near the water body; shady.	+	+	+	-	+	69.6	85.6	59.1	71.4
8	J12	Near the bank of the canal; shady.	+	+	+	-	+	76	74.9	55.5	68.8
9	J12	On the bank of canal; shady.	+	+	+	-	+	75.8	74.6	70.8	73.7
10	J15	Near the water body; shady.	+	-	+	-	+	75.3	76	75.2	75.5
11	J16	Under the forest litter; shady.	+	+	+	-	+	65.2	69.1	73.9	69.4
12	U3	On cemented wall; shady.	+	+	+	+	+	75.9	60.5	74.2	70.2
13	J13	On brick wall, exposed to sunlight.	+	+	+	-	+	77.9	55.6	65.2	66.2
14	J14	On rock; shady.	+	+	-	-	+	75.3	76	75.2	75.5
15	J12	Near the bank of the canal; shady.	+	+	+	-	+	69.6	85.6	59.1	71.4
16	J16	Under the forest litter; shady.	+	+	+	-	+	76	74.9	55.5	68.8
17	U2	Near the water body; shady.	+	-	+	-	+	75.8	74.6	70.8	73.7
18	U3	On cemented wall; shady.	+	+	+	-	+	75.3	76	75.2	75.5

Abbreviations used: +: Present -: Absent



Figs.9a-9f: Rhizoids of *Reboulia hemispherica* stained with trypan blue showing arbuscular mycorrhiza. Figs. 9a -d: Smooth walled rhizoids showing fungal hypha (10X & 40X resp.). Fig. 9e: A vesicle inside tuberculated rhizoid. Fig. 9f: Note H-connection in tuberculated rhizoid (40X). Bar:20µm

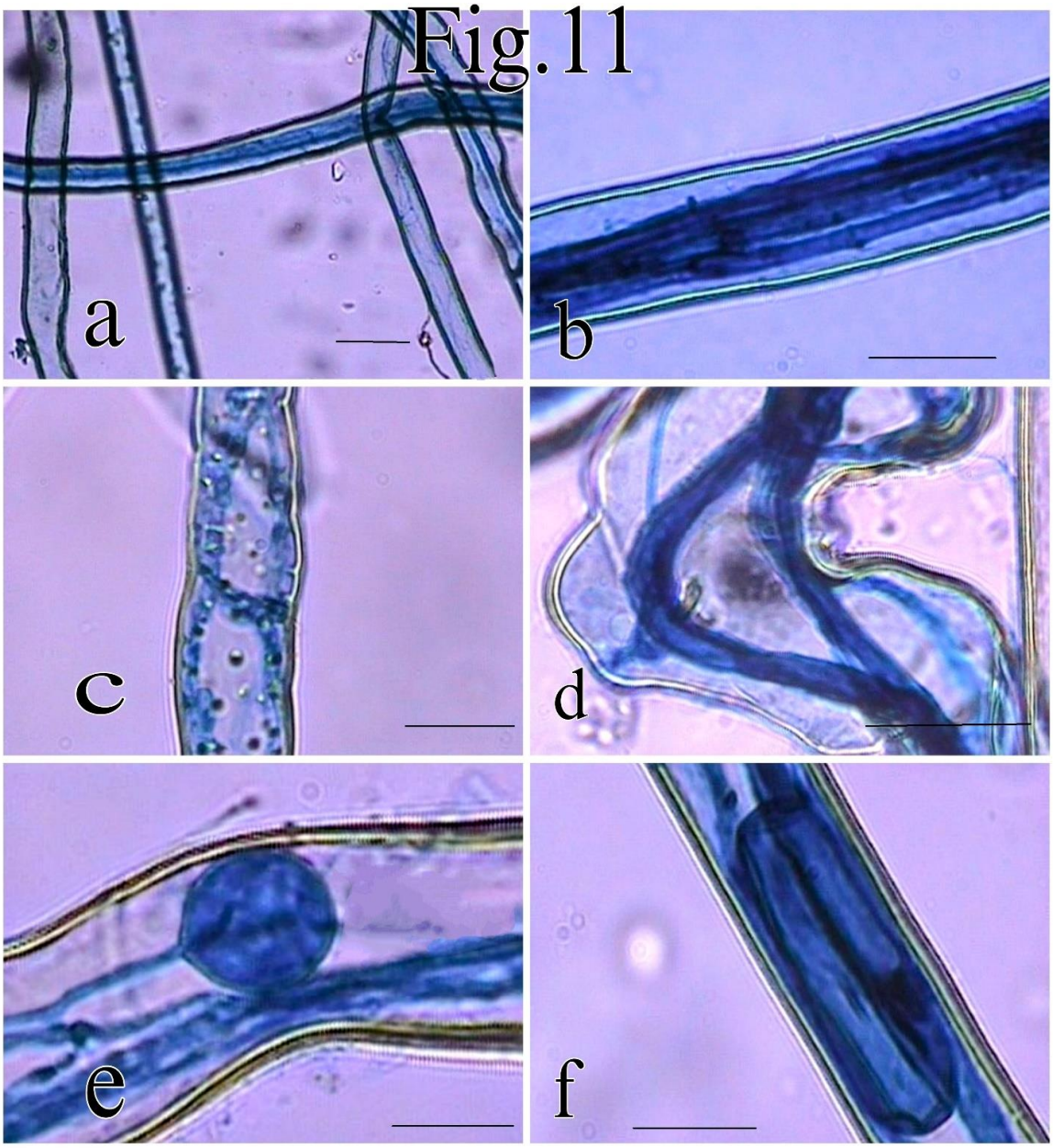


Figs.10a: Thallus of *Reboulia hemispherica* showing mycorrhizal invasion in midrib region of the plant (10X). Note fungus entering the thallus through rhizoids.Fig.10b: Fungus occupied midrib region of the thallus (40X). Fig.10d: Magnified view of a cell of midrib region of the thallus showing arbuscule (100X). Bar:20µm

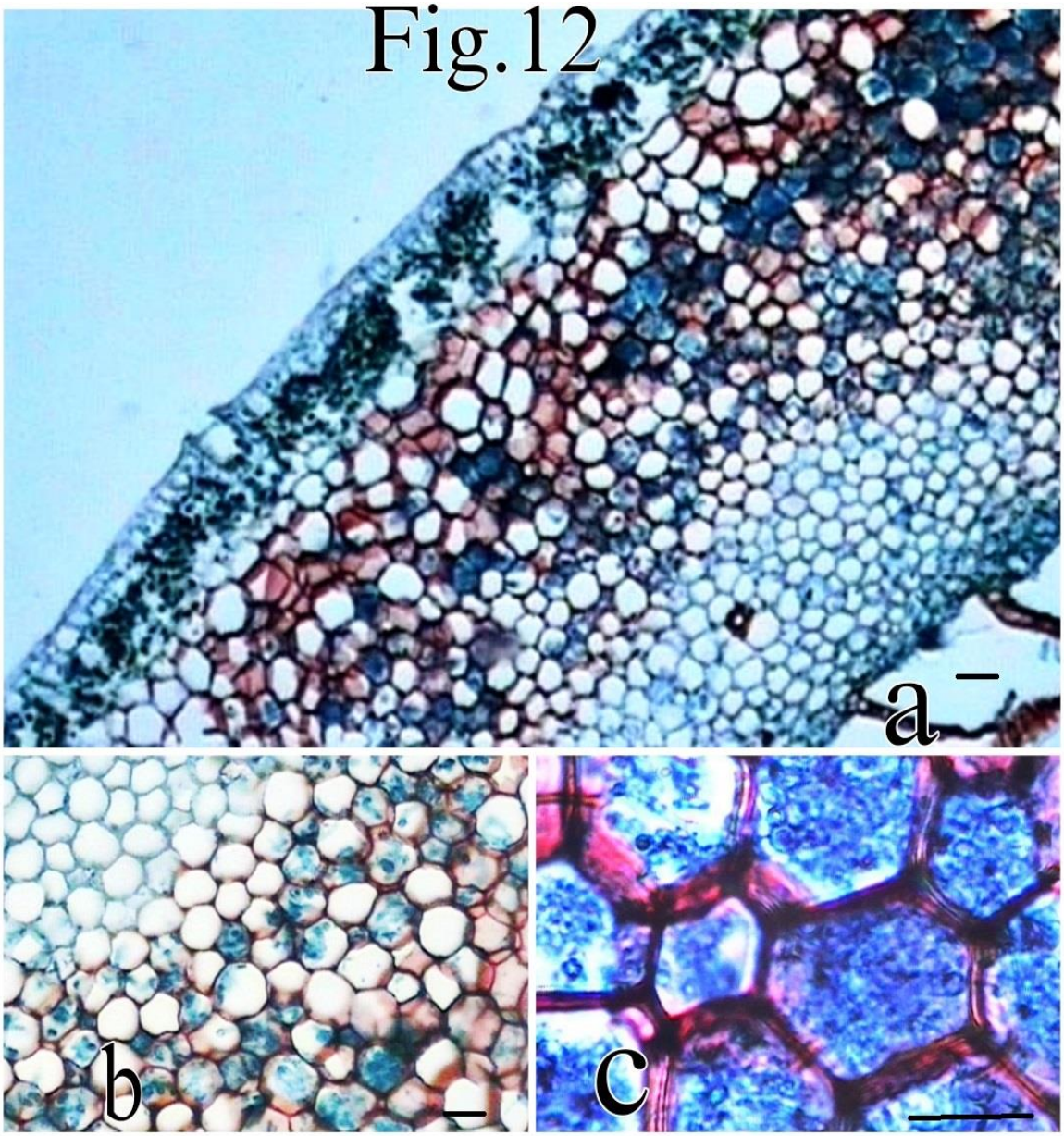
Table 8: Characteristic features of mycorrhizal hyphae associated with *Marchantia paleacea* **growing under different habitats**.

S.No.	Site No.	Habitat; Sun exposure	Fungal characteristics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal	AM associations	Population 1	Population 2	Population 3	Average PMC
1	J12	On the bank of canal; shady.	+	+	+	-	+	66.2	76	85.2	75.8
2	J12	On cemented wall, exposed to sunlight.	+	+	+	+	+	76.3	82.3	56.3	71.6
3	J4	On the bank of the canal; shady.	+	+	+	+	+	78.5	83.5	66.9	76.3
4	J9	On cemented wall, exposed to sunlight.	+	+	+	-	+	65.6	75.6	67.9	69.7
5	J12	On brick wall; shady.	+	+	+	-	+	82.1	79.1	66.8	76.0
6	J11	On cemented wall; shady.	+	+	+	+	+	85.1	60.1	76.2	73.8
7	J13	Moist sandy soil; shady.	+	+	+	-	+	74.3	84.3	79.6	79.4
8	J9	On cemented wall, exposed to sunlight.	+	+	+	+	+	64.9	84.9	86	78.6
9	K1	On rock, exposed to sunlight.	+	+	+	-	+	79.8	69.8	75.8	75.1
10	J14	On the bank of canal; shady.	+	+	+	+	+	78.4	75.4	84.3	79.4
11	K2	Moist sandy soil; shady.	+	+	+	-	+	78.6	68.6	79.2	75.5
12	K3	On the bank of the canal; shady.	+	+	+	-	+	76.2	72.2	85.9	78.1
13	J9	On the cemented wall; shady.	+	+	+	-	+	78.4	75.4	84.3	79.4
14	R1	On brick wall, exposed to sunlight.	+	+	-	-	+	66.2	76	85.2	75.8
15	R2	On the bank of canal; shady.	+	+	-	-	+	76.3	82.3	56.3	71.6
16	R3	On rock; shady.	+	+	-	-	+	78.5	83.5	66.9	76.3
17	J8	Cultivated field; shady.	+	+	-	-	+	65.6	75.6	67.9	69.7

Abbreviations used: +: Present -: Absent



Figs. 11a-11c: Rhizoids of *M. paleacea* showing growth pattern (10X & 40 X resp.). Figs. 11d: Hyphae forming coil, Note the loop inside the swollen area of a smooth walled rhizoid. Figs. 11e & 11f: Smooth walled rhizoids showing vesicles of different shapes, Note spherical (Fig. 11e) and cylindrical (Fig. 11f) vesicles (100X). Bar:20µm

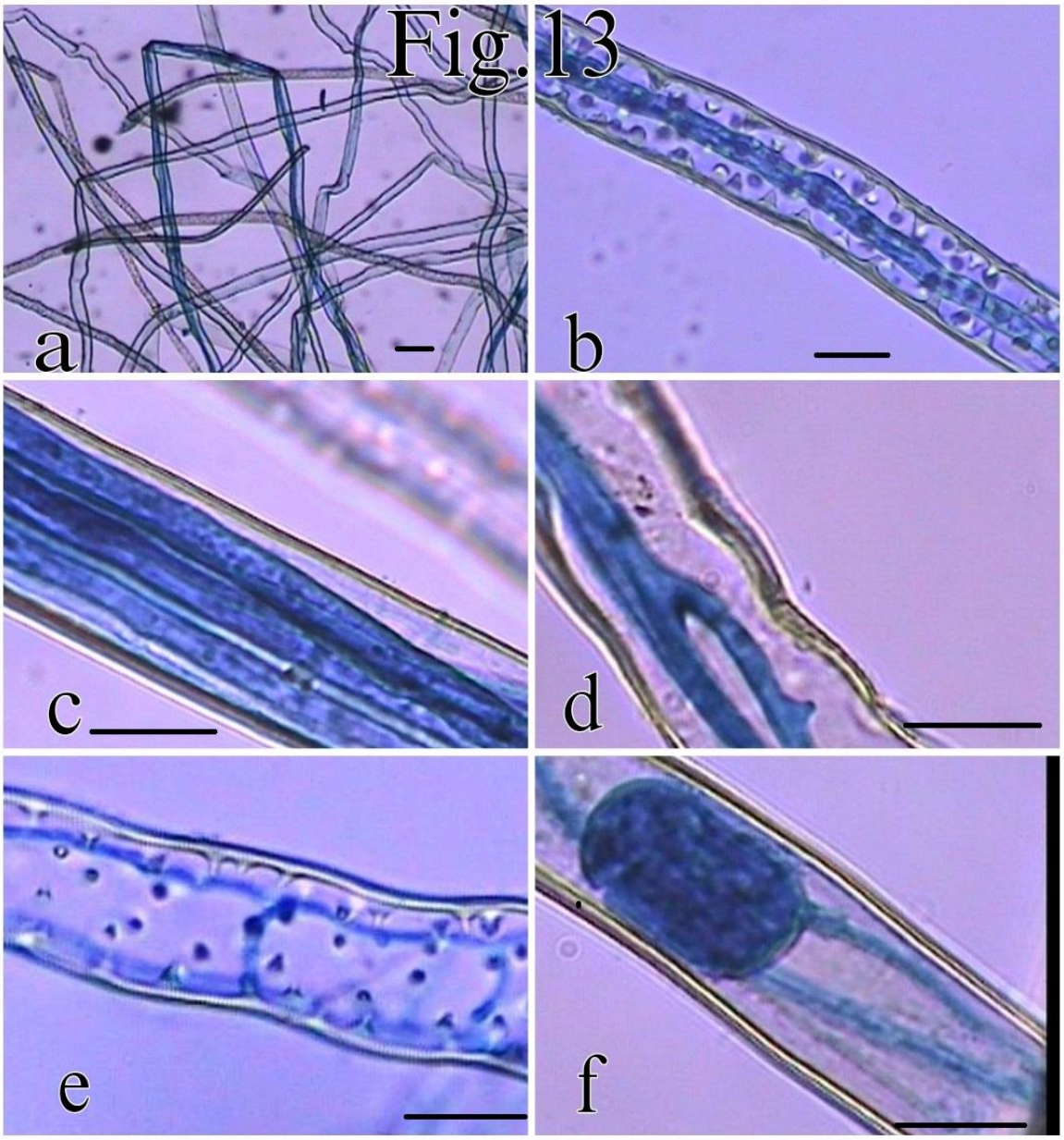


Figs. 12a -12c: V.S. of the thalli of *M. paleacea* showing fungal invasion (10X). Fig. 12b: Cells of the storage zone showing fungal invasion (40X). Fig. 12c: Magnified view of storage zone showing cells of the storage zone filled with arbuscules (60X). Bar:20µm

Table 9: Characteristic features of mycorrhizal hyphae associated with *M. papillata* growing under different habitats.

S.No.	Site No.	Habitat; Sun exposure	Fungal characteristics					Percentage mycorrhizal colonization			
			Hyphae	Arbuscules	Vesicles	Intrarhizoidal spores	AM associations	Population 1	Population 2	Population 3	Average PMC
1	J12	On the bank of. canal; shady.	+	+	+	-	+	79.2	76.2	75.2	76.9
2	J11	On cemented wall, on moist soil; shady.	+	+	+	-	+	62.3	76.3	67.3	68.6
3	R3	On rock, exposed to sunlight	+	-	+	-	+	83.5	78.5	66.9	76.3
4	J12	On the bank of canal, exposed to sunlight.	+	+	-	-	+	72.6	72.5	69.2	71.4
5	J7	On rock; shady.	+	+	-	+	+	79.1	82.1	66.8	76.0
6	J2	On the bank of canal, exposed to sunlight.	+	+	-	-	+	60.1	85.1	76.2	73.8
7	J11	On cemented wall, exposed to sunlight.	+	+	-	-	+	68.3	74.3	79.6	74.1
8	J4	On cemented wall; moist soil; shady.	+	+	-	+	+	84.9	64.9	56	68.6
9	J14	On the bank of water stream; shady.	+	+	+	-	+	69.8	79.8	75.8	75.1
10	J17	On rock; shady.	+	+	-	-	+	75.4	78.4	84.3	79.4
11	J6	On soil surface; shady.	+	+	-	-	+	74.6	73.6	79.2	75.8
12	R2	On rocks, exposed to sunlight.	+	+	+	-	+	72.2	76.2	85.9	78.1
13	J11	On cemented wall; shady.	+	+	-	-	+	60.1	85.1	76.2	73.8
14	J2	On the bank of canal; moist; shady.	+	+	-	-	+	68.3	74.3	79.6	74.1
15	K1	On the bank of water stream; shady.	+	+	-	-	+	84.9	64.9	56	68.6
16	J11	On soil surface; shady.	+	+	-	-	+	84.9	64.9	56	68.6
	J8	On the bank of water stream; shady.	+	+	+	-	+	69.8	79.8	75.8	75.1
	K3	On the bank of river; shady.	+	+	-	-	+	75.4	78.4	84.3	79.4

Abbreviations used: +: Present -: Absent



Figs. 13a-13f: Rhizoids of *Marchantia papillata* stained with trypan blue. Figs. 13a and 13b: Smooth walled (Fig. 13a) and tuberculated rhizoids (Fig. 13b) showing aseptate hyphae (10X & 40X Resp.). Note three (Fig. 13c) hyphal strands (100X). Fig. 13d & e: Y & H- connection in rhizoid. Fig. 13f: oval vesicle inside rhizoid (100X). Bar:20µm

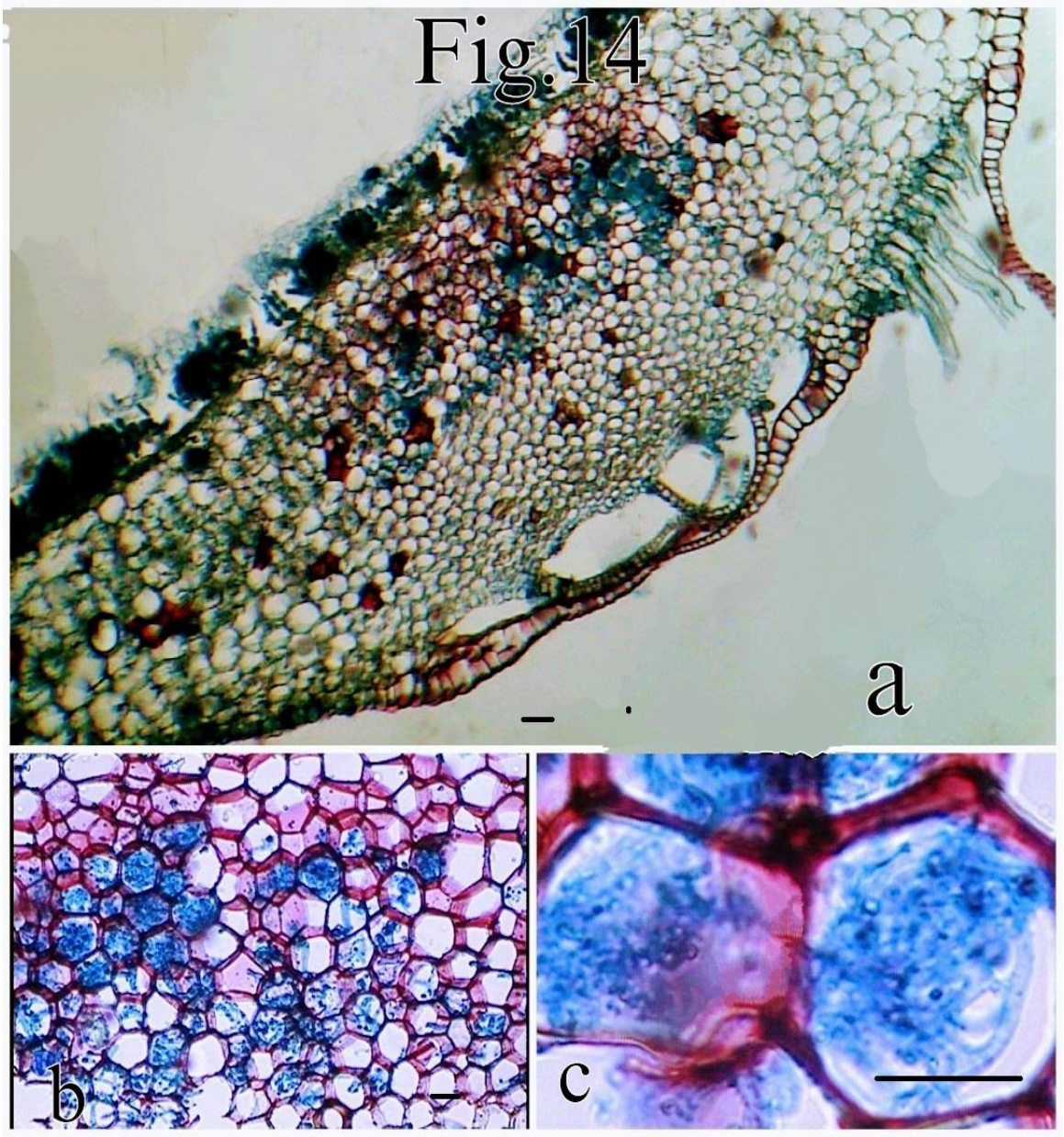


Fig. 14a: V.S. of the thalli of *M. papillata* showing fungal invasion (10X). Fig. 14b: Cells of the storage zone showing fungal invasion (40X). Fig. 14c: Magnified view of cells of the storage zone filled with arbuscules (100X). Bar:20µm

Identity of endosymbionts

All six species of liverworts' trap cultures (Fig.15a) produced spores that had similar traits.

Sporocarp: They were without peridium and have dark orange sporocarps measuring 480–1200 × 510–1600 micrometers in diameter, (Fig.15b). The interior of sporocarps was composed of interwoven hyaline thick hyphae (Fig.15c). The amorphous gel-like substance that stained orange red contained spores and hyphae. At the terminals of either the dichotomously branched primary hyphae or the perpendicular branches, spores were generated ballistically (Fig.15d).

Shape and Size of spores: Spores are typically ovoid, 36-65 x 30-52 micrometers in size, but can also be globose and 27-60 micrometers in diameter. The spores consist of two layers of spore wall (Fig.15e).

Melzer's reagent test: Layer1 took orange red colour whereas Layer 2 was light orange to orange.

Subtending hypha: The subtending hypha was 6–10 micrometers wide; light orange to orange, straight or curved and cylindrical or somewhat funnel-shaped. There are two layers of spore walls. At the base of the spores, Layer 2 of subtending hypha wall was up to three to five micrometers thick and up to 10-30 micrometers below. It was light orange to orange in colour (Fig.15e).

Pore: A pore that was typically 1-1.5 micrometers wide at the spore base and up to 30 micrometers below it was typically closed by a curved septum that continued with the innermost lamina of the laminate spore wall layer 2. Up to 5 micrometers of the septum were introduced into the lumen of the subtending hypha (Fig.15f).

In light of the facts mentioned above the fungus is identified as *Glomus aureum*.

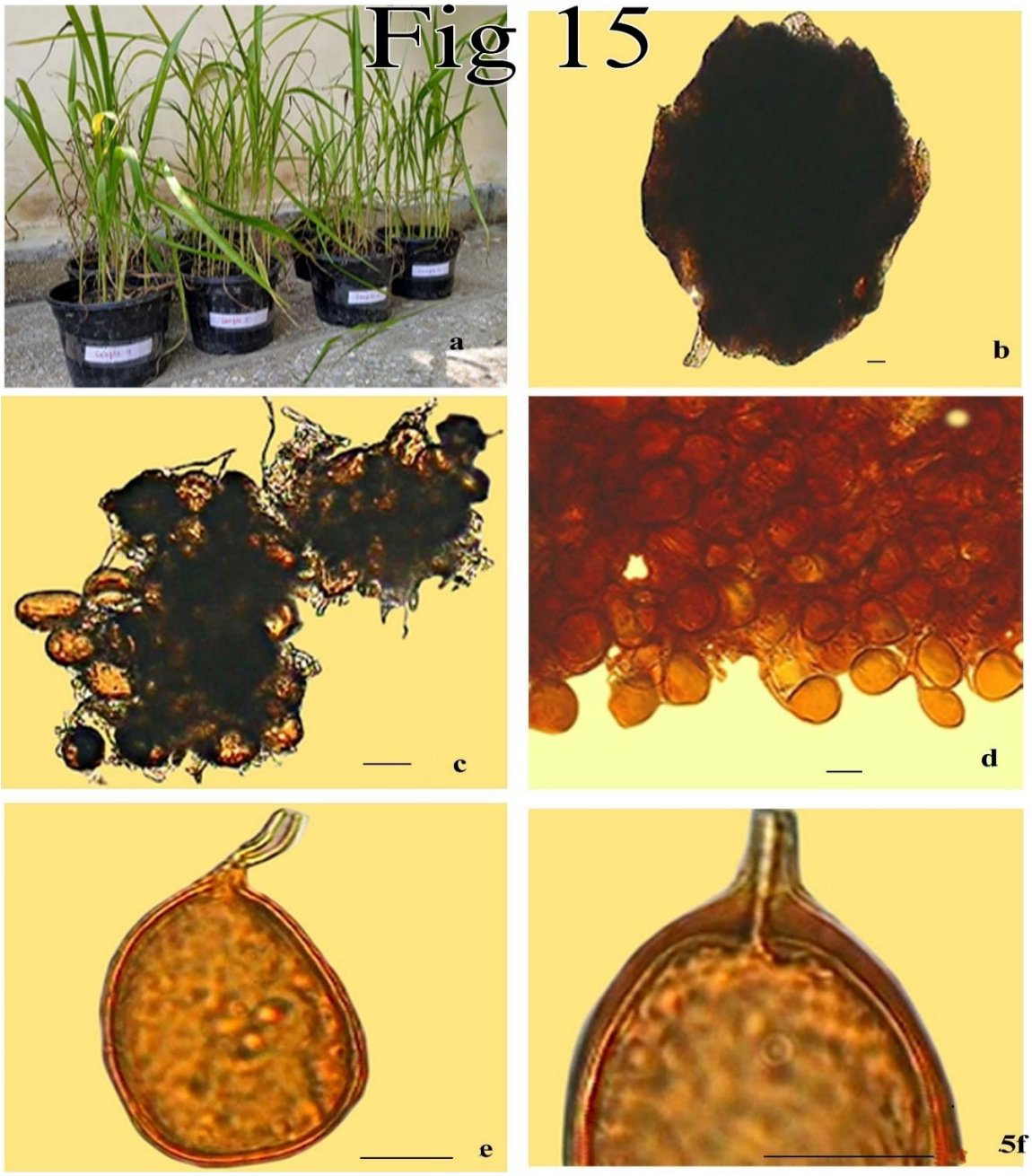


Fig.15a: Trap culture of liverworts. Fig.15b: Isolated Sporocarp of *Glomus aureum* (10X) Fig.15c: interwoven hyaline hyphae within sporocarp (10X). Fig. 15d: showing amorphous gel-like substance between spores stained red in Melzer's reagent (10X). Fig. 15e & d: Isolated spore showing wall layers, pore septum and subtending hypha of *Glomus aureum* (40X & 100X resp.). Bar: 20µm

Mycorrhizal Colonization: There were differences in the percent mycorrhizal colonization (PMC) between and within species. *Asterella multiflora (71.28), Asterella wallichiana* (69.11), *Plagiochasma appendiculatum* (77.15), *Reboulia hemispherica* (77.35), *Marchantia paleacea* (81.81) *and M. papillata* (82.11). These measurements indicate the extent of mycorrhizal colonization in these species. Let's discuss the significance of these findings:

***Asterella multiflora, Asterella wallichiana* and *Plagiochasma appendiculatum* (Class 4):** These species fall into class 4, indicating that they have moderate mycorrhizal colonization, ranging upto 75%. This suggests that mycorrhizal associations play a very important role in their ecological strategies, but they are not heavily dependent on it. They may have other mechanisms or strategies for nutrient acquisition as well (Hayman,1970).

***Reboulia hemispherica, Marchantia paleacea* and *M. papillata* (Class 5):** These species belong to class 5, with mycorrhizal colonization levels exceeding 75%. This suggests a strong dependency on mycorrhizal associations for nutrient uptake and potentially other ecological benefits (Hayman, 1986). Species with high mycorrhizal colonization are often considered obligate mycorrhizal, which means they rely heavily on these symbiotic relationships for nutrient acquisition and overall fitness (Khan, 2000). High levels of mycorrhizal colonization can be advantageous in nutrient-poor environments, as it increases the ability of plants to access essential nutrients, such as phosphorus and nitrogen, from the soil (Bucking *et al*., 2012).

Colonization in tuberculated as well as smooth walled rhizoids: During present study the mycelium was observed in association with both tuberculated and smooth walled rhizoids in all of the marchantialean taxa tested. However, smooth walled plants experienced mycorrhizal colonization more frequently than tuberculated ones. Rhizoids are believed to have a specialised function; tuberculated rhizoids create a capillary conducting system running alongside the thallus, facilitating the distribution of water to all absorptive regions of the gametophyte (McConaha, 1941) and assisting in the transportation of solutes (Kachroo, 1954). Rhizoids with smooth walls extend forward, enter the soil and absorb water and minerals. As we know that mycorrhizal connections aid

in the absorption of mineral salts and water (Kumar & Purohit, 1997–1998), the higher frequency of fungal colonization in smooth walled rhizoids makes sense.

Factors influencing the growth of AM

There are many studies in higher plants which suggest a relationship between environmental variables and mycorrhiza (O'keffe & Sylvia, 1993; Bohrer *et al.*, 2001; Klironomos & Kendrick, 1995 and Lutgen *et al*., 2003), however, information on lower plants is scarced. In this study we tried to investigate the influence of various abiotic factor on the Percent Mycorrhizal colonization of different liverworts species. Numerous abiotic variables, including habitat, temperature, soil water content and pH, have an impact on the development of arbuscular mycorrhizae.

Habitat: The degree of colonization of arbuscular mycorrhizal (AM) fungi varies among populations of six different taxa (*Asterella multiflora, A. wallichiana, Plagiochasma appendiculatum, Reboulia hemispherica*, *Marchantia paleacea* and *M. papillata)* depending on the type of habitat they are growing in (clay soil, stone wall, sandy soil and cemented wall). Here, we can discuss the observations made in Graph 1, which indicate that maximum AM colonization higher than 80 % occurred in populations growing in clay soil, while minimum colonization, less than 65 % was observed in populations growing on stone walls. PMC was also observed higher in the habitats that were exposed to sunlight as compared to the habitats which remain in shade for most of the time in a year.

Maximum AM Colonization: The observation that populations in clay soil exhibit the highest degree of AM colonization (more than 80%) could be attributed to several factors. Clay soil typically has a higher water-holding capacity and nutrient content compared to other habitats mentioned. This can create favorable conditions for AM fungi to establish symbiotic relationships with the host plants. Additionally, the physical structure of clay soil may provide more space for fungal hyphae to spread and interact with plant roots.

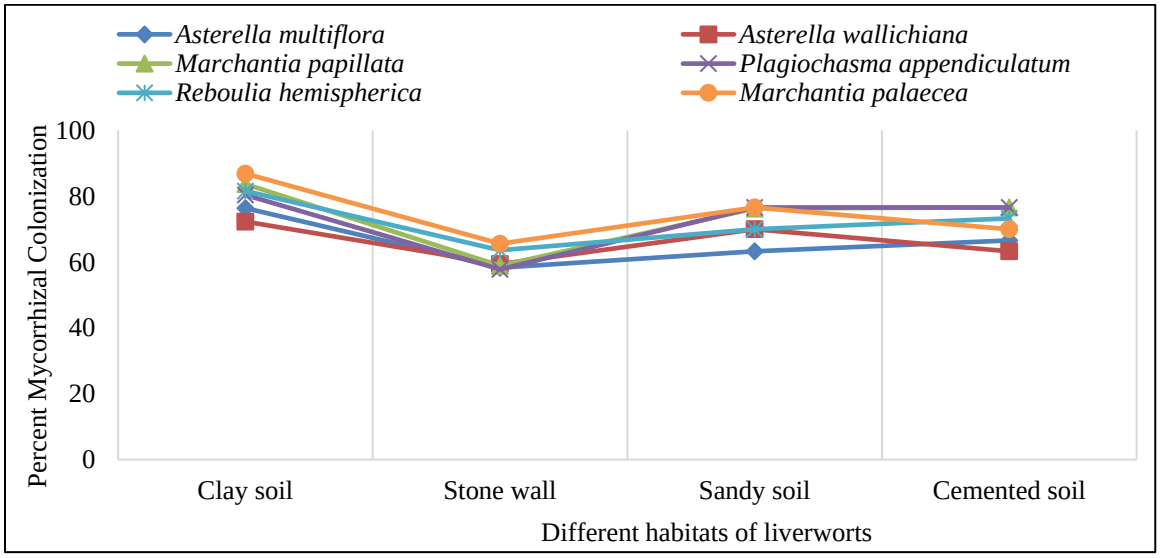
Minimum AM Colonization: The populations growing on stone walls show the lowest degree of AM colonization (less than 65%). This could be due to several reasons:

Lack of Nutrients: Stone walls are generally nutrient-poor environments and AM fungi may struggle to provide significant benefits to the host plants in such conditions.

Physical Constraints: The hard and compact nature of stone walls may limit rhizoid growth and mycorrhizal colonization, as the fungi may find it difficult to penetrate and colonize the roots effectively.

Moisture Levels: Stone walls might not retain moisture as well as soil, leading to drier conditions that are less conducive to AM fungal growth and symbiosis.

Other Habitats: The degree of AM colonization in sandy soil and cemented wall habitats likely falls between the extremes observed in clay soil and stone wall. Sandy soil may offer better drainage and aeration, while a cement wall may have some physical constraints similar to a stone wall.



Graph 1: Relationship between different habitats and Percent mycorrhizal colonization

Temperature: Just like habitat, temperature is an equally important factor influencing AM colonisation of host plants. Several workers have studied the effect of temperature on growth responses of AM fungi (Lumini *et al.*, 2010., Heinmeyer & Fitter, 2004., Camenzind *et al.*, 2014 and Botnen *et al.*, 2015). From ecological point of view, these variations are important for mycorrhizal functioning . Seasonal variation in the response of plants to mycorrhizal associations may result in development of adaptations required for

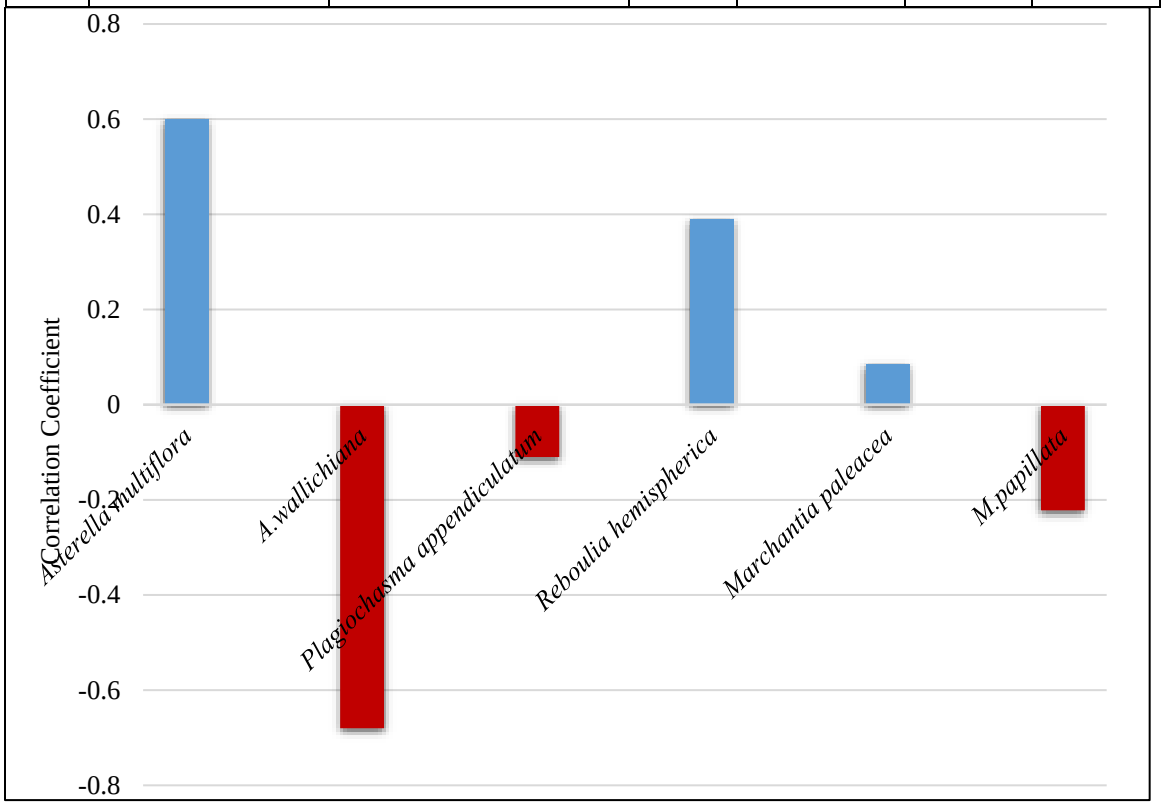
acquiring nutrients in different times of the year. Lugo *et al*. (2003) reported a marked seasonal variation in endomycorrhizal colonisation whereas Liu *et al.* (2011) found a significant correlation between AM fungal colonisation and sampling month. Similar to these observations, present study also revealed a seasonal variation in mycorrhizal colonisation in the hepatic taxa at different temperatures. Kumari (2015) recorded maximum colonisation in liverwort populations collected from Dhanolti in September with mean temperature 35°C whereas maximum fungal endophytes in mosses were observed in samples of Dayara (20°C) followed by those of Almora (25°C) and Mukteshwar (20°C) indicating that 35°C temperature was most favourable for mycorrhizal associations in hepatics and 20-25°C in mosses.

Minimum and maximum Percentage of mycorrhizal colonisation (PMC) for all the six taxa recorded presently have been arranged in the Table 10. Perusal of the data reveals enormous variability in mycorrhizal associations during different months of the year. In *Asterella multiflora*, minimum degree of colonization (54.6%) was recorded during January (5.4°C/20.5°C) and maximum in July (3.4C°/37.8C°). However, in *Asterella wallichiana* the trend was reversed, as minimum and maximum degree of colonization were recorded during June (40.8°C/20.4C) and November (25.4°C/6°C) respectively. In *Plagiochasma appendiculatum* minimum (34.5%) as well as maximum (80.8%) percent degree of colonization was observed in September (22.8°C /35.1°C). In both the species of *Marchantia* maximum PMC (85.9%) was recorded in the month of December (6°C /25.4°C), whereas minimum 56.3% and 56% in *M. paleacea* and *M. Papillata* during the month of February (7°C /25.4°C) and August (22.8°C /37.8°C) respectively. Maximum (85.6%) and minimum (42.8%) PMC observed in July (23.4°C /37°C) and March (11°C /33.5°C) resp. in case of *Reboulia hemispherica*.

Table 10: Relationship between Min/Max. temperature and Percent Mycorrhizal Colonization (PMC).

S.No.	Species	Correlation coefficient with Average temperature	PMC	Month	Min. Temp. (°C)	Max. Temp. (°C)
1.	*Asterella multiflora*	0.60	54.6.	January	5.4	20.5
			75.62	October	15.6	32
		-0.68	43.9	August	22.8	37.8

	Asterella wallichiana		77	November	9.5	29.6
3.	*Plagiochasma appendiculatum*	-0.11	34.5	September	22.8	35.1
			80.8	September	22.8	35.1
4.	*Reboulia hemispherica*	0.390	42.8	March	11	33.5
			85.6	July	23.4	37
5.	*Marchantia paleacea*	0.086	56.3	February	7	25.4
			85.9	December	6	25.4
6.	*M. papillata*	-0.222	56.0	August	22.8	37.8
			85.9	December	6	25.4



Graph 2: Correlation coefficient (r) between Percent Mycorrhizal Colonization (PMC) and Average temperature in six liverworts species.

Let's discuss the interpretation of the correlation coefficients for each species individually (Graph 2):

***Asterella multiflora* (r = 0.60):**

A positive correlation coefficient of 0.60 suggests a moderately strong positive relationship between temperature and mycorrhizal colonization in *Asterella multiflora*. As temperature increases, the mycorrhizal colonization in this species tends to increase.

***Asterella wallichiana* (r = -0.68):**

A negative correlation coefficient of -0.68 indicates a strong negative relationship between temperature and mycorrhizal colonization in *Asterella wallichiana*. As temperature increases, mycorrhizal colonization in this species tends to decrease significantly.

***Plagiochasma appendiculatum* (r = -0.11):**

A correlation coefficient of -0.11 suggests a weak negative relationship between temperature and mycorrhizal colonization in *Plagiochasma appendiculatum*. The relationship is not very strong, indicating that temperature has a minor influence on mycorrhizal colonization in this species.

***Reboulia hemispherica* (r = 0.390):**

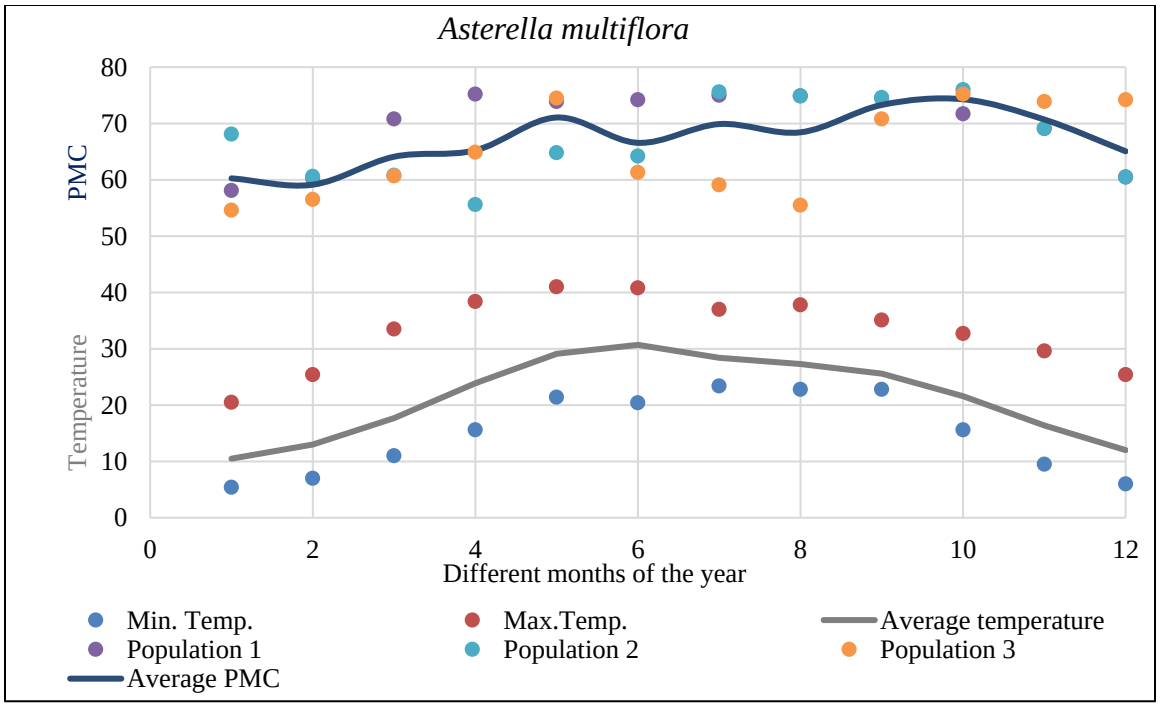
A positive correlation coefficient of 0.390 represents a moderate positive relationship between temperature and mycorrhizal colonization in *Reboulia hemispherica*. As temperature increases, mycorrhizal colonization in this species tends to increase, but the relationship is not as strong as in *Asterella multiflora*.

***Marchantia paleacea* (r = 0.086):**

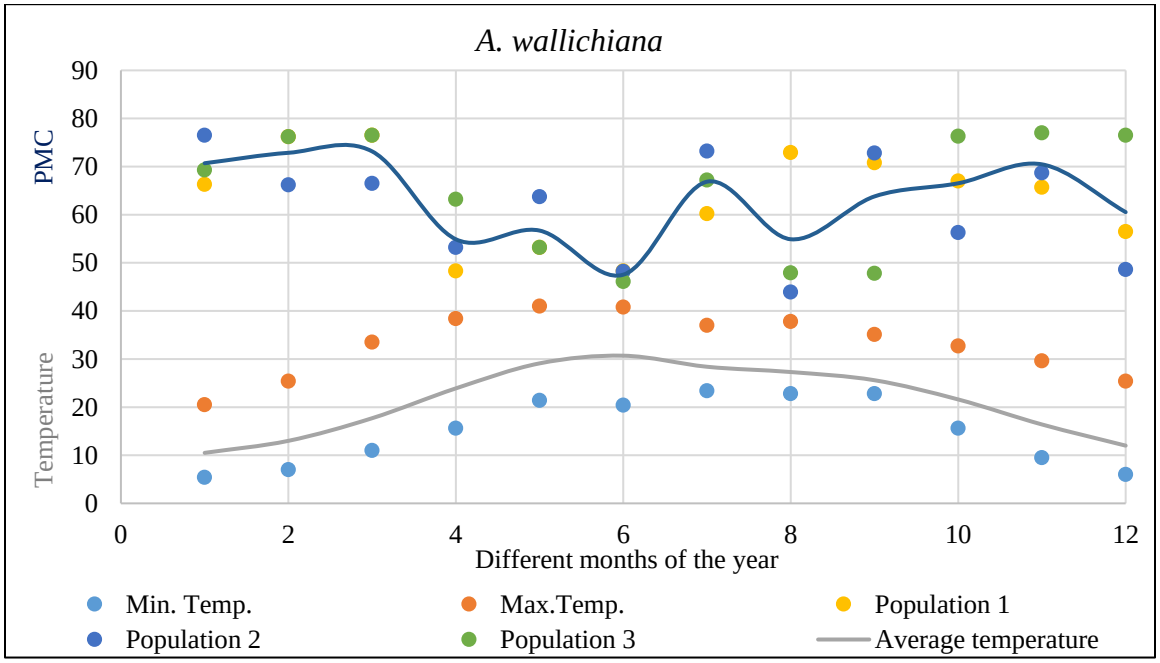
A very low positive correlation coefficient of 0.086 indicates an extremely weak positive relationship between temperature and mycorrhizal colonization in *Marchantia paleacea*. The correlation is almost negligible, suggesting that temperature has almost no impact on mycorrhizal colonization in this species.

***M. papillata* (r = -0.222):**

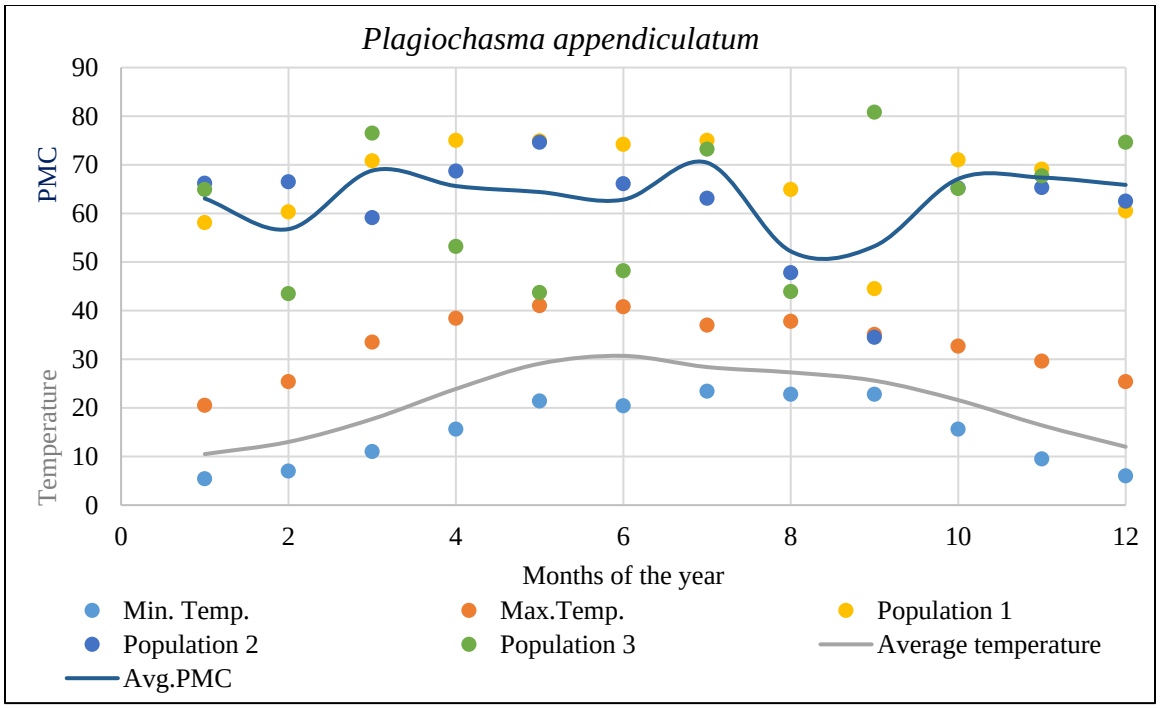
A negative correlation coefficient of -0.222 suggests a weak negative relationship between temperature and mycorrhizal colonization in *M. papillata*. As temperature increases, mycorrhizal colonization in this species tends to decrease slightly, but the relationship is not very strong.



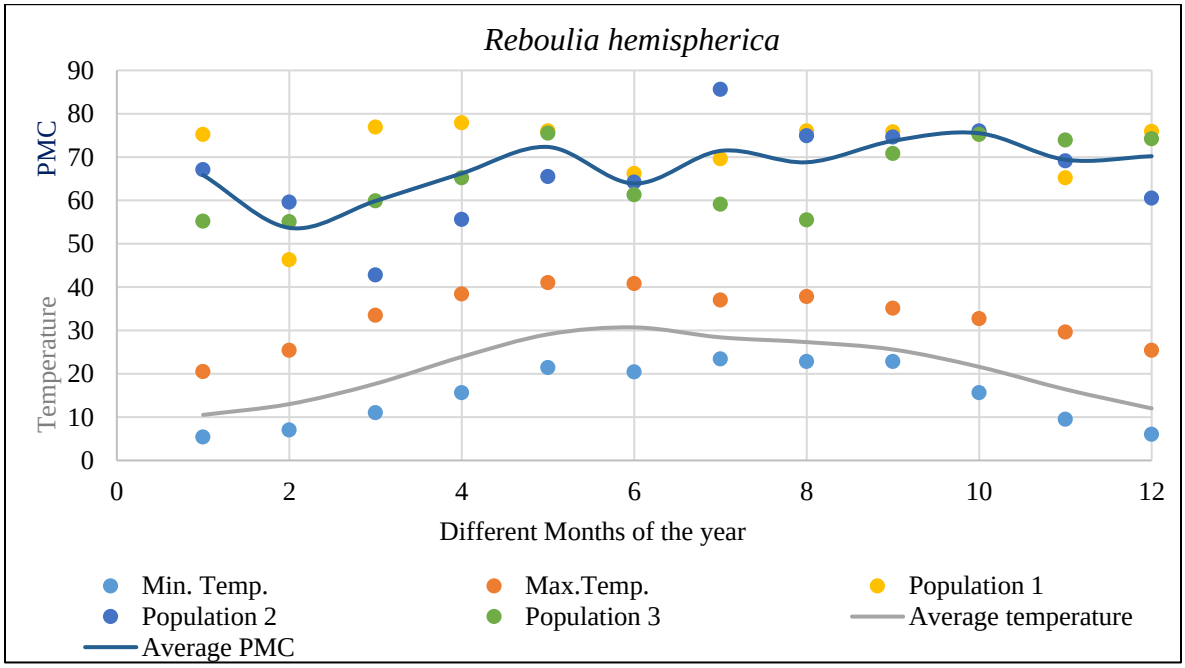
Graph 3a: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *Asterella multiflora* during different months of year.



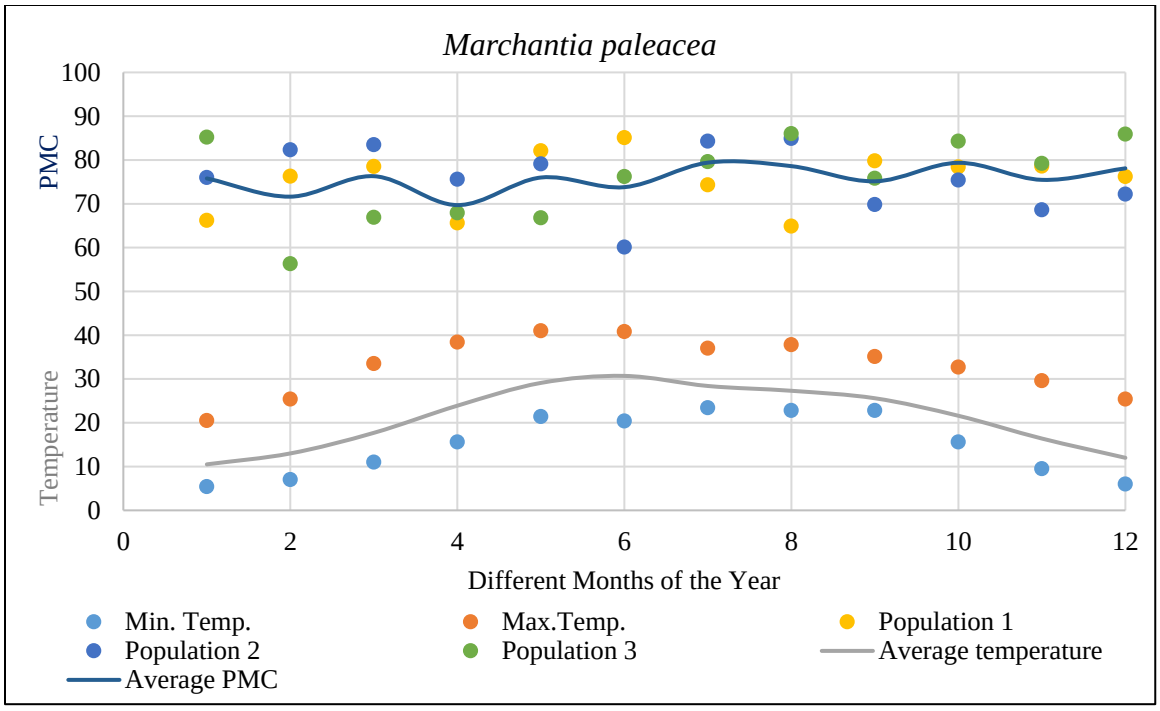
Graph 3b: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *A. wallichiana* during different months of the year.



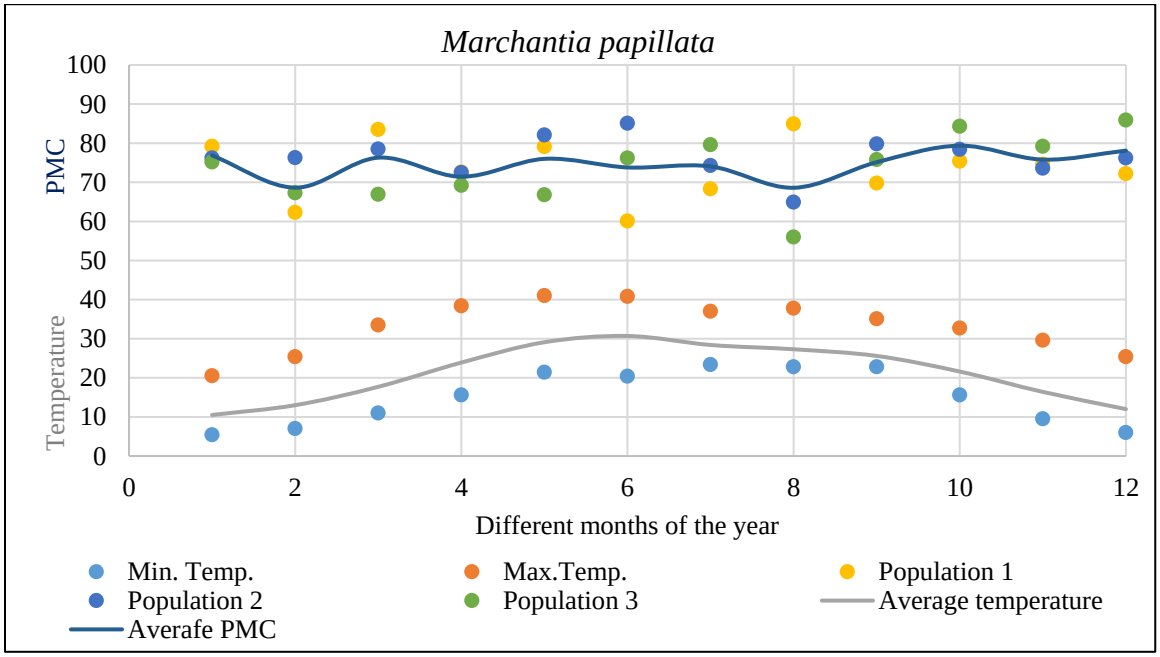
Graph 3c: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *Plagiochasma appendiculatum* during different months of the year.



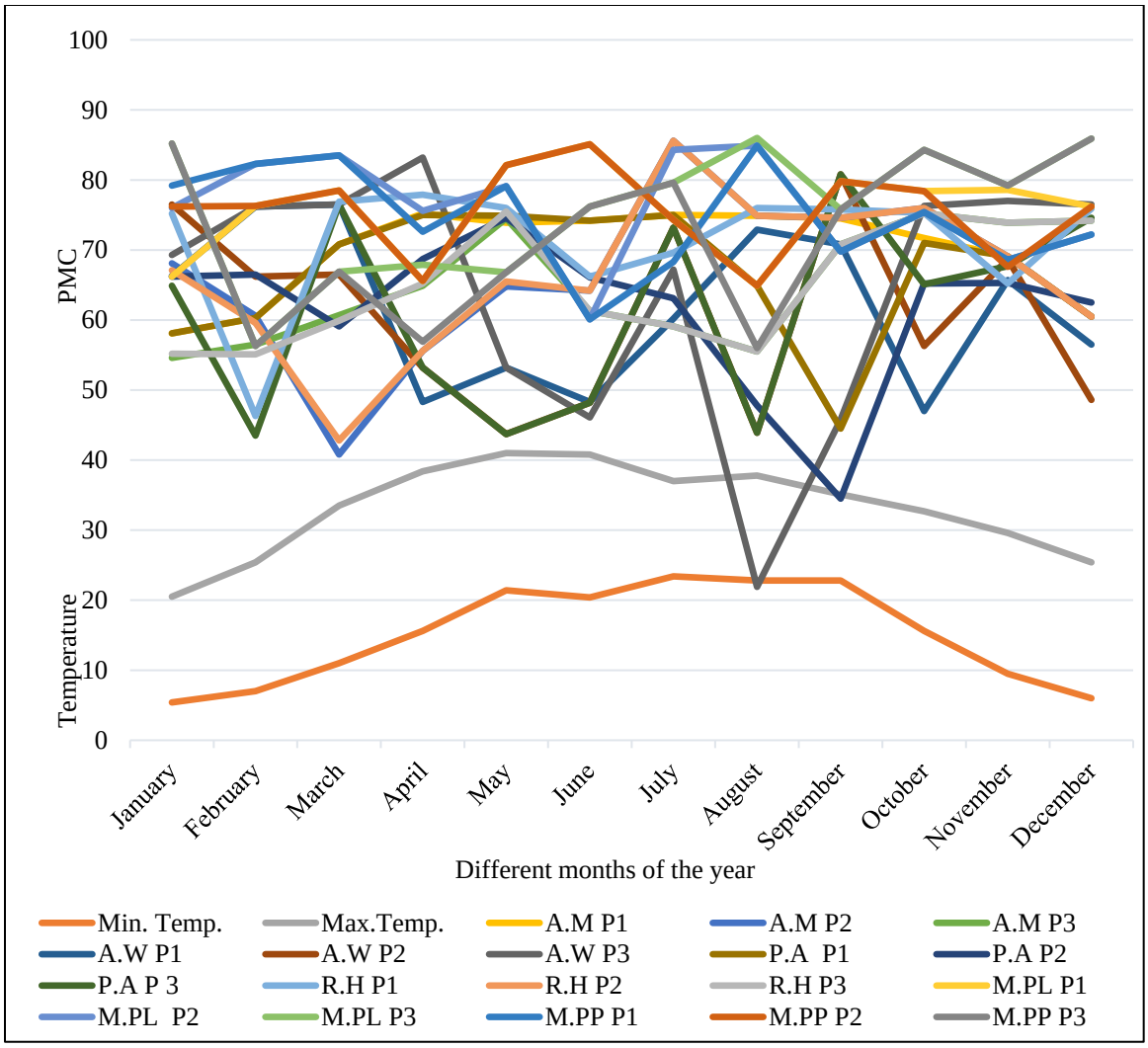
Graph 3d: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *Reboulia hemispherica* during different months of the year.



Graph 3e: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *M. paleacea* during different months of the year.



Graph 3f: Scattered Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and Temperature in *M. papillata* during different months of the year.



Graph 4: Graph representing relationship of temperature and PMC in different populations (P1, P2 and P3) of *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. p apillata* (M.PP) during different months of the year.

In summary, temperature has varying effects on mycorrhizal colonization in different liverwort species. It has a significant positive impact on *Asterella multiflora* and *Reboulia hemispherica* a strong negative impact on *Asterella wallichiana* and minor influences on *Plagiochasma appendiculatum*, *Marchantia paleacea* and *M. papillata*. It seems there might be a complex relationship between temperature and PMC, as different species respond differently to temperature variations (Graph 3 & 4). These results highlight the species-specific nature of the relationship between temperature and mycorrhizal colonization in liverworts.

In summary, there is no universal trend among these liverwort species regarding PMC and temperature. Some species show an increase in PMC with rising temperatures, some remain stable and others decrease (Graph5). Other factors might be at play, including moisture levels, soil composition and specific species traits, which could be influencing the relationship between temperature and mycorrhizal colonization differently in each species.

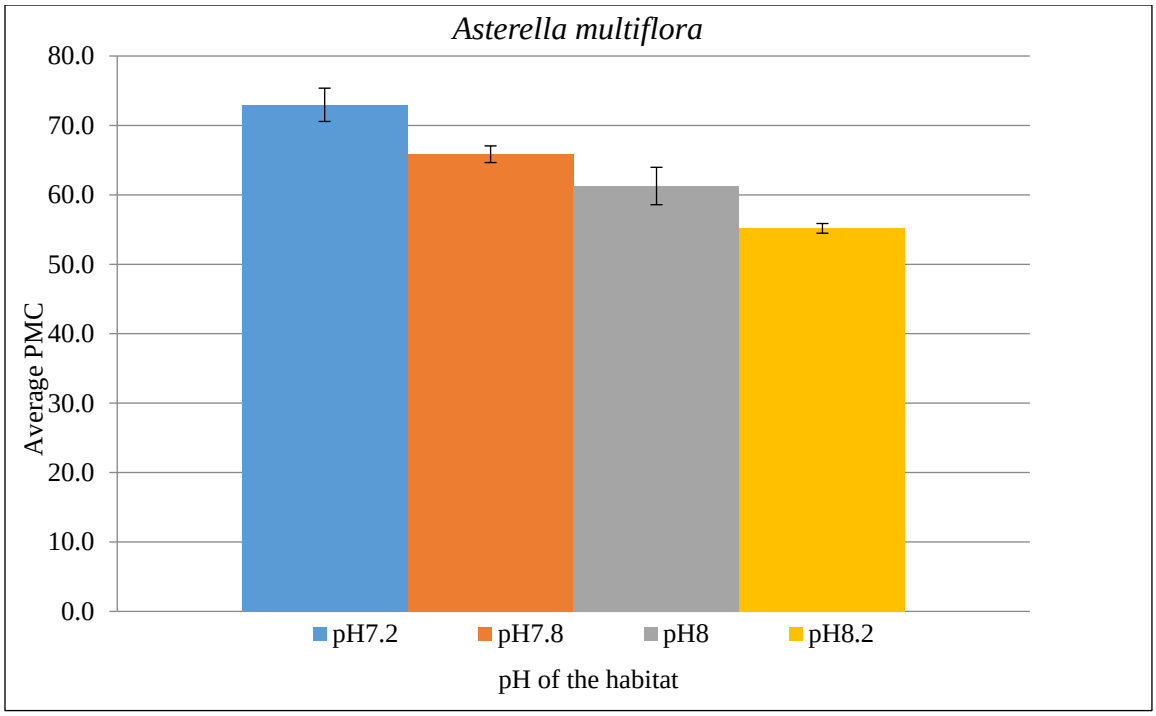
pH Tolerance: Most liverwort species are reported to grow on slightly alkaline soil with pH between 7.5 and 8.0. However, during present investigation liverworts have also been collected from substrates with a wide pH range, from slightly acidic (pH 6.3) to slightly alkaline (pH 8.5) as evident from Table11.

Table 11: Table showing pH range of collection sites of various liverworts.

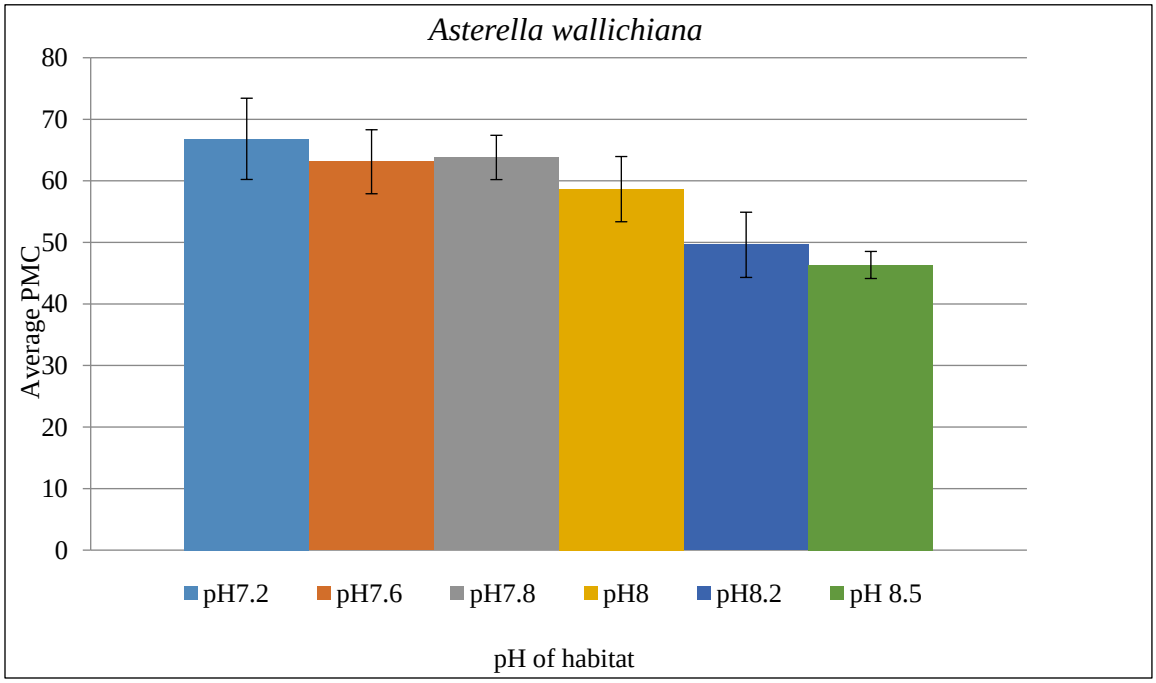
S.No.	Species	pH range of collection sites
1.	*Asterella multiflora*	7.3-8.2
2.	*Asterella wallichiana*	7.2-8.5
3.	*Plagiochasma appendiculatum*	6.3-8.2
4.	*Reboulia hemispherica*	5.3-8.0
5.	*Marchantia paleacea*	6.9-8.5
6.	*M. papillata*	7.2-8.5

Effect of pH on Mycorrhizal Colonization:

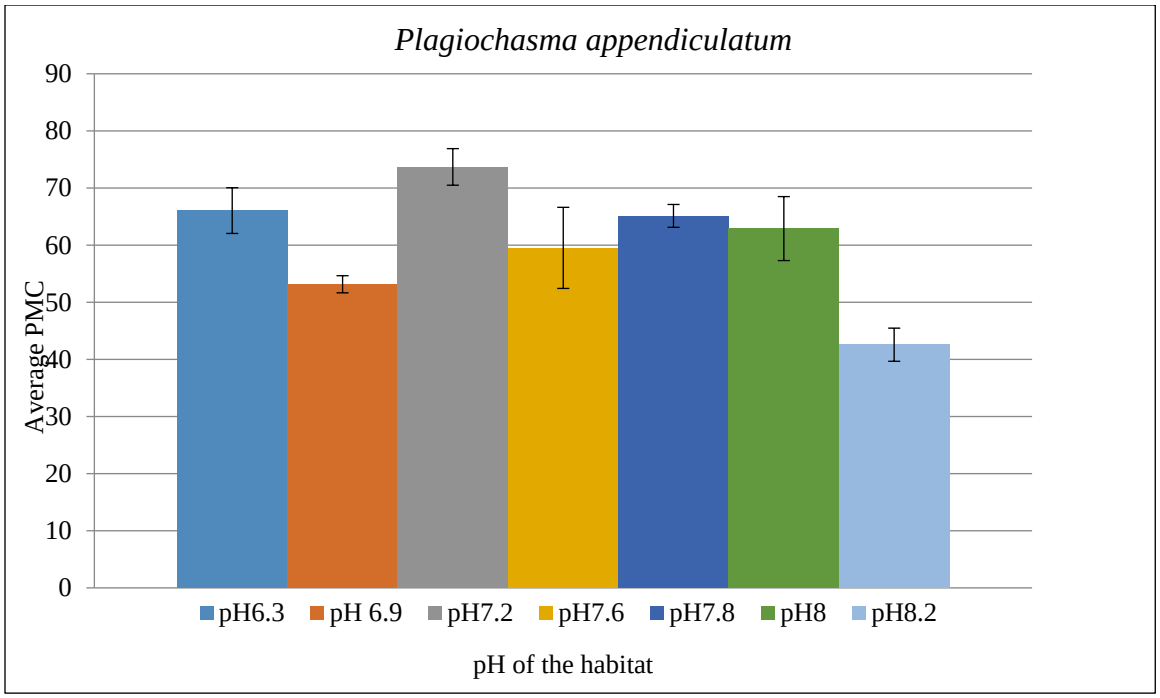
In vitro studies have suggested that optimal germination of AM spores occurs between pH 6 and 7, with lower germination rates at pH values below 5 and above 8 (Siqueira *et al.,* 1982*)*. A study implies that temperature and pH have an effect on the niche space that arbuscular mycorrhizal fungi are able to occupy. This finding lends credence to the notion that pH also plays a significant part in the habitat that these fungi inhabit (Vasar *et al*., 2021). As observed in the plots of all the six taxa, maximum mycorrhizal colonization in most of the species was observed in populations growing on pH 7.2 to 8.0, whereas minimum mycorrhizal colonization was observed in most of the populations growing on acidic soil.



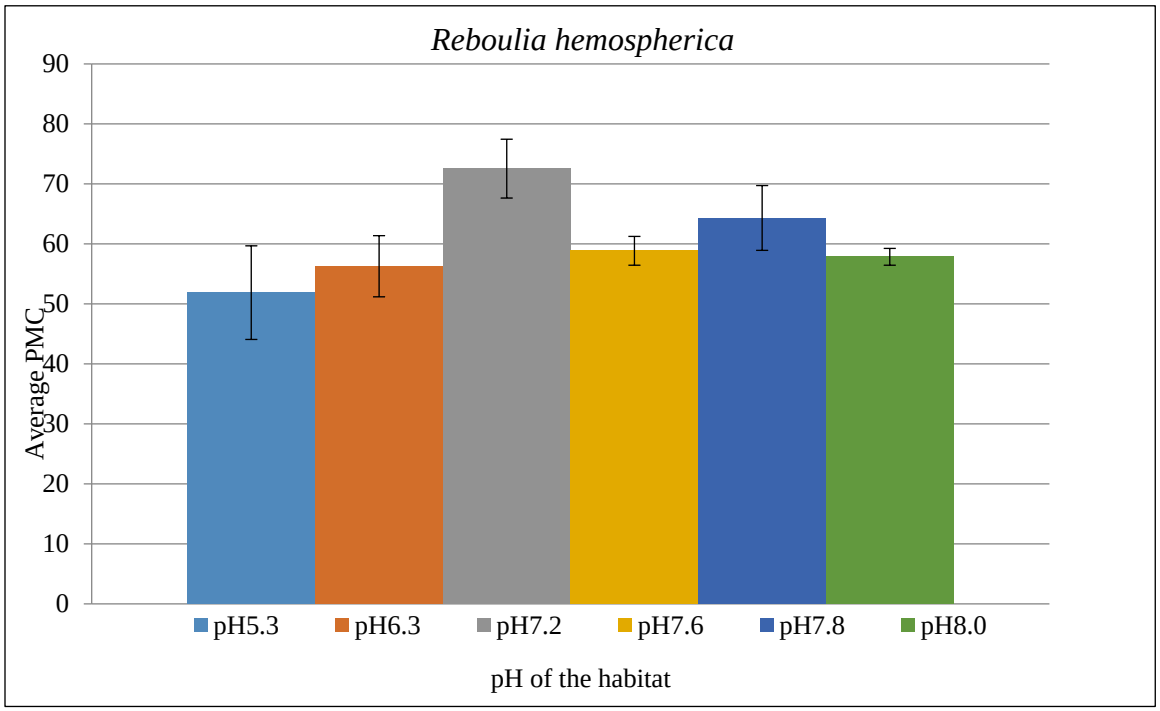
Graph 5a: Graph showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *Asterella multiflora*.



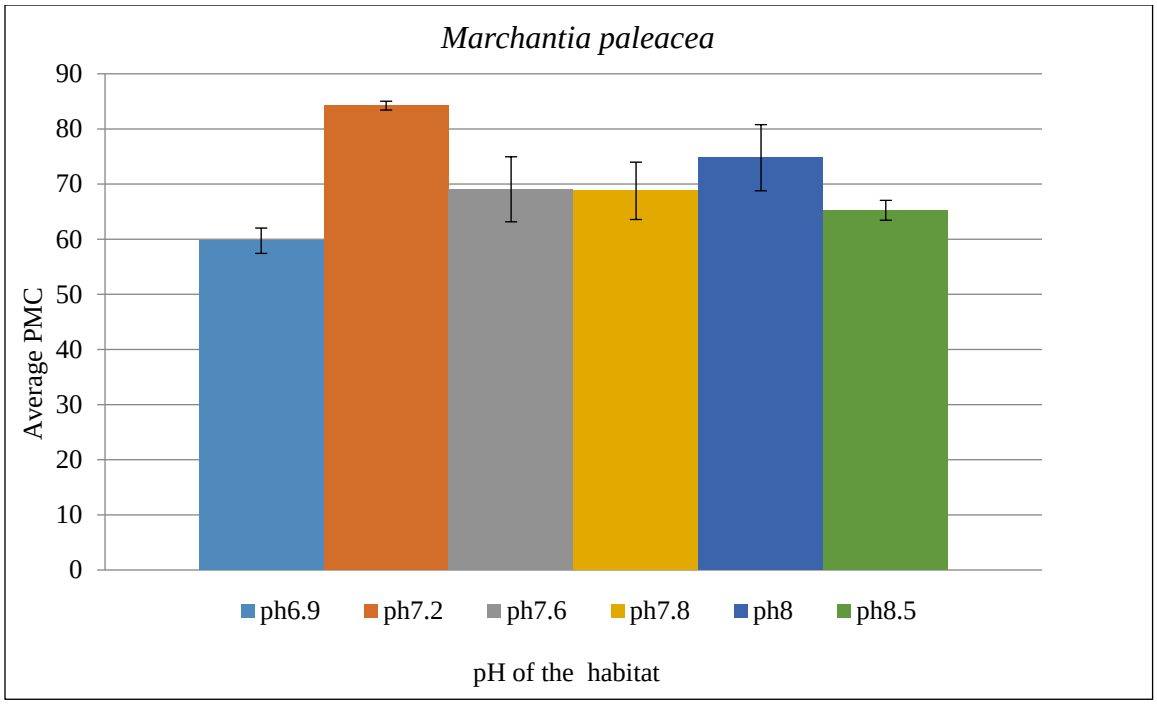
Graph 5b: Graph showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *A. wallichiana*.



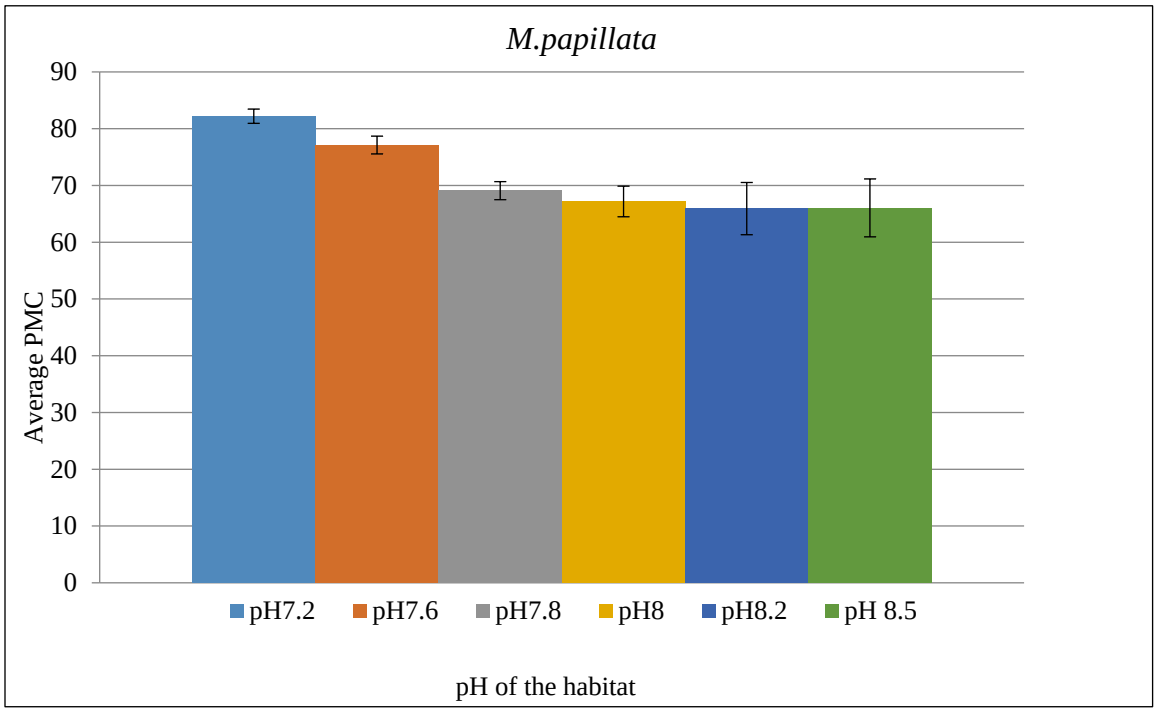
Graph 5c: Diagram showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *Plagiochasma appendiculatum*.



Graph 5d: Graph showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *Reboulia hemispherica*.

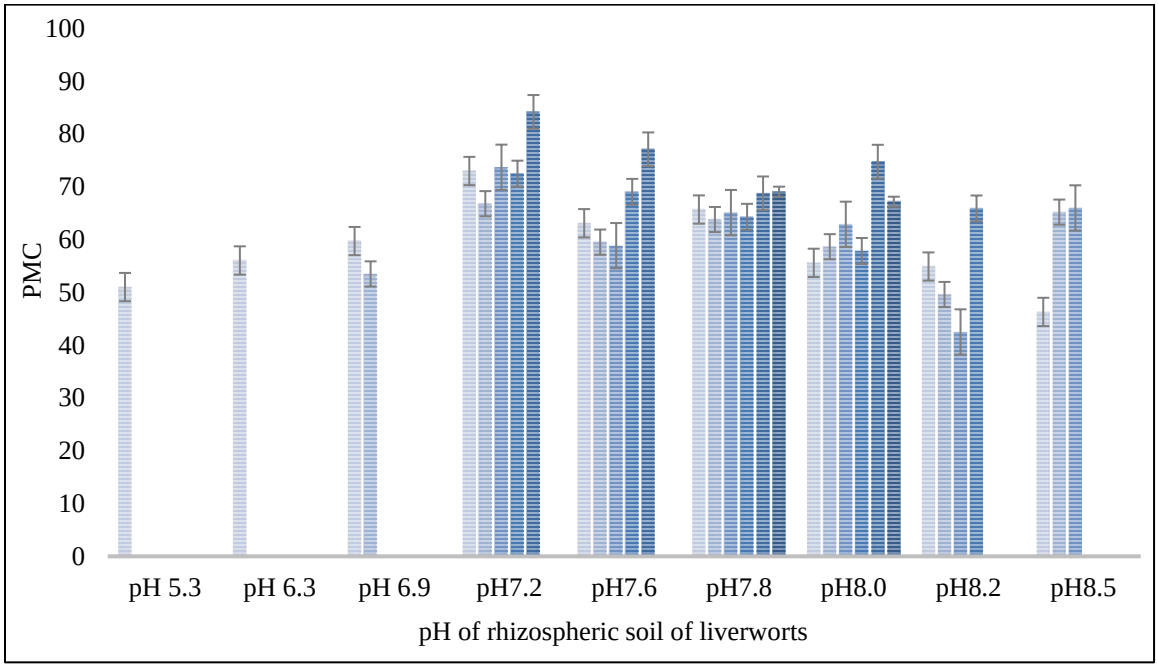


Graph 5e: Graph showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *Marchantia paleacea.*



Graph 5f: Graph showing relationship between Percent Mycorrhizal Colonization (PMC) and pH of habitat in *M. papillata*.

The relationship presents a graph (Graph 6) that illustrates the effect of pH on mycorrhizal colonization in liverworts. It shows that maximum mycorrhizal colonization was recorded in plants inhabiting substrates with pH values 7.2. Deviation from this pH values in any direction (either more acidic or more alkaline) leads to a decrease in mycorrhizal colonization. However, it was also observed that higher mycorrhizal colonization was found in the populations growing on alkaline soil as compared to acidic soil. Mycorrhiza was found to be absent in most of the plants inhabiting soil with pH above 8.5 and below 5.0 (*Riccia discolor*). The absence of mycorrhiza in most of the samples collected from the soils with pH above 8.5 and below 5 suggests that liverworts may have specific pH requirements for successful mycorrhizal colonization.



Graph 6: Graph representing relationship of pH of habitat with PMC in various populations of studied liverworts.

The findings of this study shed light on the fact that this symbiotic connection is extremely sensitive to variations in pH. There are ecological repercussions associated with the pH-dependent colonization of liverworts by mycorrhizal fungi for liverworts. The results of this study reveal that the pH levels in the soil can have an effect on the distribution and abundance of liverwort species, as well as the breadth of their mycorrhizal relationships. Understanding these pH-dependent interactions can have broader

implications for ecosystem dynamics, especially in regions where liverworts play a significant ecological role (van Der Heijden *et al*., 2015)

Moisture Content

According to several studies (Rabatin, 1980; Reid & Bowen, 1979), soil moisture levels may have an impact on AM colonization. According to research by Osonubi *et al.* (1991) & Mizoguchi (1992), AM fungus can help plants adapt to dry conditions by promoting the growth of tree seedlings when they are treated to dryness in a nursery setting. Numerous impacts of soil water stress on mycorrhizal invasion and growth promotion have been documented. Water stress induces AM fungi to produce more spores, which increases the number of AM spores in the soil (Hiremath *et al*., 1990). Jinger *et al*., 2023 performed a survey in Rajasthan. They discovered that both root colonization and AM sporulation were influenced by soil moisture. Root colonization and spore population decreased because of excessive soil moisture. However, current investigations under varied moist conditions have shown varying colonization levels (Table 13). A perusal of the table reveals that:

1) *Plagiochasma appendiculatum* had the highest degree of colonization, with a moisture level of 16.3% and *Reboulia hemispherica* had the lowest, with a moisture level of 12.3%.

2) The lowest degree of colonisation for *Asterella multiflora, Plagiochasma appendiculatum* and Marchantia paleacea had higher moisture content, whereas the highest degree had reduced moisture content. But with *A. wallichiana, Reboulia hemispherica* and *Marchantia papillata*, the pattern was the opposite.

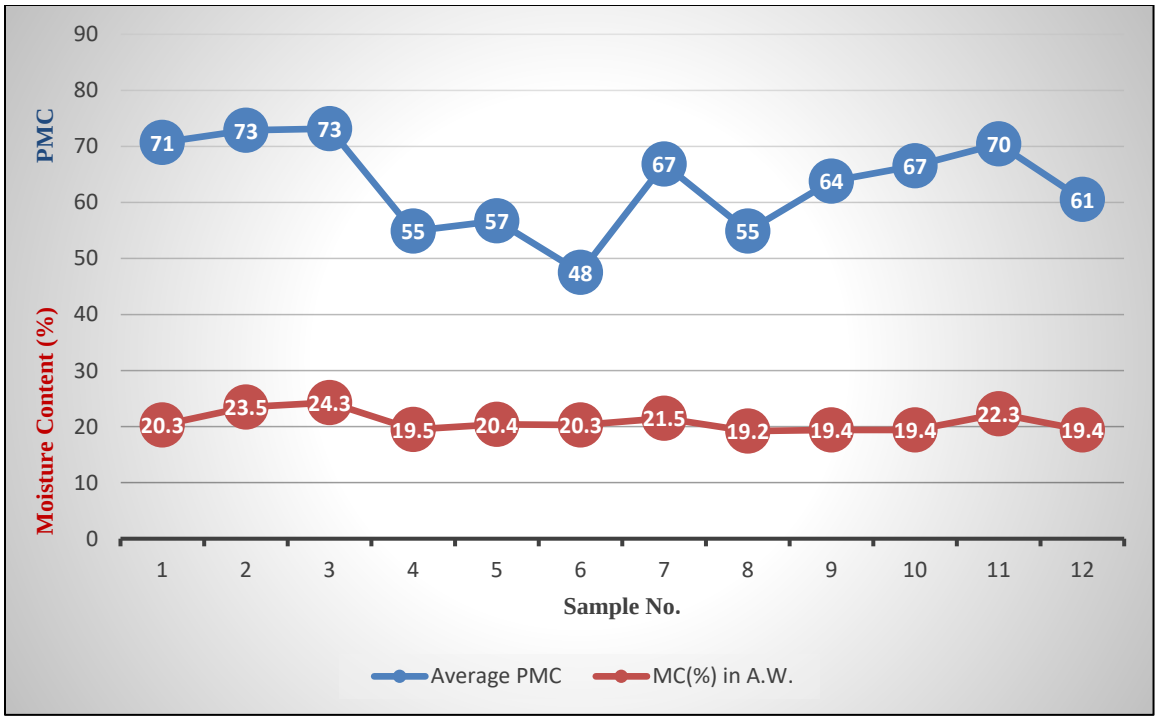
Most of the populations of *Marchantia paleacea* exhibited moisture levels in the range of 21-30%, whereas the majority of populations of *Plagiochasma appendiculatum* were collected from soils having moisture levels between 16-28%. Populations of *Reboulia hemispherica* with the highest levels of colonisation had moisture levels between 11-24%. Populations of *Asterella multiflora* with the highest degree of colonisation were gathered at moisture levels between 21-36%. *Asterella wallichiana* (19-24%) and *Asterella multiflora* (19-24 %,) had rather small ranges. Populations of *M. paleacea* and *Marchantia papillata* were gathered from soils that ranged in moisture content from 26-30%,

respectively. Kumari (2015) discovered that plants had higher colonization levels in humid and wet environments. Additionally, she also observed that AM colonization increased as moisture levels increased. In all of his experiments, the highest colonisation was seen when the soil's available water content continued to range between 30 and 60%. However, Tommerup (1983) and Mejstrik (1965) expressed different opinions, believing that dry soils with low moisture content tend to support AM spore germination. Our experiment data also suggests a positive correlation between mycorrhiza and soil water content (Graph 7).

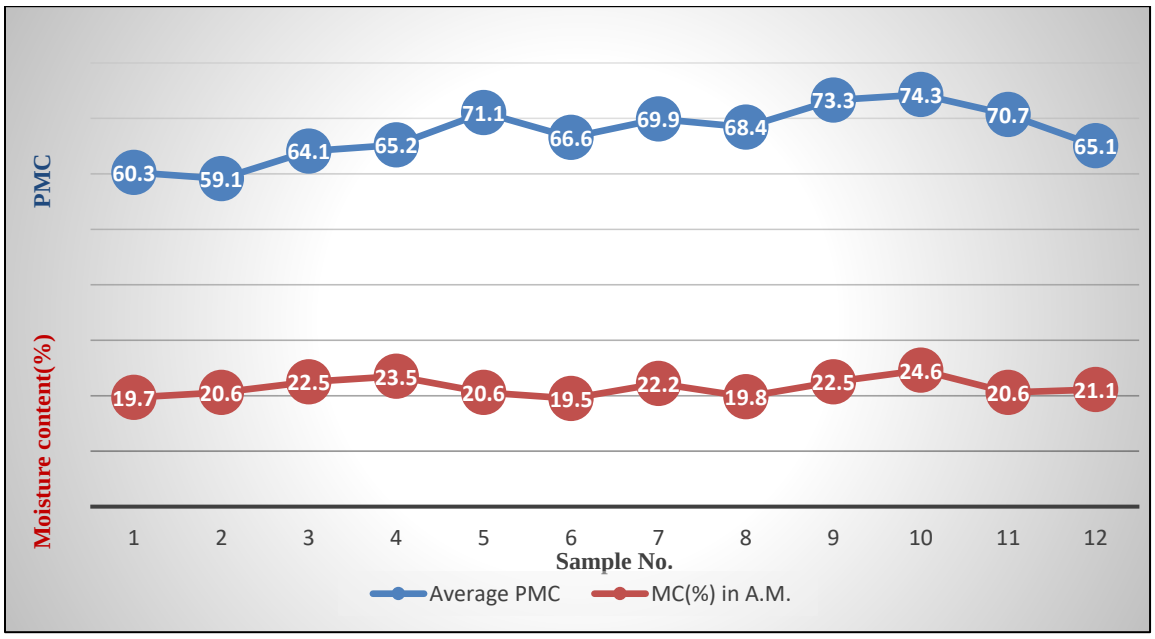
Table 12: Data regarding the % Moisture Content (MC) of the rhizospheric soils and the Percent Mycorrhizal Colonization (PMC) in different liverwort species.

PMC (%) in A.M	MC (%) in A.M.	PMC (%) in A. W	MC (%) in A.W.	PMC (%) in P. A	MC (%) in P. A	PMC (%) in R.H	MC (%) in R.H	PMC (%) in M.PL	MC (%) in M.PL	PMC (%) in M.PP	MC (%) in M.PP
60.3	19.7	71	20.3	63.1	19.4	65.8	18.5	76	21.2	76.8	28.3
59.1	20.6	73	23.5	56.8	18.9	53.7	19.4	71	21.3	69	26
64.1	22.5	73	24.3	68.8	20.6	59.9	18.7	76	28.3	76	28
65.2	23.5	55	19.5	65.6	20.2	66.2	20.6	70	28.3	71	27.5
71.1	20.6	57	20.4	64.4	20.1	72.3	17.9	76	28.2	76	28
66.6	19.5	48	20.3	62.8	18.5	63.9	19.4	74	20.6	74	29
69.9	22.2	67	21.5	70.4	28.6	71.4	18.9	79	30.5	74	30
68.4	19.8	55	19.2	52.2	16.4	68.8	20.6	79	30.6	69	31.5
73.3	22.5	64	19.4	53.3	17.3	73.7	20.2	75	29.8	75	29
74.3	24.6	67	19.4	67.1	18.7	75.5	20.1	79	30.5	79	30.5
70.7	20.6	70	22.3	67.4	20.6	69.4	19.4	75	29.4	76	29
65.1	21.1	61	19.4	65.9	20.6	70.2	18.9	78	28.7	78	28

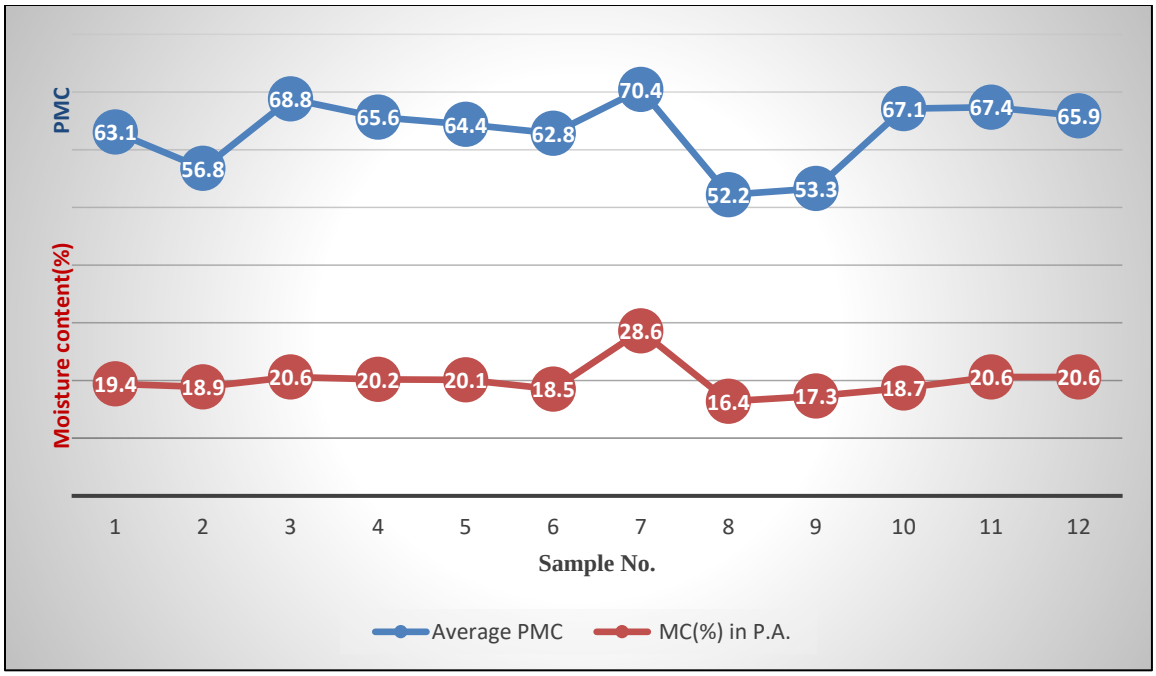
Abbrrevation used PMC: Percent Mycorrhizal Colonization; MC: Moisture content in soil*; Asterella multiflora* (A.M); *A. wallichiana* (A.W); *Plagiochasma appendiculatum* (P.A); *Reboulia hemispherica* (R.H); *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).



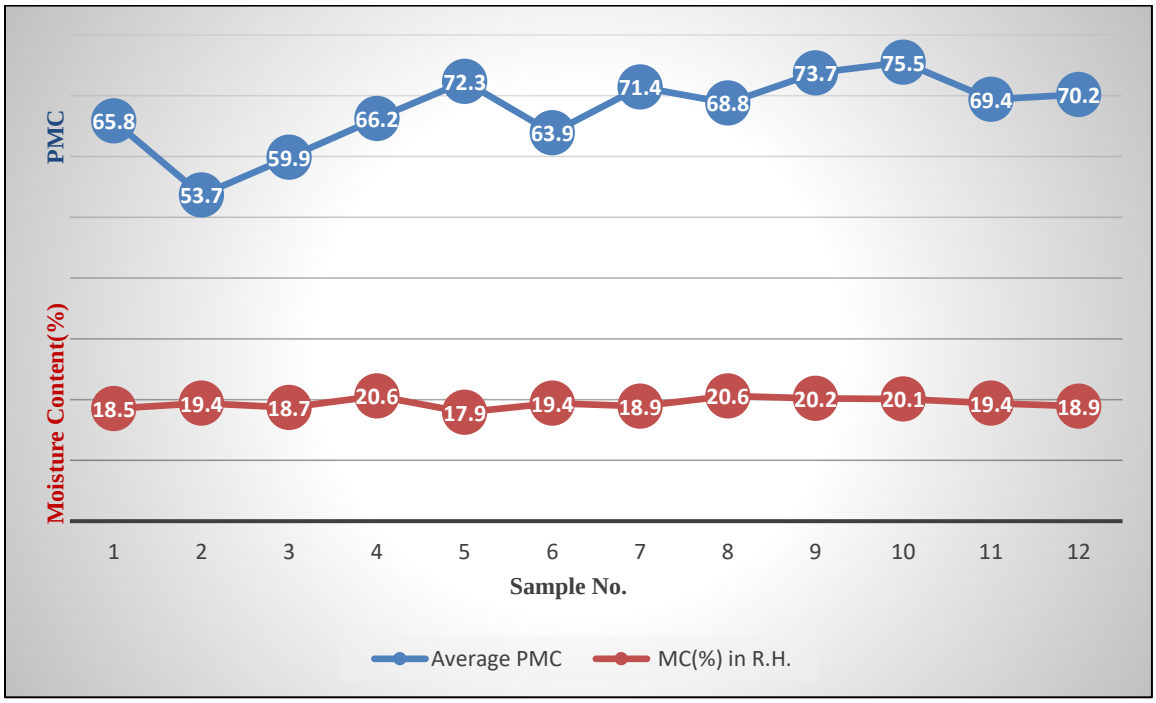
Graph 7a: Scattered diagram showing relationship between Moisture content (%) and Percent Mycorrhizal Colonization (PMC) in *Asterella wallichiana* (A.W).



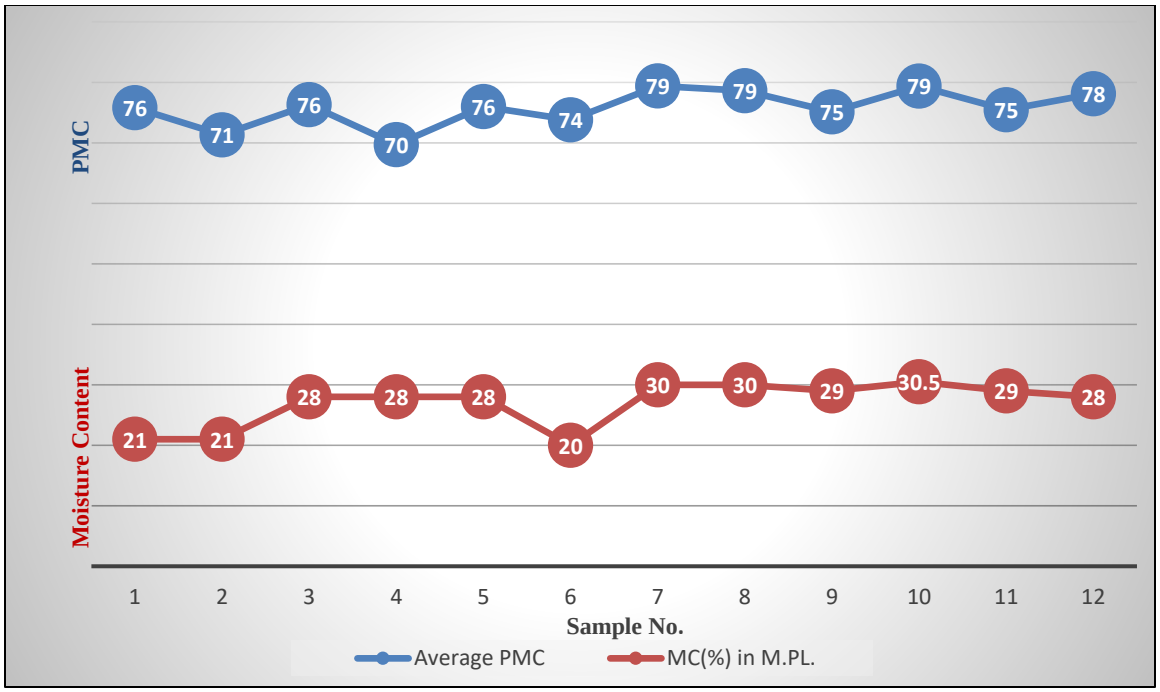
Graph 7b: Scattered diagram showing relationship between Moisture content (MC) and Percent Mycorrhizal Colonization in *Asterella multiflora* (A.M).



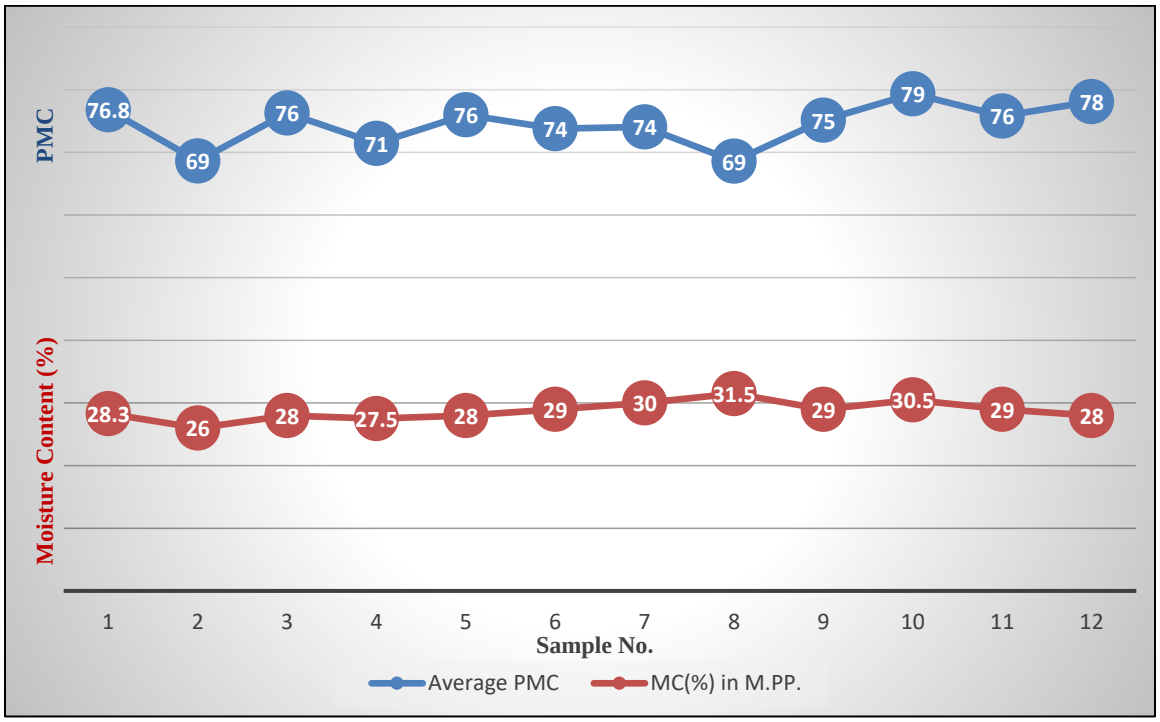
Graph 7c: Scattered diagram showing relationship between Moisture content (MC) and Percent Mycorrhizal Colonization in *Plagiochasma appendiculatum* (P.A).



Graph 7d: Scattered Diagram showing relationship between Moisture content (MC) and Percent Mycorrhizal Colonization in *Reboulia hemispherica* (R.H).



Graph 7e: Scattered diagram showing relationship between Moisture content (MC) and Percent Mycorrhizal Colonization in *Marchantia paleacea* (M.PL).



Graph 7f: Scattered diagram showing relationship between Moisture content (MC) and Percent Mycorrhizal Colonization in M. *papillata* (M.PP).

Antioxidants

In the present study, six species of liverworts, *A. multiflora*, *A. wallichiana*, *P. appendiculatum*, *R. hemispherica*, *M. paleacea* and *M. papillata* were explored and investigated for the antioxidant potential.

Protein, Superoxide Dismutase (SOD), Guaiacol Peroxidase (GPOX), Catalase (CAT), Ascorbate Peroxidase activity (APOX), Glutathione reductase activity (GR), Ascorbic acid (ASA), Total Phenol content (TPC), Proline, Glycine-betaine (GB), MDA (Malondialdehyde), Glutathione (GSH), Chlorophyll a (Chl a), Chlorophyll (Chl b) and Carotenoids (CAR) content of these liverworts were estimated.

In this study we estimated antioxidants which are non-enzymatic antioxidants including Ascorbic acid (ASA), Proline content, Glutathione (GSH), Glycine-betaine (GB) and Total Phenol content (TPC), along with the enzymatic antioxidants like Superoxide Dismutase (SOD), Catalase (CAT), Guaiacol Peroxidase (GPOX), Ascorbate Peroxidase activity (APOX), Glutathione reductase activity (GR).

Biochemical estimations in different liverwort species

Results of biochemical analysis of six liverwort species including *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP) are summarized in Table 13.

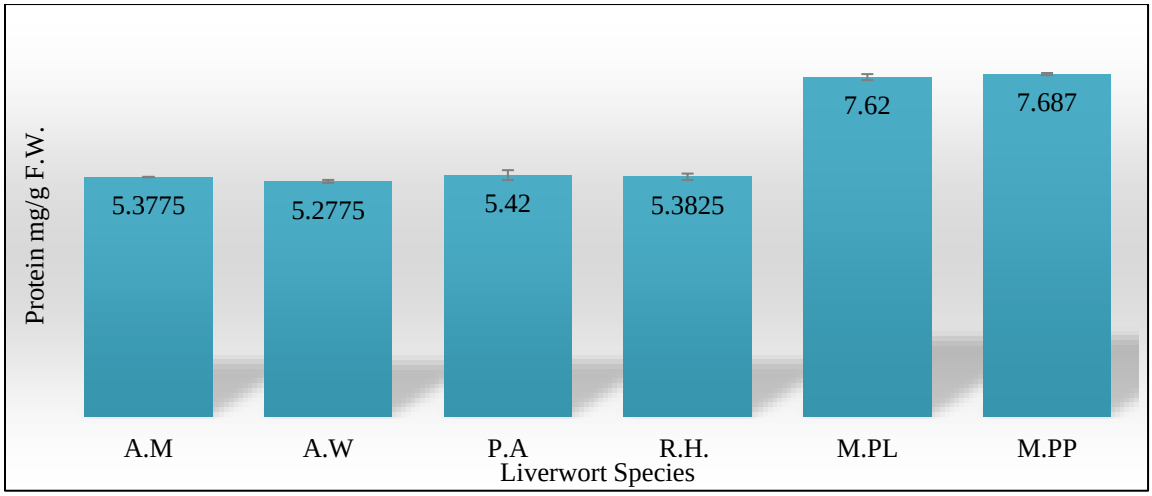
Table 13: Data of Biochemical estimations of liverworts.

S.No.	Biochemicals	*Asterella multiflora (A.M)*	*A. wallichiana (A.W)*	*Plagiochasma appendiculatum (P.A)*	*Reboulia hemispherica (R.H)*	*Marchantia paleacea (M.PL)*	*M. papillata (M.PP)*
1	**Protein**	5.38	5.28	5.42	5.45	7.62	7.69
2	**Superoxide Dismutase UA/mg protein**	0.05	0.05	0.08	0.08	0.1	0.1
3	**Guaiacol Peroxidase UA/mg protein**	0.26	0.27	0.28	0.27	0.29	0.29
4	**Catalase UA/mg protein**	0.014	0.016	0.015	0.015	0.018	0.018
5	**Ascorbate Peroxidase UA/mg protein**	1.99	1.67	1.89	1.84	2.14	2.16
6	**Glutathione Reductase UA/mg protein**	1.18	1.25	1.9	1.92	2.28	2.29
7	**Ascorbic acid mg/gF.W.**	14.79	14.9	14.72	14.34	17.34	17.8
8	**Total Phenols mgGAE/gF.W.**	14.74	14.9	11.95	14.3	17.27	17.04
9	**Proline mg/gF.W.**	10.41	9.94	7.57	8	4.55	4.76
10	**Glycine-betaine mg/gF.W.**	5.57	7.2	4.03	3.92	8.94	9.03
11	**MDA content µmol/ gF.W.**	0.72	0.84	2.24	2.14	0.61	0.82
12	**Total glutathione mg/gF.W.**	2.29	2.38	2.65	2.59	4.13	3.92
13	**Chlorophyll a mg/gF.W.**	0.14	0.15	0.18	0.16	0.23	0.23
14	**Chlorophyll b mg/gF.W.**	0.11	0.13	0.12	0.16	0.17	0.17
15	**Total Caratenoids mg/gF. W**	0.12	0.135	0.16	0.165	0.17	0.18

These observations provide insights into the biochemical composition and antioxidant defense mechanisms of these liverworts, indicating their ability to cope with stress and environmental conditions. The levels of antioxidant compounds in liverwort species can provide information about their stress tolerance and adaptive strategies (Gahtori &

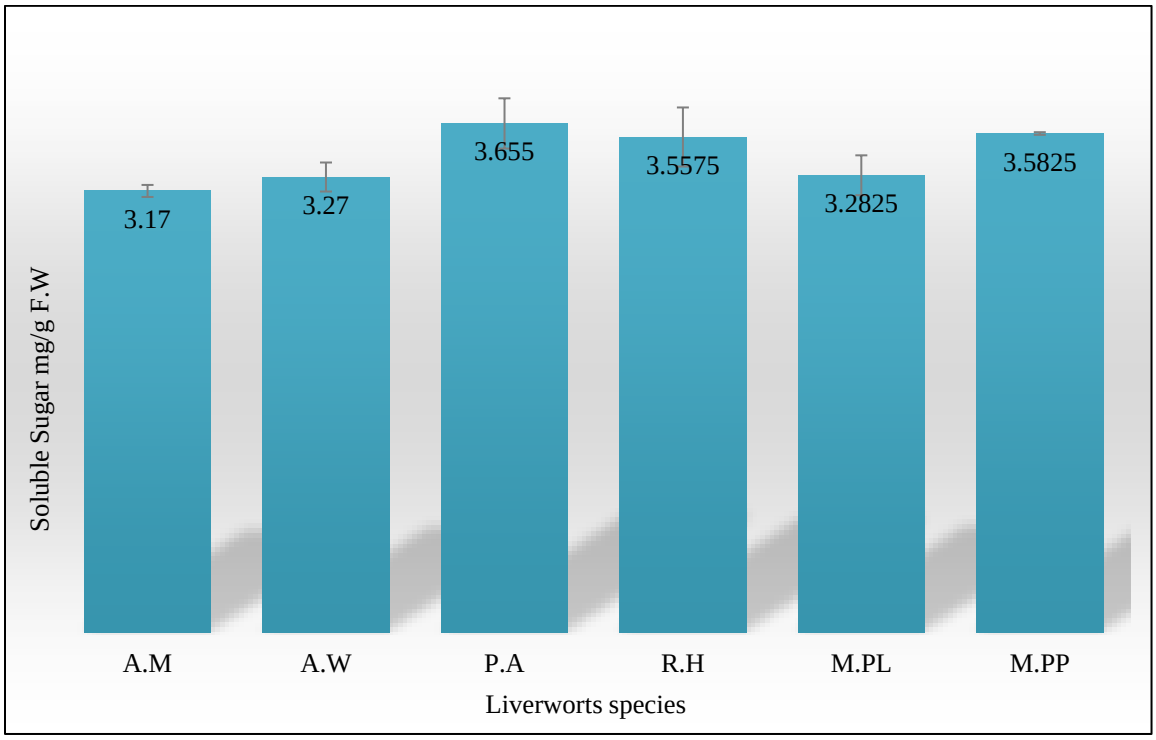
Chaturvedi, 2019). Higher levels of amino acids may indicate a better capacity to withstand osmotic and oxidative stress, which can be crucial for the survival of these plants in challenging environments (Kurutas, 2015). Based on the data of all the six liverworts here are the observations on the individual biochemical:

1. Protein Content: The value of protein content is 5.28-7.69 mg/g fresh weight (F.W.), protein content varies among the different liverwort species. *Marchantia papillata* (M.PL) *and M. paleacea* (M.PP) have the highest protein content, followed by *Reboulia hemispherica* (R.H), *Asterella multiflora* (A.M), *Asterella wallichiana* (A.W) and *Plagiochasma appendiculatum* (P.A.) (Graph 8). Different liverwort species have varying genetic compositions, which can influence their ability to synthesize and accumulate proteins. Some species may have evolved to produce higher levels of certain proteins as a response to specific ecological or physiological demands (Spenedi *et al*., 2021). This genetic diversity can explain why some species, like *Marchantia papillata (M.PL*) *and M. paleacea (M.PP)*, have higher protein content compared to others. This variation may reflect differences in their metabolic activities and growth strategies. Similarly, the values reported by Sharma *et al*. in 2015 for *Plagiochasma appendiculatum* and *Pellia endiviifolia* are higher than those reported in our experiment. This could be due to differences in measurement techniques, environmental conditions, or the genetic makeup of the liverwort populations studied in their research.



Graph 8: Protein content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

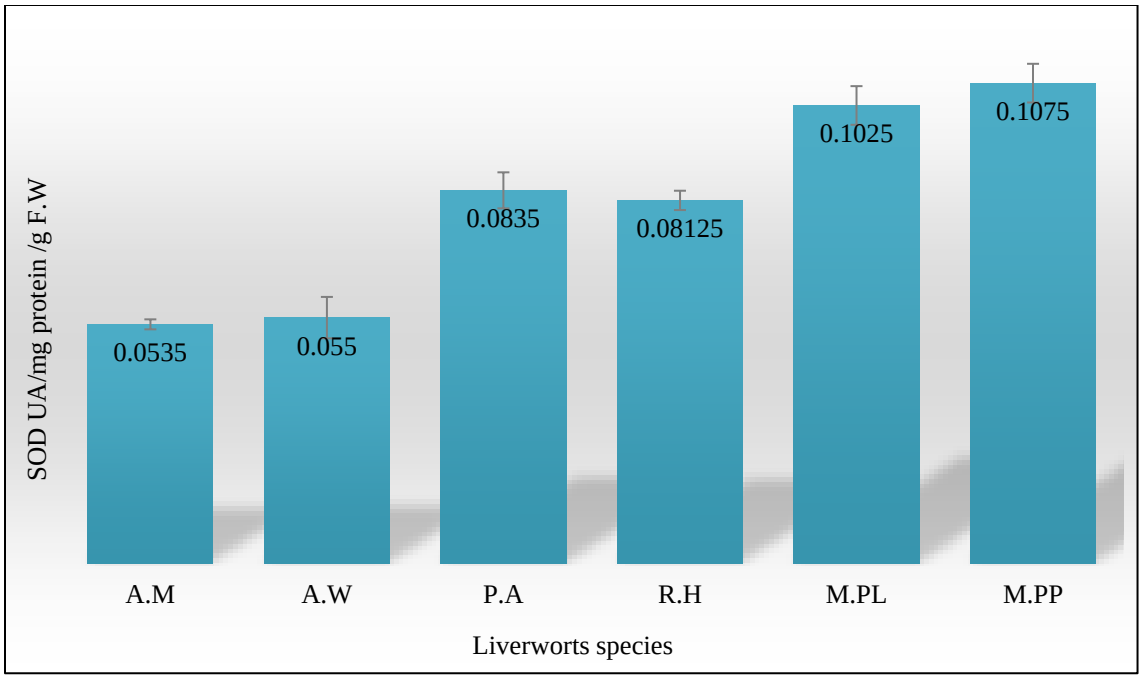
2. Soluble Sugar Content: The soluble sugar content is between 3.17-3.65 mg/g F.W., suggesting the presence of sugars in the plant, which may serve as a source of energy and carbon (Graph 9). The soluble sugar content also varies among the liverwort species. P. A has the highest soluble sugar content, followed by M.PP, M.PL, R.H, A.W and A.M. Soluble sugars serve as an energy source and osmoprotectants and these variations may indicate differences in their stress tolerance and energy requirements.



Graph 9: Soluble sugar content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

3. Enzyme Activities:

3a. **Superoxide Dismutase (SOD)**: The study on Superoxide Dismutase (SOD) activities provides valuable insights into the antioxidant and oxidative stress responses of different samples. The SOD activities observed in various samples indicate their potential to detoxify superoxide radicals, a crucial process in maintaining cellular health. The range of SOD activity in the given samples varies from 0.05UA/mg protein to 0.1 UA/mg protein (Graph 10).



Graph 10: Unit activity of superoxide dismutase (SOD) in liverworts species, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

1. A.M and A.W both exhibit an SOD activity of 0.05 UA/mg protein. This suggests that these samples have similar capabilities in neutralizing superoxide radicals. This similarity might be indicative of a shared source or composition between A.M and A.W.

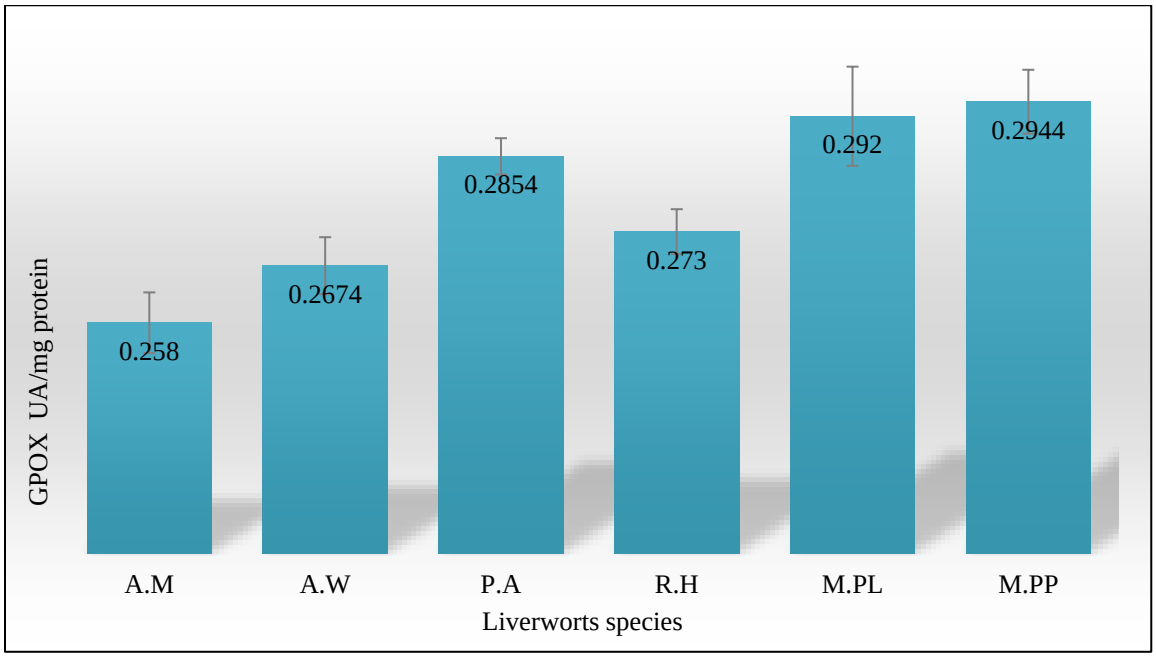
2. P.A &R.H, with a SOD activity of 0.08 UA/mg protein, demonstrates higher SOD activity than P.A but is still within the range observed in A.M and A.W. This suggests that R.H possesses a moderate antioxidant capacity.

3. M.PL and M.PP exhibit the highest SOD activity among all the samples, both with a SOD activity of 0.1 UA/mg protein. This is a significant finding as it indicates a robust antioxidant defense system against superoxide radicals in M.PL and M.PP.

The variations in SOD activities across these samples reflect differences in their oxidative stress responses. Higher SOD activity generally implies a more efficient defense against oxidative damage caused by superoxide radicals. This may be particularly relevant in the

context of skin diseases, where oxidative stress plays a significant role in disease development and progression (Okayama, 2005). *Plagiochasma sp*. used by a tribe in the Himachal Pradesh for treating diseases of skin. The study found that this extract not only prevents lipid peroxidation but also increases SOD (Superoxide Dismutase) and CAT (Catalase) activity (Kumar *et. al*., 2008). The increase in SOD activity suggests that the extract enhances the detoxification of superoxide radicals, contributing to its antioxidant properties. This is highly relevant in the context of skin health, as oxidative stress is a common factor in skin diseases.

3b. Guaiacol Peroxidase (GPOX): The results of our experiment indicate the presence of guaiacol peroxidase activity in the liverwort species under investigation, with a range of 0.26 to 0.29 UA/mg protein (Graph 11) This suggests the involvement of an enzyme that plays a role in peroxidation reactions and, notably, in the detoxification of hydrogen peroxide. Guaiacol peroxidase is known for its significance in protecting cells from oxidative stress by catalyzing the breakdown of hydrogen peroxide into water and oxygen (Noctor *et al.*, 2012).

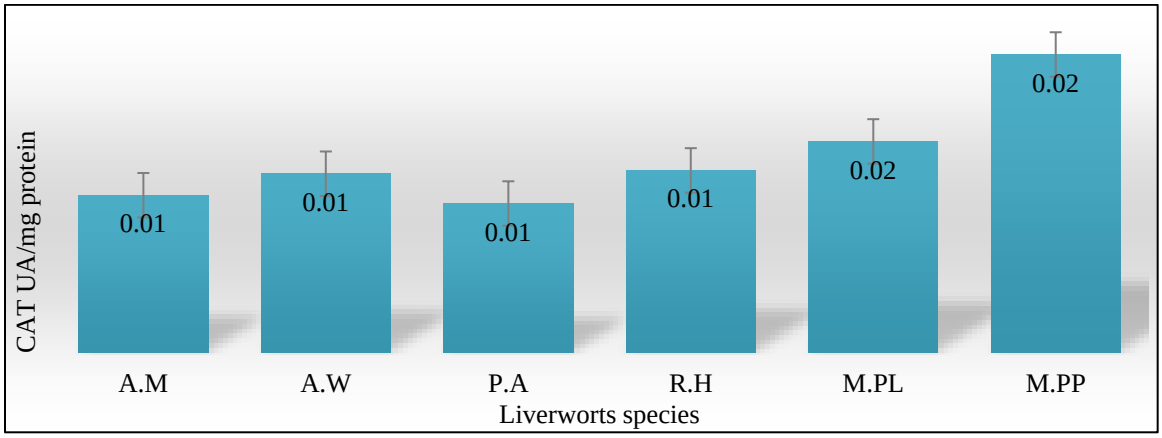


Graph 11: Unit activity of Guaiacol Peroxidase in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Interestingly, the observed guaiacol peroxidase activity is quite consistent among the liverwort species, with only slight variations. Notably, M.PL and M.PP exhibit slightly higher activity at 0.29 UA/mg protein. These findings suggest that guaiacol peroxidase may be a conserved enzyme across liverwort species, possibly reflecting its importance in these plants' defense mechanisms against oxidative damage.

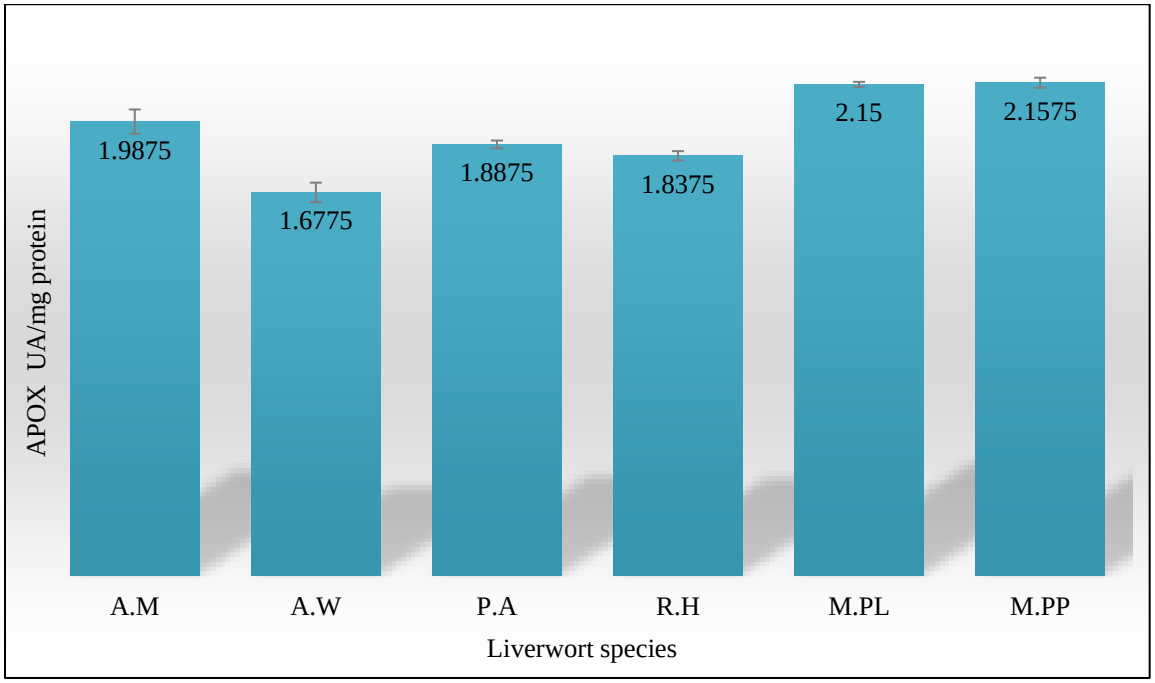
It's essential to acknowledge the reference values provided by Sharma *et al*. (2005), which reported higher guaiacol peroxidase activity (0.93 UA/mg protein in *Plagiochasma appendiculatum* and 0.12 UA/mg protein in another liverwort, *Pellia endiviifolia*). These differences between our results and those of the previous study could be attributed to various factors, including variations in experimental conditions, environmental factors or genetic diversity among liverwort populations. It is important to note that enzyme activity can be influenced by a multitude of variables and the specific conditions under which the enzyme is assayed can have a significant impact on the results.

3c. Catalase: The catalase activity is 0.0154-0.018 UA/mg protein (Graph 12), indicating the presence of an enzyme involved in the breakdown of hydrogen peroxide. Catalase activity is also similar across the species, with M.PL and M.PP having slightly higher activity. Catalase is essential for hydrogen peroxide breakdown. Sharma *et al*. (2015) conducted research comparing catalase activity in two liverwort species, *Plagiochasma sp*. and *Pellia* sp. They found that *Plagiochasma* sp. had significantly higher catalase activity (0.014 UA/mg Prot. g-1 FW) compared to *Pellia* sp. (0.0045 UA/mg Prot. g-1 FW). Our results also confirmed the similar catalse activity in liverworts.



Graph 12: Unit activity of catalase in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

3d. Ascorbate Peroxidase (APOX): The ascorbate peroxidase activity is 1.67 - 2.16UA/mg protein (Graph13), suggesting the presence of an enzyme involved in the detoxification of hydrogen peroxide.

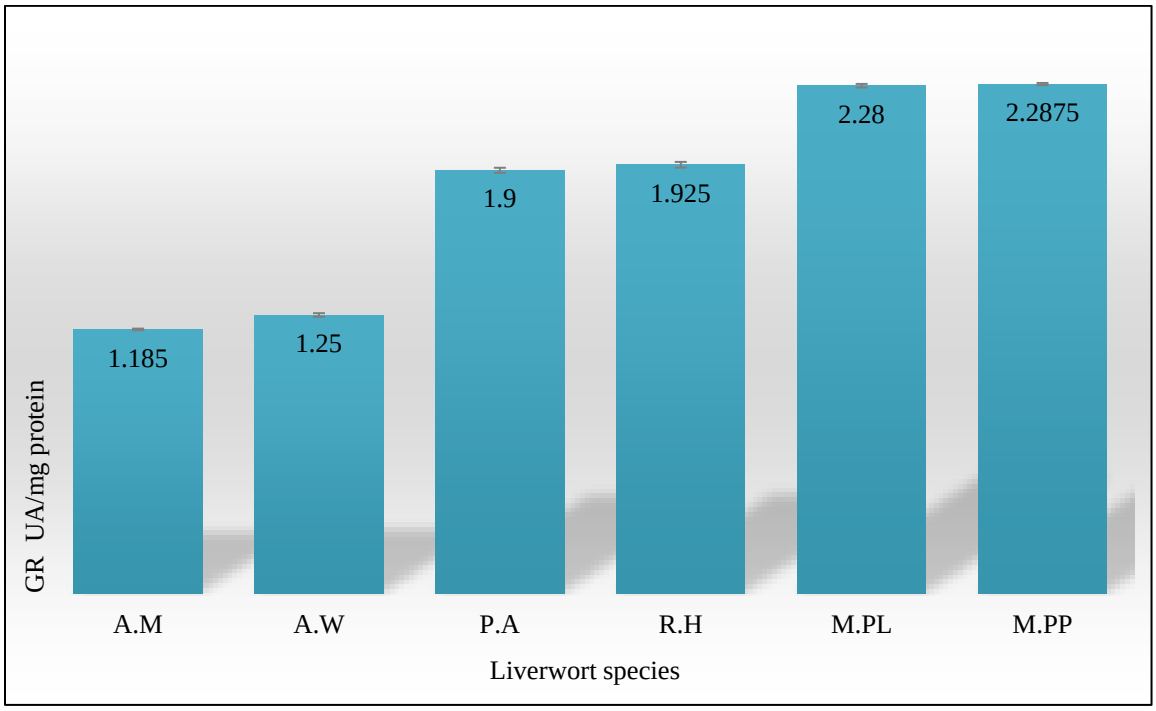


Graph 13: Unit activity of Ascorbate Peroxidase in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Ascorbate peroxidase activity varies among the species, with M.PL and M.PP showing the highest activity. This enzyme plays a role in the ascorbate-glutathione cycle, important for ROS detoxification. Yadav *et al.* (2022) similar APX activity (2.5 UA per mg protein) in *Dumortiera hirsuta* from Khasi hills, Shillong, Meghalaya. This finding suggests that liverworts possess the capacity to scavenge reactive oxygen species (ROS) using APX.

3e. Glutathione Reductase (UA/mg protein): Glutathione reductase (GR) is an enzyme involved in the maintenance of cellular redox balance and plays a crucial role in the reduction of glutathione (GSH), a major antioxidant molecule in cells. (Rao *et al*., 2008). It is evident that the Glutathione reductase activity varies among different liverwort species. M.PL and M.PP have the highest activity levels among the mentioned liverworts, with values of 2.28 and 2.29 UA\mg protein, respectively (Graph 14). This suggests that

these two liverwort species may have a higher capacity for reducing glutathione compared to the others. However, *Dumortiera hirsuta* from Shillong, Meghalaya, stands out with a GR activity of 25 micro moles per minute per mg protein (Yadav *et al*., 2022). This is notably higher than the values observed in the Jammu liverworts. The differences in GR activity among liverwort species could be attributed to various factors, including their natural habitats, exposure to different stressors and genetic variations.



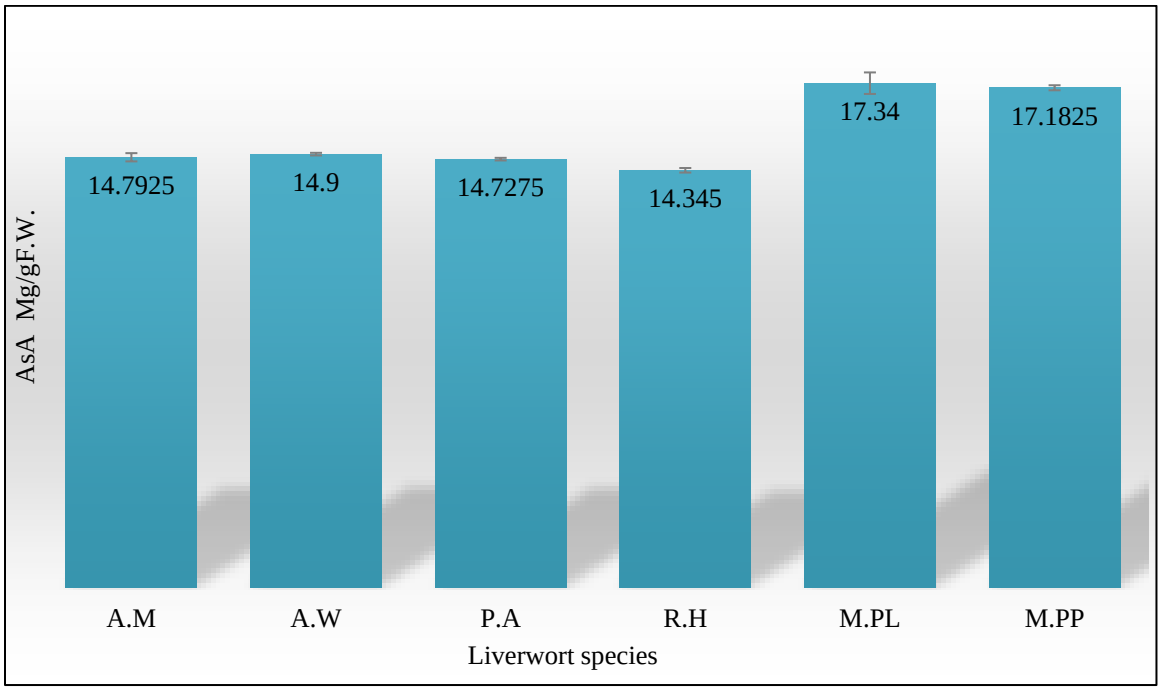
Graph 14: Unit activity of Glutathione Reductase in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

4. Antioxidants

4a. Ascorbic Acid (mg/g F.W.): The ascorbic acid content in liverworts may have ecological or nutritional significance, depending on their role in the ecosystem and their potential use in traditional medicine or dietary supplements.

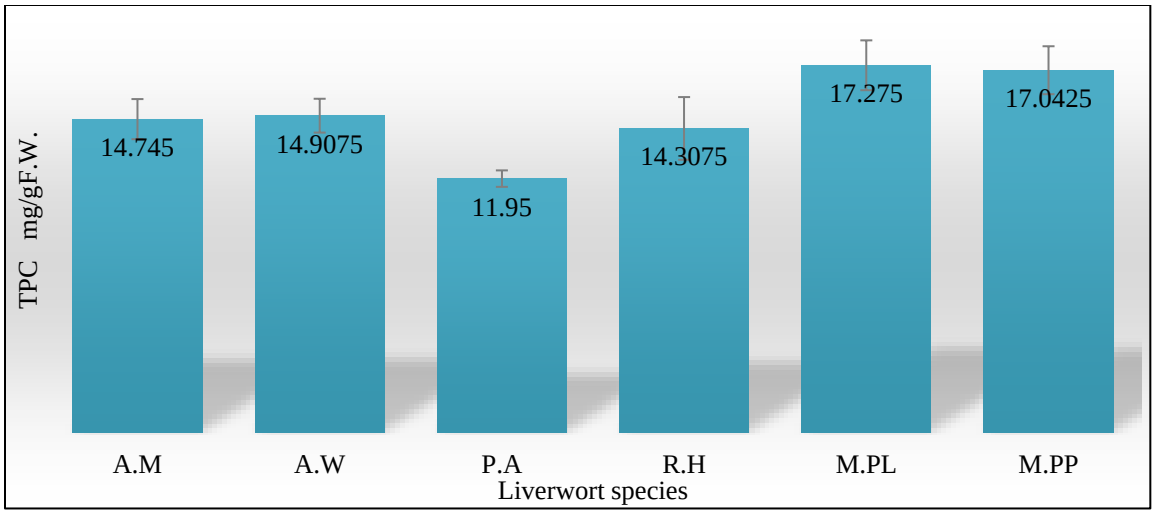
Looking at the data, we can observe that M.PL (*Marchantia paleacea)* and M.PP *(M. papillata*) have the highest levels of ascorbic acid, with values of 17.27 and 17.04 mg/g F.W., respectively (Graph 15). On the other hand, R.H (*Reboulia hemispherica*) has the

lowest level at 14.30 mg/g F.W. Sharma *et al*. in (2015) reported a lower ascorbic acid content (10.12 mg/g) in *Pellia endiviifolia* (P.E) compared to the values we obtained for liverworts. However, it's pertinent to mention here that there is variation in ascorbic acid content among different species of liverworts. Environmental factors, growing conditions and geographical locations can also influence the ascorbic acid content in plants (Gahtori & Chaturvedi, 2020).



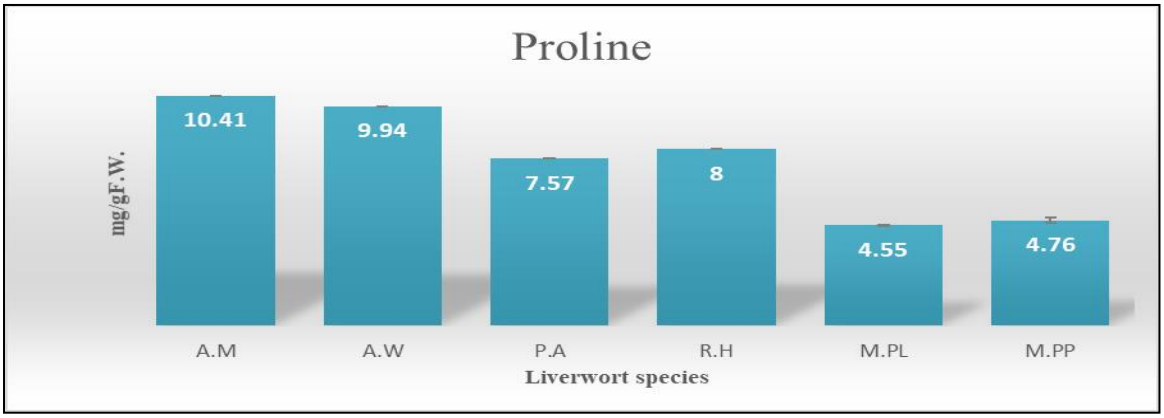
Graph 15: Ascorbic acid content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

4b. Total Phenols (mg GAE/g F.W.): Total phenols are a group of compounds known for their antioxidant properties and potential health benefits. Total phenol content of bryophytes in bryophytes, *Thuidium tamariscinum*, *Plathypnidium riparioides*, *Pellia endiviifolia* had already been known (Aslanba *et al*., 2017, Sharma *et al.*, 2015). During present investigation, M.PP (*M. papillata*), M.PL (*Marchantia paleacea*) and R.H (*Reboulia hemispherica*) have the highest levels of total phenols, with values of approx. 17 &14 mg GAE/g F.W., respectively. On the other hand, P.A has the lowest level at 11.95 mg GAE/g F.W. (Graph 16).



Graph 16: Total Phenol content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

4c. Proline (mg/g F.W.): Proline acts as an osmoprotectant in plants. This organic compound enhances plant resilience against non-living environmental factors by increasing photosynthesis, boosting both enzymatic and non-enzymatic antioxidant activities, regulating osmolyte levels and maintaining sodium and potassium balance (Hosseinifard *et al.*, 2022). The three liverworts *Plagiochasma appendiculatum*, *Pellia endiviifolia* (Sharma *et al*., 2015) and *Dumortiera hirsuta* (Yadav *et al.*, 2022) has already been known to be explored for proline content. Graph 17 represents the estimated proline content in different liverwort species during present investigation. Let's analyze the results:



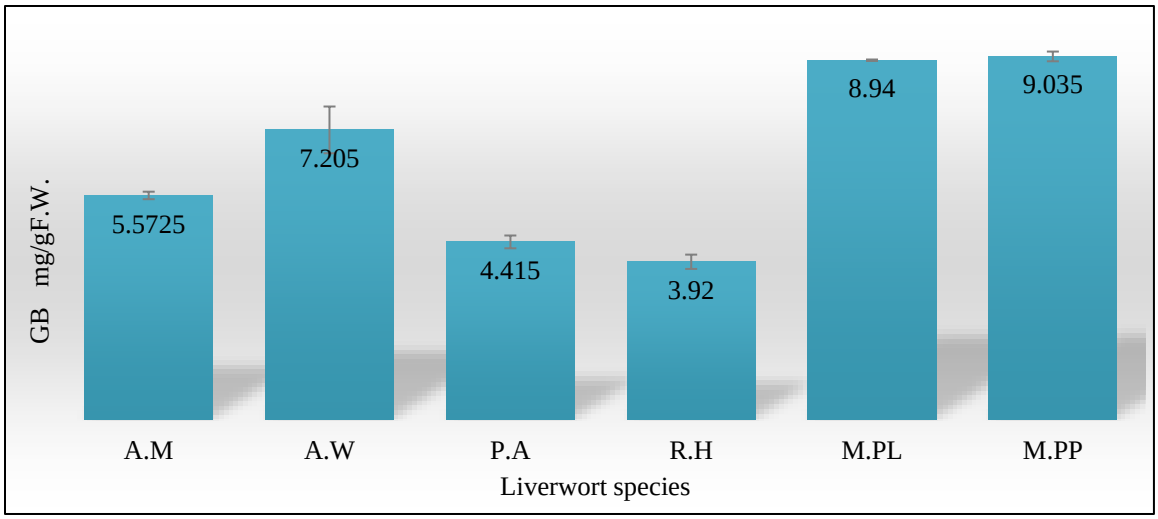
Graph 17: Proline content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Asterella multiflora* (10.41) and *A. wallichiana (9.94): These species have the highest proline content among the examined liverworts. High proline content often indicates plant stress, possibly due to environmental factors such as drought, high salinity, or extreme temperatures. This could suggest that *Asterella multiflora* is experiencing environmental stressors in its habitat.

***Plagiochasma appendiculatum* (7.57) *and Reboulia hemispherica* (8):** These liverwort species have a lower proline content compared to the *Asterella* species. While it still has a considerable amount of proline, it might be experiencing less severe stress compared to *Asterella multiflora* and *A. wallichiana.*

***Marchantia paleacea* (4.55) and *M. papillata* (4.76):** These two species have the lowest proline content among the given liverworts. It might indicate that these species are under relatively less stress compared to the others.

4d. Glycine-Betaine (mg/g F.W.): Glycine betaine is an osmoprotectant and a compatible solute, meaning it helps plants cope with various environmental stressors, such as salinity, drought and extreme temperatures. It plays a crucial role in protecting plants from these stresses by stabilizing proteins and membranes, regulating osmotic balance and acting as a reactive oxygen species (ROS) scavenger (Ali *et al.*, 2020).

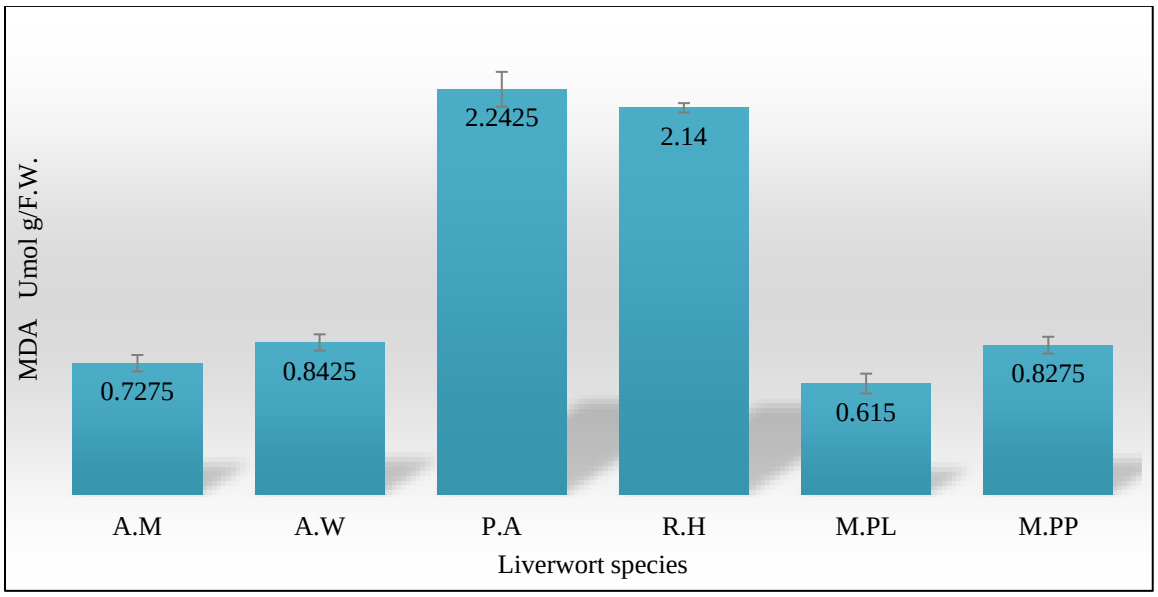


Graph 18: Glycine Betaine content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Graph 18 represents the results of estimated Glycine-Betaine content during present study. Liverworts M.PP and M.PL have the highest glycine betaine contents, suggesting that these liverwort species are potentially better adapted to tolerate environmental stressors due to their ability to accumulate higher levels of this osmoprotectant. Conversely, liverworts P.A and R.H have lower glycine betaine contents, which may indicate that they are less capable of coping with environmental stressors that could lead to osmotic stress (Ahmed & Prashad, 2011). The variation in glycine betaine content among different liverwort species could be due to genetic factors, environmental conditions, or a combination of both (Gahtori & Chaturvedi, 2020).

5. Oxidative Stress Markers:

5a. MDA Content: The malondialdehyde (MDA) content is 0.61-2.13 µmol/g F.W., which is often used as an indicator of lipid peroxidation and oxidative stress. The low MDA content indicates that *Marchantia* species have effective mechanisms to prevent lipid peroxidation, which can be harmful to cell membranes under stress conditions (Ayala *et al*., 2014). Graph 19 represents malondialdehyde (MDA) content in different liverwort species. MDA is a marker for lipid peroxidation, which occurs when cells are under oxidative stress.



Graph 19: MDA content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

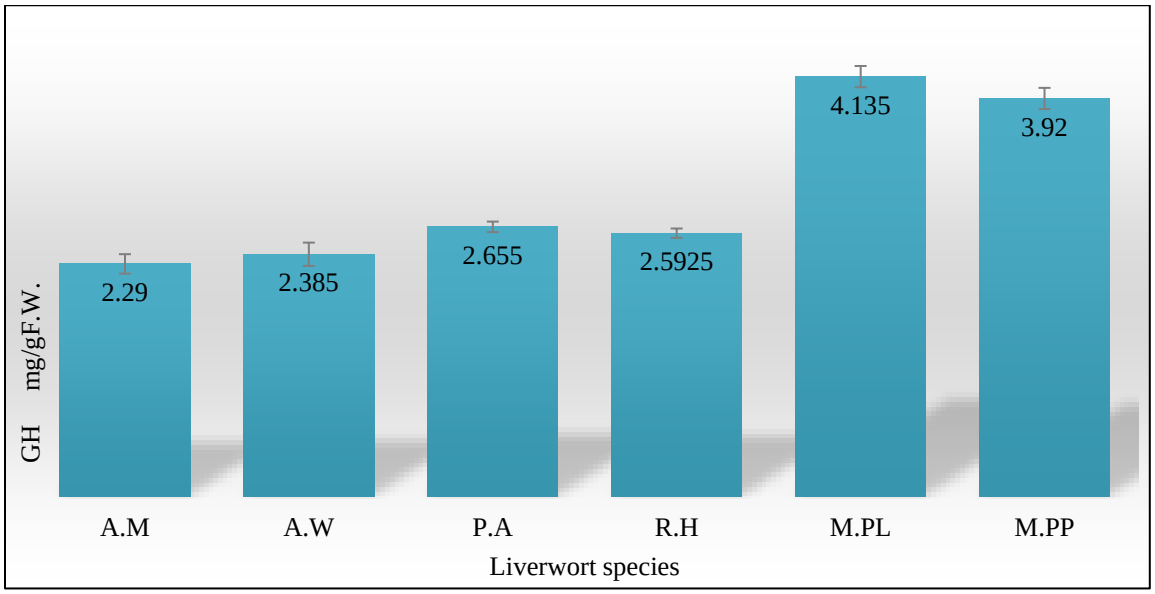
Here's an analysis of the estimated MDA content values (Graph 22):

***Asterella multiflora* (0.72) and *A. wallichiana* (0.84):** These species have relatively low MDA content compared to the other liverworts. Lower MDA levels suggest that these plants have lower lipid peroxidation, indicating less oxidative stress. It could be indicative of these species having efficient antioxidant defense mechanisms (Zhu *et al*., 2011).

***Plagiochasma appendiculatum* (2.24) and *Reboulia hemispherica* (2.13):** These liverwort species have significantly higher MDA content compared to *Asterella* species. Elevated MDA levels indicate higher lipid peroxidation and oxidative stress (Zhu *et al*., 2011).

***Marchantia paleacea* (0.61) and *M. papillata* (0.62):** Like *Asterella* species, these liverworts have relatively low MDA content. This suggests that *Marchantia paleacea* and *M. papillata* might have efficient antioxidant systems, helping them combat oxidative stress effectively (Zhu *et al*., 2011; Verma *et al*., 2024).

5b. Total Glutathione Content: Glutathione is an important antioxidant and plays an important role in cellular redox balance.

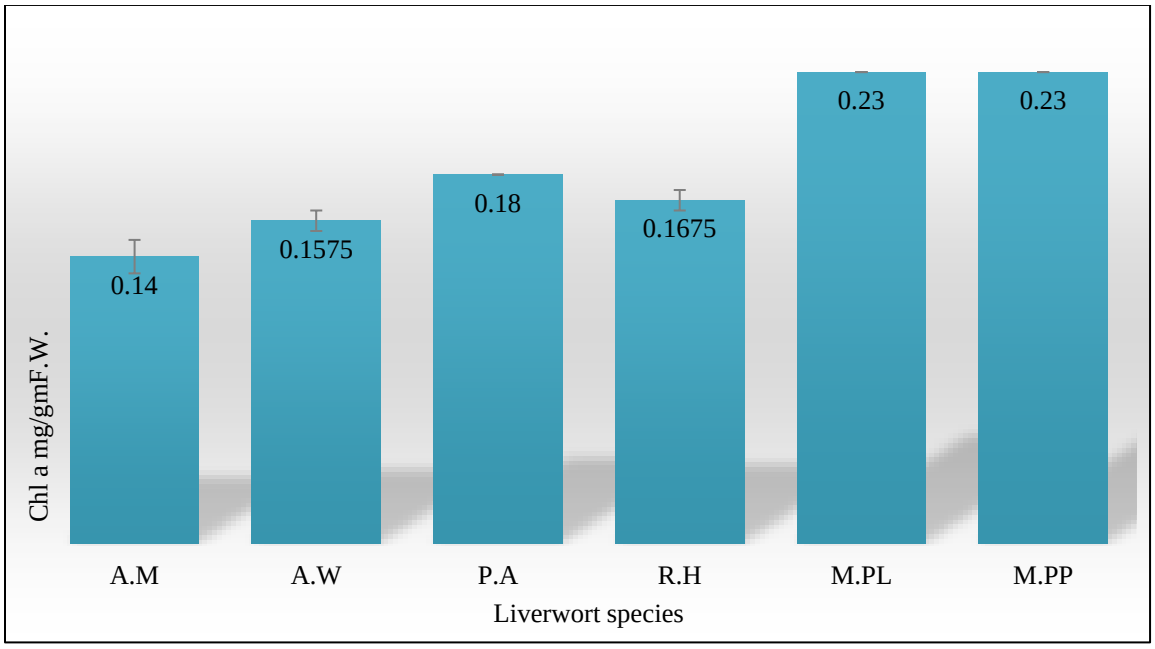


Graph 20: Total Glutathione content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Glutathione was identified as a crucial antioxidant compound in the land moss, *Pseudoscleropodium purum*. It serves as a valuable biomarker for monitoring pollution levels. (Varela *et al.*, 2018). The total glutathione content value was estimated between 2.38-4.13 mg/g F.W. during present study (Graph 20) indicating the presence of the antioxidant glutathione.

6. Chlorophyll and Carotenoids:

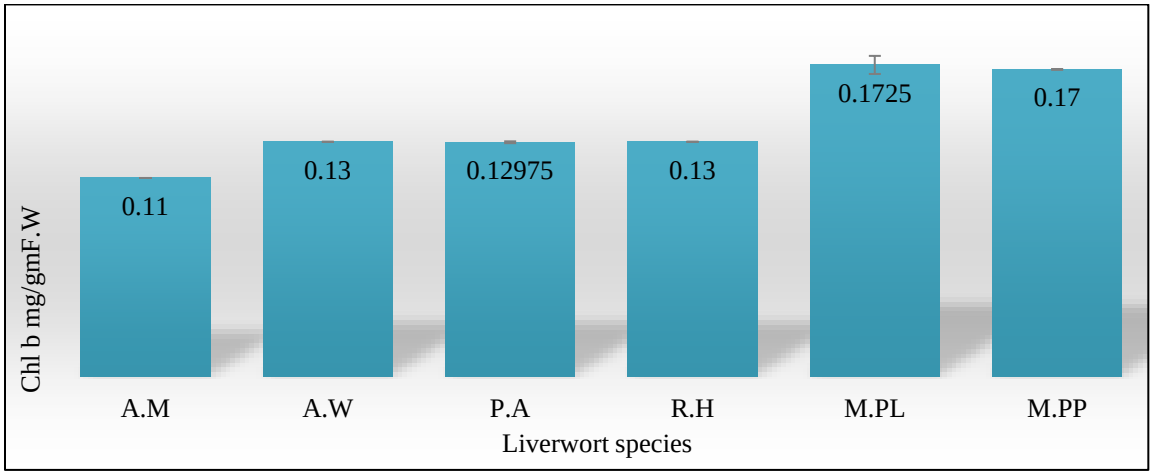
6a. Chlorophyll a: Chlorophyll a is a critical pigment involved in photosynthesis, primarily responsible for capturing light energy and converting it into chemical energy. The content of chlorophyll a was calculated between 0.14-0.23 mg/g F.W. (Graph 21), suggesting the presence of this pigment involved in photosynthesis.



Graph 21: Chlrophyll a content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

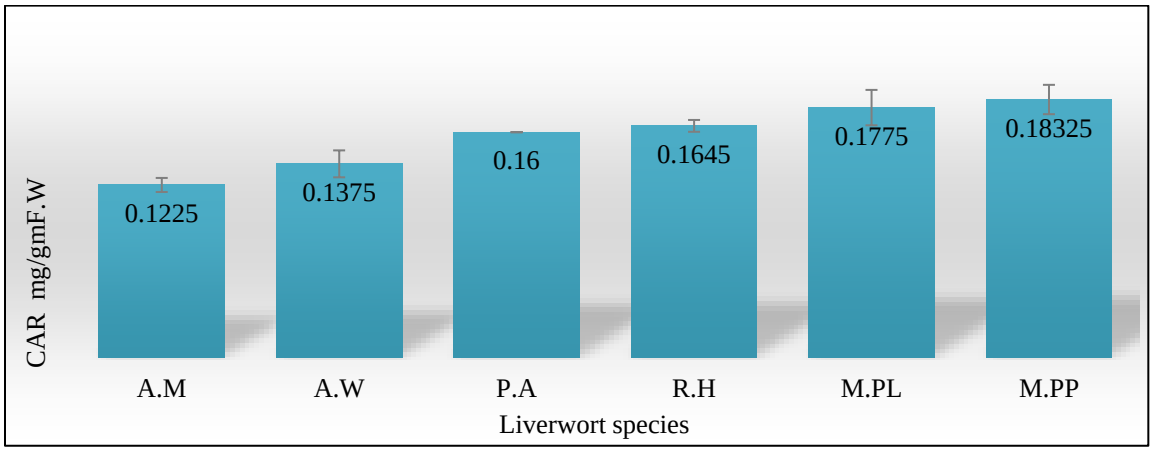
6b. Chlorophyll b: Chlorophyll b is another essential photosynthetic pigment that complements chlorophyll a by absorbing light at slightly different wavelengths. The content of chlorophyll b was estimated between 0.11 -0.17mg/g F.W. (Graph 22), indicating the presence of another photosynthetic pigment.

The balance between chlorophyll a and chlorophyll b is crucial for efficient light absorption and energy conversion during photosynthesis. The variations in chlorophyll b content may reflect adaptations to different light conditions or environmental factors (Khalid *et al*., 2023).



Graph 22: Chlorophyll b content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

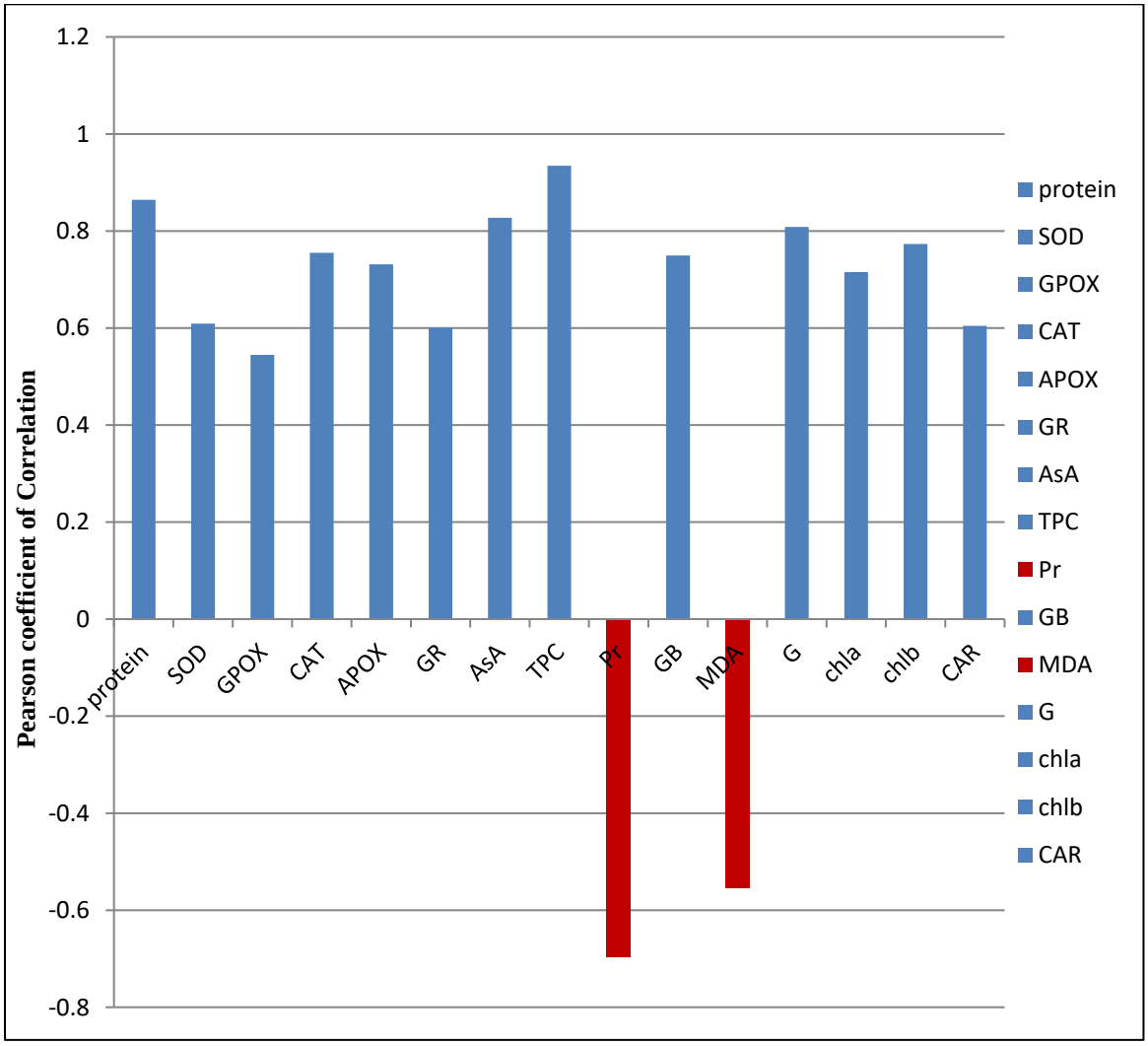
6c. Total Carotenoids: Carotenoids are pigments that have potential antioxidant functions and play a role in light-harvesting and photoprotection. The content of total carotenoids is 0.12-0.18 mg/g F.W. (Graph 23) suggesting the presence of these pigments with potential antioxidant functions.



Graph 23: Total Carotenoids content in liverworts, *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP).

Higher total carotenoid content in some samples may indicate a greater need for photoprotection against excess light or oxidative stress in their environments. Carotenoids can help dissipate excess light energy and scavenge reactive oxygen species (Murthy *et al*., 2005).

Relationship between mycorrhizal colonization and Antioxidants.



Graph 24: Graph representing Pearson coefficient of correlation between Percent Mycorrhizal Colonization (PMC) and Protein, Superoxide Dismutase (SOD), Guaiacol Peroxidase (GPOX), Catalase (CAT), Ascorbate Peroxidase activity (APOX), Glutathione reductase activity (GR), Ascorbic acid (ASA), Total Phenol content (TPC), Proline, Glycine-betaine (GB), MDA (Malondialdehyde), Glutathione (GSH), Chlorophyll a (Chl a), Chlorophyll (Chl b) and Carotenoids (CAR) content in studied liverworts species.

The correlation coefficients provided show the relationship between different antioxidants and mycorrhizal colonization in liverworts. Let's analyse the results:

Protein: There is a strong positive correlation (0.864) between protein content and mycorrhizal colonization (Graph 24). This suggests that mycorrhizal colonization positively influences the protein content in liverworts. Mycorrhizal fungi enhance the plant's nutrient absorption capabilities, especially in the case of phosphorus and nitrogen. Adequate nitrogen is essential for protein synthesis in plants (Marschner & Dell, 1994). By improving nitrogen uptake and assimilation, mycorrhizal associations can contribute to increased protein content in plants (Garg & Chandel, 2011; Verma *et al*., 2024).

Enzyme Activities:

Superoxide dismutase (SOD): SOD shows a moderate positive correlation (0.609) with mycorrhizal colonization. SOD is an enzyme that helps in detoxifying superoxide radicals, indicating an important role of arbuscular mycorrhizal colonization in oxidative stress regulation. Mycorrhizal plants often show increased activities of many antioxidant enzymes, including SOD,

Glutathione peroxidase (GPOX): GPOX exhibits a moderate positive correlation (0.545) with mycorrhizal colonization. GPOX is involved in the reduction of hydrogen peroxide, indicating a potential role in antioxidant defence during mycorrhizal colonization.

Catalase (CAT):CAT shows a strong positive correlation (0.755) with mycorrhizal colonization. It catalyses the decomposition of H_2O_2 to water and oxygen, suggesting an active antioxidant defence mechanism in mycorrhizal liverworts.

Ascorbate peroxidase (APOX): APOX displays a strong positive correlation (0.731) with mycorrhizal colonization. APOX is involved in the ascorbate-glutathione cycle, which is crucial for scavenging reactive oxygen species, indicating an efficient antioxidant system during mycorrhizal colonization.

Glutathione reductase (GR): GR shows a moderate positive correlation (0.601) with mycorrhizal colonization. GR plays an important role in the regeneration of reduced

glutathione, suggesting the importance of glutathione-related antioxidant processes in mycorrhizal liverworts.

In plants, mycorrhizal symbiosis has the potential to boost the activities of antioxidant enzymes, which in turn assists in the detoxification of reactive oxygen species (ROS). This is due to the fact that mycorrhizal fungi have the ability to enhance the nutritional status of plants, hence increasing the availability of vital nutrients to the plant that is being nourished (Marschner & Dell, 1994). In order for enzymes to be synthesized and activated, getting the right nourishment is really necessary. When plants receive nutrients through mycorrhizal networks, their overall antioxidant enzyme activity can increase, so, the enhanced enzyme activity can be attributed to the improved overall health (Yang *et al.*, 2023), moreover, mycorrhizal symbiosis can enhance the antioxidant defense system of plants, as it often enhances plant's tolerance to various stresses, such as drought, salinity and diseases (Singh *et al*., 2011; Mitra *et al*., 2021). According to Balestrasse and Tomaro (2001), plants may increase the production of enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPOX), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) in response to stress.

Ascorbic acid (AsA): AsA exhibits a strong positive correlation (0.827) with mycorrhizal colonization. AsA is a powerful antioxidant and its high correlation indicates an active antioxidant defence system in mycorrhizal liverworts. Ascorbic acid is a key component of the plant's antioxidant defence system, helping to neutralize reactive oxygen species (ROS) and protect cellular structures from oxidative damage (Das & Roychoudhury, 2014). The positive correlation suggests that mycorrhizal liverworts might be more efficient in producing or utilizing Ascorbic acid when they are colonized by mycorrhizal fungi (Singh *et al*., 2011; Mitra *et al*., 2021). This enhanced antioxidant defence system could be one of the mechanisms through which mycorrhizal associations contribute to the stress tolerance and overall health of liverworts in their natural environments (Chakraborty & Pradhan, 2012; Hussain *et al*., 2019).

Total phenolic content (TPC): TPC shows a very strong positive correlation (0.935) with mycorrhizal colonization. Phenolic compounds are known antioxidants and the high correlation suggests a significant role of phenolics in the antioxidant defence system during

mycorrhizal colonization. Mycorrhizal symbiosis can help plants cope with various stress factors, such as drought, salinity, or pathogen attacks (Avio *et al*., 2017., Singh *et al*., 2011).

Proline: Proline shows a moderate negative correlation (-0.697) with mycorrhizal colonization. Proline is an amino acid that acts as an osmoprotectant and antioxidant. The negative correlation might indicate a decrease in proline levels during mycorrhizal colonization. Mycorrhizal symbiosis can enhance the plant's tolerance to various stresses, including drought and salinity (Mitra *et al*., 2021). In stressful conditions, plants tend to accumulate proline as it acts as an osmolyte, helping plants maintain cell turgor and protect cellular structures (Aslanpour *et al*., 2016). If mycorrhizal fungi help the plant cope with stress more efficiently, the plant might accumulate less proline (Zhu *et al.*, 2011).

Glycine betaine (GB):GB exhibits a strong positive correlation (0.750) with mycorrhizal colonization. GB is a compatible solute and osmoprotectant, suggesting its role in stress tolerance during mycorrhizal colonization. Mycorrhizal fungi enhance the plant's nutrient uptake, including essential nutrients like nitrogen (Marschner & Dell, 1994). Better nutrient status in the plant can influence its overall metabolism, potentially leading to increased production of compounds like glycine betaine (Aslanpour *et al.*, 2016)

Malondialdehyde (MDA): MDA shows a moderate negative correlation (-0.555) with mycorrhizal colonization. MDA is a marker for oxidative stress and the negative correlation suggests a decrease in oxidative stress levels during mycorrhizal colonization. Mycorrhizal fungi can enhance a plant's antioxidant defense system. They can scavenge ROS directly or induce the plant to produce antioxidant enzymes, which help in reducing oxidative stress (Singh *et al*., 2011; Mitra *et al*., 2021). When oxidative stress is reduced, there is less damage to cell membranes, leading to lower MDA production (Zhu *et al.*, 2011).

Glutathione: Glutathione (GSH) displays a strong positive correlation (0.808) with mycorrhizal colonization. GSH is a key antioxidant molecule, indicating an active glutathione-related antioxidant defense system during mycorrhizal colonization. Mycorrhizal fungi can improve the plant's nutrient uptake, including essential elements like

sulfur. Glutathione is synthesized from cysteine, which is a sulfur-containing amino acid. By enhancing the plant's sulfur uptake, mycorrhizal fungi indirectly support the synthesis of glutathione (Ruiz-Sánchez *et al.*, 2010; Verma *et al*., 2024).

Photosynthetic Pigments:

Chlorophyll a (0.716): Chlorophyll a show a moderate positive correlation with mycorrhizal colonization, indicating a potential influence on photosynthetic processes in mycorrhizal liverworts.

Chlorophyll b (0.773): Chlorophyll b exhibits a strong positive correlation with mycorrhizal colonization, suggesting a potential enhancement of photosynthetic activity in mycorrhizal liverworts.

Carotenoids (0.604): Carotenoids show a moderate positive correlation with mycorrhizal colonization, indicating their potential role in photoprotection and antioxidant defence during mycorrhizal colonization.

Mycorrhizal associations can improve photosynthetic efficiency, ensuring that the plant produces energy more effectively (Ruiz-Sánchez *et al.*, 2010). This can lead to a reduction in oxidative stress, as efficient photosynthesis means less formation of ROS (Aslanpour *et al.*, 2016).

In summary, mycorrhizal colonization in liverworts appears to be associated with a robust antioxidant defence system, including enzymes like CAT, APOX and GSH, as well as phenolic compounds, chlorophylls and carotenoids. These antioxidants likely play a crucial role in stress tolerance and overall plant health during mycorrhizal interactions. The negative correlation with proline and MDA suggests a potential reduction in oxidative stress markers and osmoprotectants during mycorrhizal colonization.

CHAPTER-5

SUMMARY AND CONCLUSION

The present study is based on the 21 liverwort species belonging to two orders (Metzgeriales & Marchantiales) and 13 genera i.e. *Pellia endiviifolia, Asterella multiflora, A. wallichiana, Mannia foreaui, M. indica, Plagiochasma appendiculatum, P.intermedium, Reboulia hemispherica, Athalamia pusilla, Wiesnerella denudata, Marchantia paleacea, M. papillata, M. polymorpha, M. subintegra, Preissia quadrata, Riccia billardieri, R. cruciata, R. crystallina, R. discolor, R. melanospora* and *Targionia hypophylla*. The sites explored presently falls between an altitude of 287-720m in Jammu region (UT of Jammu & Kashmir). The liverwort species were examined for the arbuscular mycorrhizal fungi (AMF) in rhizoids and thallus. Only six i.e., *Asterella multiflora* (A.M), *A. wallichiana* (A.W), *Plagiochasma appendiculatum* (P.A), *Reboulia hemispherica* (R.H), *Marchantia paleacea* (M.PL) and *M. papillata* (M.PP) out of the 21 species of liverworts six have showed AM associations. The antioxidant potential of these six liverworts species were also estimated.

The aseptate AM fungi was frequently observed growing by forming H- and Y-connections in the smooth as well as tuberculated rhizoids. The recognition of Arbuscular Mycorrhizal Symbiosis relies on identifying characteristic intercellular hyphae, intracellular hyphae, vesicles, arbuscules and rhizosphere spores (Brundrett, 2002; Peterson *et al.*, 2004). Throughout the current research, these structures were commonly seen in six liverwort species, apart from intercellular hyphae in anatomical sections. The absence of intercellular hyphae is attributed to the liverwort's anatomy as the cells of the storage zone of the thallus are very compactly arranged and secondly, rhizoids are single celled. *Glomus aureum* spores were also isolated from the trap culture of the liverworts. AM was found to be influenced by several abiotic factors such as habitat, temperature and pH.

There were differences in the average percent mycorrhizal colonization (PMC) between and within species i.e., *Asterella multiflora (71.28), Asterella wallichiana* (69.11), *Plagiochasma appendiculatum* (77.15), *Reboulia hemispherica* (77.35), *Marchantia paleacea* (81.81) *and M. papillata* (82.11). Percent mycorrhizal colonization was observed between 69.11 to 82.11%. Maximum colonization (above 80%) was recorded in both species of *Marchantia.*

The six species of liverworts, *A. multiflora*, *A. wallichiana*, *P. appendiculatum*, *R. hemispherica*, *M. paleacea* and *M. papillata* were explored and investigated for the antioxidant potential. Protein, Superoxide Dismutase (SOD), Guaiacol Peroxidase (GPOX), Catalase (CAT), Ascorbate Peroxidase activity (APOX), Glutathione reductase activity (GR), Ascorbic acid (ASA), Total Phenol content (TPC), Proline, Glycine-betaine (GB), MDA (Malondialdehyde), Glutathione (GSH), Chlorophyll a (Chl a), Chlorophyll (Chl b) and Carotenoids (CAR) content of these liverworts were estimated. The antioxidant s of liverworts exhibited a good titrant of biochemicals. There is a significant correlation between Arbuscular Mycorrhiza and antioxidant activities of the liverworts. On the basis of present observations, following conclusions can be drawn:

Liverwort Species and Mycorrhizal Associations:

- Out of the 21 liverwort species studied, only six species showed associations with Arbuscular Mycorrhizal (AM) fungi.
- These mycorrhizal associations were found in the rhizoids and thallus of the liverwort species.
- The presence of AM associations varied between species, with the highest colonization observed in *Marchantia* species (*M. paleacea* and *M. papillata*) at above 80%.

Mycorrhizal Structures and Adaptations:

- Aseptate AM fungi were frequently observed, forming connections in both smooth and tuberculated rhizoids.
- Typical mycorrhizal structures like vesicles and arbuscules were found in association with liverwort rhizoids and thallus cells.
- Smooth walled rhizoids exhibited higher frequency of mycorrhizal colonization as compared to tuberculated rhizoids.

- Ventral scales were also devoid of any invasion by fungus.
- Sporophytic tissue was not invaded by fungus.
- Fungus invaded rhizoids and entered the thallus from ventral epidermis through rhizoids, where it grew intracellularly in the cells of the thalli and formed arbuscules in case of AM.
- Intercellular hyphae were absent in liverwort anatomy, likely due to the compact arrangement of thallus storage zone cells and the single-cell nature of rhizoids.
- *Glomus aureum* was isolated from the trap culture of rhizospheric soil of liverworts.

Influence of Abiotic Factors:

- AM presence was influenced by abiotic factors such as habitat, temperature and pH indicating the sensitivity of liverwort mycorrhizal associations to environmental conditions.
- The maximum AM colonization occurred in populations growing in clay soil and minimum on population growing on stone wall.
- There is no universal trend among these liverwort species regarding PMC and temperature. Some species show an increase in PMC with rising temperatures, some remain stable and others decrease.
- Alkaline soil seems to harbour higher mycorrhizal colonization.

Antioxidant Potential:

- The antioxidant potential of six liverwort species (*A. multiflora, A. wallichiana, P. appendiculatum, R. hemispherica*, *M. paleacea* and *M. papillata*) were investigated.
- Various biochemical parameters like Protein, Superoxide Dismutase (SOD), Guaiacol Peroxidase (GPOX), Catalase (CAT), Ascorbate Peroxidase activity (APOX), Glutathione reductase activity (GR), Ascorbic acid (ASA), Total Phenol

content (TPC), Proline, Glycine-betaine (GB), MDA (Malondialdehyde), Glutathione (GSH), Chlorophyll a (Chl a), Chlorophyll (Chl b) and Carotenoids (CAR) were estimated to assess the antioxidant system.

- The value of protein content was 5.28-7.69 mg/g fresh weight (F.W.). It varies among the different liverwort species. *Marchantia papillata* (M.PL) *and M. paleacea* (M.PP) have the highest protein content, followed by *Reboulia hemispherica* (R.H), *Asterella multiflora* (A.M), *Asterella wallichiana* (A.W) and *Plagiochasma appendiculatum* (P.A.).

- The range of unit activity of SOD, GPOX, CAT, APOX and GR in the liverworts varies from 0.05-0.1, 0.26-0.29, 0.015-0.018, 1.67-2.16 and 2.28-2.29 UA/mg protein resp.

- The values of biochemicals like Ascorbic Acid, Total Phenol, Glycine betaine, Glutathione and Carotenoids were observed to be significantly high in both species of *Marchantia.*

Correlation between Mycorrhiza and Antioxidant Activities:

- A significant correlation was found between the presence of AM associations and the antioxidant activities of the liverworts.

- SOD, GPOX, CAT, APOX, GR, ASA, TPC, GB, GSH, Chl a, Chl b and CAR were observed to be positively correlated with mycorrhiza, whereas Proline and MDA content negatively.

- Mycorrhizal colonization in liverworts appears to be associated with a robust antioxidant defense system, including enzymes like CAT, APOX and GSH as well as phenolic compounds, chlorophylls and carotenoids. These antioxidants likely play a crucial role in stress tolerance and overall plant health during mycorrhizal interactions.

- The negative correlation with proline and MDA suggests a potential reduction in oxidative stress markers and osmoprotectants during mycorrhizal colonization.

- This correlation suggests a potential interplay between mycorrhizal interactions and the liverwort's antioxidant defense mechanisms.

Implications and Future Research:

- The study underscores the importance of mycorrhizal associations in liverwort species particularly concerning their antioxidant capabilities.
- Further research can explore the specific mechanisms through which mycorrhizal interactions enhance the antioxidant potential of liverworts.
- Understanding these interactions could have broader ecological implications, shedding light on the symbiotic relationships between plants and fungi in various environmental contexts.

These conclusions highlight the intricate relationship between liverwort species, arbuscular mycorrhizal fungi and their antioxidant defense systems providing valuable insights for both ecological and botanical research.

BIBLIOGRAPHY

Aalipour, H., Nikbakht, A., Ghasemi, M., & Amiri, R. (2020). Morpho-physiological and biochemical responses of two turfgrass species to arbuscular mycorrhizal fungi and humic acid under water stress condition. *Journal of Soil Science and Plant Nutrition*, *20*, 566-576.

Aebi, H. (1984). Catalase in vitro. *Methods in enzymology*, *105*, pp.121-126.

Ahmad, P. & Prasad, M.N.V. eds., (2011). *Environmental adaptations and stress tolerance of plants in the era of climate change*. Springer Science & Business Media.

Alam, A. (2021). Potential of bryophytes in prevention and medication of COVID-19. *Ann Phytomedicine*, *10*, 121-29.

Ali, S., Abbas, Z., Seleiman, M. F., Rizwan, M., YavaŞ, İ., Alhammad, B. A., & Kalderis, D. (2020). Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. *Plants*, *9*(7), 896.

Allen, M. F. (2007). Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal*, *6*(2), 291-297.

Antoine, S., Hériché, M., Boussageon, R., Noceto, P.A., van Tuinen, D., Wipf, D., Courty, P.E. (2021). A historical perspective on mycorrhizal mutualism emphasizing arbuscular mycorrhizas and their emerging challenges. *Mycorrhiza*, 31, 637–653.

Asakawa, Y., Ludwiczuk, A., & Nagashima, F. (2013a). Phytochemical and biological studies of bryophytes. *Phytochemistry*, *91*, 52-80.

Asakawa, Y., Ludwiczuk, A., & Nagashima, F. (2013b). *Chemical constituents of bryophyta* (pp. 563-605). Springer Vienna.

Aslanba, B., Yilmaz, S., Yayintaş, Ö. T., Özyurt, D., & Öztürk, B. D. (2017). Total phenol content and antioxidant activity of mosses from Yenice forest (Ida Mountain). *Journal of Scientific Perspectives*, *1*(1), 1-12.

Aslanpour, M., Baneh, H. D., Tehranifar, A., & Shoor, M. (2016). The effect of mycorrhizal fungi on the amount of glycine betaine, soluble sugar, proline, leaf

water content and leaf chlorophyll of the white seedless grape under drought stress conditions. *International Journal of Advanced Biotechnological Research*, *7*, 1119-1133.

Atwood, J. J., Buck, W. R., & Brinda, J. C. (2024). Recent literature on bryophytes—127 (1). *The Bryologist*, *127*(1), 123-156.

Avio, L., Sbrana, C., Giovannetti, M., & Frassinetti, S. (2017). Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. *Scientia Horticulturae*, *224*, 265-271.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, *2014*.

Bagyaraj, D. J. (1991). Ecology of vesicular-arbuscular mycorrhizae. In: *Handbook of Applied Mycology: Soil and Plants* (eds DK Arona, B Rai, KG Mukerjii & GR Knudsen), 3–34. Marcel Dekker, New York, USA.

Balestrasse, K. B., Gardey, L., Gallego, S. M., & Tomaro, M. L. (2001). Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. *Functional Plant Biology*, *28*(6), 497-504.

Basile, A., Giordano, S., López-Sáez, J. A., & Cobianchi, R. C. (1999). Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry*, *52*(8), 1479-1482.

Bates, L.S., Waldren, R.P. and Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), pp.205-207.

Beaverie, K. (1902). E¨tudesurles symbiose bryophytes. *Ann.Jard. Bot.Buitenz*.4:107-138.

Begara-Morales, J. C. (2018). GSNOR regulates VND7-mediated xylem vessel cell differentiation. *Plant and Cell Physiology*, *59*(1), 5-7.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N. &Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science*, *10*, p.1068.

Berdaguer, R., van der Wielen, N., Lorenzo, Z.C., Testerink, C. and Karlova, R., 2024. The bryophyte rhizoid-sphere microbiome responds to water deficit. *Plant, Cell & Environment*. 2024 Jun 30.

Beyschlag, W. (2024). Bryophytes. In *Biology of Algae, Lichens and Bryophytes* (pp. 475-603). Berlin, Heidelberg: Springer Berlin Heidelberg.

Bharathi, S., & Varier, D. (2024). Mycorrhiza and its Ecological Significance. In *Industrial Applications of Soil Microbes* (pp. 104-114). Bentham Science Publishers.

Bhattarai, H.D., Paudel, B., Lee, H.K., Oh, H. & Yim, J.H. (2009). In vitro antioxidant capacities of two Benzonaphthoxanthenones: ohioensins F and G, isolated from the antarctic moss *Polytricha strumalpinum*. *Zeitschrift für Naturforschung C*, *64*(3-4), pp.197-200.

Bhattarai, H.D., Paudel, B., Lee, H.S., Lee, Y.K.& Yim, J.H. (2008). Antioxidant activity of *Sanionia uncinata*, a polar moss species from King George Island, Antarctica. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 22(12), pp.1635-1639.

Bidartondo, M.I., Bruns, T.D., Weiß, M., Sérgio, C.& Read, D.J. (2003). Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1517), pp.835-842.

Bidartondo, M. I., Read, D. J., Trappe, J. M., Merckx, V., Ligrone, R., & Duckett, J. G. (2011). The dawn of symbiosis between plants and fungi. *Biology letters*, *7*(4), 574-577.

Bohrer, G., Kagan-Zur, V., Roth-Bejerano, N., & Ward, D. (2001). Effects of environmental variables on vesicular-arbuscular mycorrhizal abundance in wild populations of *Vangueria infausta. Journal of Vegetation Science*, 12(2), 279-288.

Botnen, S., Kauserud, H., Carlsen, T., Blaalid, R., & Høiland, K. (2015). Mycorrhizal fungal communities in coastal sand dunes and heaths investigated by pyrosequencing analyses. *Mycorrhiza*, *25*, 447-456.

Boullard, B. (1988). Observations on the coevolution of fungi with hepatics. *Coevolution of fungi with plants and animals/*edited by KA Pirozynski and DL Hawksworth. 107 – 124.AcademicPress, London, UK.

Bowman, J. L., Araki, T., Arteaga-Vazquez, M. A., Berger, F., Dolan, L., Haseloff, J. & Kohchi, T. (2016). The name of names: guidelines for gene nomenclature in *Marchantia*. *Plant and Cell Physiology*, *57*(2),257-261.

Brito, I., Carvalho, M., Alho, L., & Goss, M. J. (2014). Managing arbuscular mycorrhizal fungi for bioprotection: Mn toxicity. *Soil Biology and Biochemistry*, *68*, 78-84.

Brundrett, M. C., & Abbott, L. K. (1994). Mycorrhizal fungus propagules in the jarrah forest: I. Seasonal study of inoculum levels. *New Phytologist*, *127*(3), 539-546.

Brundrett, M. (1994). Spores of glomalean fungi. *Practical methods in mycorrhiza research*, 35-40.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *NewPhytologist*,154, 275–304.

Bucher, M. (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytologist*, 173(1), 11-26.

Bücking, H., Liepold, E. & Ambilwade, P. (2012). The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying these transport processes. *Plant Sciences*, *4*, pp.108-132.

Burrows, R. L. (2014). Glomalin production and infectivity of arbuscular-mycorrhizal fungi in response to grassland plant diversity. *American Journal of Plant Sciences, 2014*.

Bush, J. K. (2008). The potential role of mycorrhizae in the growth and establishment of Juniperus seedlings. In *Western North American Juniperus Communities: A Dynamic Vegetation Type* (pp. 111-130). New York, NY: Springer New York.

Cairney, J.W.G. (2000). Evolution of mycorrhiza systems. *Naturwissenschaften*, *87*(11), 467-475.

Cakmak, I. & Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant physiology*, *98*(4), pp.1222-1227.

Camenzind, T., Hempel, S., Homeier, J., Horn, S., Velescu, A., Wilcke, W., & Rillig, M. C. (2014). Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology*, *20*(12), 3646-3659.

Carafa, A., Duckett, J. G., & Ligrone, R. (2003). Subterranean gametophytic axes in the primitive liverwort *Haplomitrium* harbour a unique type of endophytic association with aseptate fungi. *New Phytologist*, 160(1), 185-197.

Cavers, F. (1904). On the structure and biology of *Fegatella conica*. *Annals of Botany*, 18(69), 87-120.

Cázares, E., & Trappe, J. M. (1993). Vesicular endophytes in roots of the Pinaceae. *Mycorrhiza*, *2*, 153-156.

Chahal, A. K., Chandan, G., Kumar, R., Chhillar, A. K., Saini, A. K., & Saini, R. V. (2020). Bioactive constituents of Emblica officinalis overcome oxidative stress in mammalian cells by inhibiting hyperoxidation of peroxiredoxins. *Journal of Food Biochemistry*, *44*(2), e13115.

Chahal, K., Rana, B., & Jha, M. (2022). Arbuscular Mycorrhizal Fungi: A Potentially Sustainable Alternative in Agriculture and other Ecosystems. *Strad Res*, 9, 296-307.

Chakraborty, U., & Pradhan, B. (2012). Oxidative stress in five wheat varieties (*Triticum aestivum* L.) exposed to water stress and study of their antioxidant enzyme defense system, water stress responsive metabolites and H_2O_2 accumulation. *Brazilian Journal of Plant Physiology*, 24, 117-130.

Chanda, D., Sharma, D., & Talukdar, A. (2024). Study on the diversity of Arbuscular Mycorrhizal (AM) Fungi in some medicinal plants of Meghalaya. *Indian J. Applied & Pure Bio. Vol*, *39*(2), 822-832.

Chandrasekaran, M., Chanratana, M., Kim, K., Seshadri, S., & Sa, T. (2019). Impact of arbuscular mycorrhizal fungi on photosynthesis, water status and gas exchange of plants under salt stress–a meta-analysis. *Frontiers in Plant Science*, *10*, 457.

Chaudhari, H.& Rajaram, K. (1925). A study of the fungal endophyte of *Marchantia nepalensis*. *Proceedings Plant Sciences*, 13(4),255-260.

Chen, W., Koide, R.T. &Eissenstat, D.M. (2018). Nutrient foraging by mycorrhizas: from species functional traits to ecosystem processes. *Functional Ecology*, *32*(4), pp.858-869.

Chassagne, F., Cabanac, G., Hubert, G., David, B., & Marti, G. (2019). The landscape of natural product diversity and their pharmacological relevance from a focus on the Dictionary of Natural Products. *Phytochemistry Reviews*, *18*, 601-622.

Cianciullo, P., Maresca, V., Sorbo, S., & Basile, A. (2021). Antioxidant and Antibacterial Properties of Extracts and Bioactive Compounds in Bryophytes. *Applied Sciences*, *12*(1), 160.

Christenhusz, M. J., & Byng, J. W. (2016). The number of known plants species in the worldanditsannual increase. *Phytotaxa*, *261*(3),201-217.

Cooke, R. (1977). *The biology of symbiotic fungi*. John Wiley and Sons.

Dagar, J.C.& Dagar, H.S. (1999). *Ethnobotany of aborigines of Andaman-Nicobar Islands*. Surya International Publications.

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in environmental science*, *2*, 53.

Davies Jr, F. T., Puryear, J. D., Newton, R. J., Egilla, J. N., & Grossi, J. A. S. (2001). Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). *Journal of Plant Physiology*, *158*(6), 777-786.

De, A. B. (2017). Association of *Glomus tenue* with the hepatic *Mannia fragrans* (Balb.) Frye & Clark. *International Journal of Advanced Research in Biological Sciences,* 4(8), 51-58.

Deepika, S., & Kothamasi, D. (2015). Soil moisture—a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. *Mycorrhiza*, *25*, 67-75.

Deepika, S., & Kothamasi, D. (2021). Plant hosts may influence arbuscular mycorrhizal fungal community composition in mangrove estuaries. *Mycorrhiza*, 31, 699-711.

Delaux, P.M. (2017). Comparative phylogenomics of symbiotic associations. *NewPhytologist*, 213(1), 89-94.

Desiro, A., Duckett, J.G., Pressel, S., Villarreal, J.C., & Bidartondo, M.I. (2013). Fungal symbioses in hornworts: a chequered history. *Proceedings of the Royal Society B: Biological Sciences*, 280(1759), 20130207.

Dey, M., & Ghosh, S. (2022). Arbuscular mycorrhizae in plant immunity and crop pathogen control. *Rhizosphere*, *22*, 100524.

Dey, A. and Mukherjee, A., 2015. Therapeutic potential of bryophytes and derived compounds against cancer. *Journal of acute disease*, 4(3), 236-248.

Dixit, P. (2021). Bryophytes, An Extensive Source of Antioxidants. In *Antioxidant-Based Therapies for Disease Prevention and Management* (pp. 203-219). Apple Academic Press.

Duckett, J. G., & Ligrone, R. (2005). A comparative cytological analysis of fungal endophytes in the sporophyte rhizomes and vascularized gametophytes of Tmesipteris and Psilotum. *Botany*, 83(11), 1443-1456.

Duckett, J. G., & Read, D. J. (1995). Ericoid mycorrhizas and rhizoid-ascomycete associations in liverworts share the same mycobiont: isolation of the partners and resynthesis of the associations in vitro. *New Phytologist*, 129(3), 439-447.

Duckett, J.G., and Pressel, S. (2018). The evolution of the stomatal apparatus: intercellular spaces and sporophyte water relations in bryophytes—two ignored dimensions. Philosophical Transactions of the Royal Society B: *Biological Sciences*, 373(1739),20160498.

Duckett, J.G., Carafa, A., and Ligrone, R. (2006). A highly differentiated glomeromycotean association with the mucilage-secreting antipodean liverwort *Treubia*; clues to the origins of mycorrhizas. *American Journal of Botany*.93, 797–813.

Duh, P.D., Du, P.C. and Yen, G.C., (1999). Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology*, 37(11), pp.1055-1061.

Dulger, B., Ergul, C.C., & Gucin, F. (2002). Antimicrobial activity of the macrofungus *Lepista nuda*. *Fitoterapia* 73:695–697.

Dziwak, M., Wróblewska, K., Szumny, A., & Galek, R. (2022). Modern use of bryophytes as a source of secondary metabolites. *Agronomy*, *12*(6), 1456.

El-Beltagi, H. S., & Mohamed, H. I. (2013). Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 41(1), 44-57.

Entry, J. A., Watrud, L. S., & Reeves, M. (1999). Accumulation of 137Cs and 90Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. *Environmental Pollution*, 104(3), 449-457.

Ertürk, Ö., Sahin, H., Ertürk, E.Y., Hotaman, H.E., Koz, B. & Özdemir, Ö. (2015). The antimicrobial and antioxidant activities of extracts obtained from some moss species in Turkey. *Herba Polonica*, 61(4), pp.52-65.

Fernández, I., Cosme, M., Stringlis, I. A., Yu, K., De Jonge, R., Van Wees, S. M., ... & Van Der Heijden, M. G. (2019). Molecular dialogue between arbuscular mycorrhizal fungi and the nonhost plant *Arabidopsis thaliana* switches from initial detection to antagonism. *New Phytologist*, 223(2), 867-881.

Feijen, F. A., Vos, R. A., Nuytinck, J., & Merckx, V. S. (2018). Evolutionary dynamics of mycorrhizal symbiosis in land plant diversification. *Scientific Reports*, *8*(1), 10698.

Fitter, A. H., Heinemeyer, A., & Staddon, P. L. (2000). The impact of elevated CO2 and global climate change on arbuscular mycorrhizas: a mycocentric approach. *The New Phytologist*, 147(1), 179-187.

Frank, B. (1885). Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. (Ber. Deutsch). *Bot. Ges*. 3:128-145.

Friese, C. F., & Allen, M. F. (1991). The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia*, *83*(4), 409-418.

Gahtori, D., & Chaturvedi, P. (2019). Bryophytes: A potential source of antioxidants (pp. 53-64). London, UK: *IntechOpen.*

Gallaud, I. **(**1905). E¨tudessur les mycorhizes endotrophes. Rev. Ge¨n.Bot.17,7-43.

Garg, N., & Chandel, S. (2011). Arbuscular mycorrhizal networks: process and functions. *Sustainable agriculture volume* 2, 907-930.

Gemma, J. N., Koske, R. E., & Flynn, T. (1992). Mycorrhizae in Hawaiian pteridophytes: occurrence and evolutionary significance. *American Journal of Botany*, 79(8), 843-852.

Glime, J.M., & Pócs, T. (2020). Volume4, Chapter8-6: Tropics: Epiphylls.

Glime, J. M., 2024. Roles of Bryophytes in Forest Sustainability—Positive or Negative?. *Sustainability, 16*(6), p.2359.

Golenkin, M. (1901). Die Mycorrhiza-ähnlichenBildungen de Marchantiales. Flora. 88, 348-350.

Gordon, M. (1990). The mechanism of antioxidant action in vitro. In *Food antioxidants* (pp. 1-18). Springer, Dordrecht.

Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for determination of water-soluble quaternary ammonium compounds. *Plant and soil*, 70, 303-307.

Han, Y., Zveushe, O. K., Dong, F., Ling, Q., Chen, Y., Sajid, S., ... & de Dios, V. R. (2021). Unraveling the effects of arbuscular mycorrhizal fungi on cadmium uptake and detoxification mechanisms in perennial ryegrass (*Lolium perenne*). *Science of The Total Environment*, *798*, 149-152.

Han, Y., Zhang, W., Xu, T., & Tang, M. (2022). Effect of arbuscular mycorrhizal fungi and posphorus on drought-induced oxidative stress and 14-3-3 proteins gene expression of *Populus cathayana*. *Frontiers in Microbiology*, 13, 934-964.

Han, S., Wang, X., Cheng, Y., Wu, G., Dong, X., He, X., & Zhao, G. (2023). Multidimensional analysis reveals environmental factors that affect community dynamics of arbuscular mycorrhizal fungi in poplar roots. *Frontiers in Plant Science*, 13, 1068527.

Hawksworth, D. L. (1988). The variety of fungal-algal symbioses, their evolutionary significance and the nature of lichens. *Botanical journal of the Linnean Society*, *96*(1), 3-20.

Harley, J. L. (1984). The Mycorrhizal Associations. In *Cellular interactions* (pp. 148-186). Berlin, Heidelberg: Springer Berlin Heidelberg.

Harper, C. J., Taylor, T. N., Krings, M., & Taylor, E. L. (2015). Arbuscular mycorrhizal fungi in a voltzialean conifer from the Triassic of Antarctica. *Review of Palaeobotany and Palynology*, 215, 76-84.

Hayman, D. S. (1970). Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season ans soil treatment. *Transactions of the British Mycological Society*, 54(1), 53-62.

Hayman, D. S. (1986). Mycorrhizae of nitrogen-fixing legumes. *American journal of Applied Microbiology and Biotechnology*, 2, 121-145.

Heath, R.L. &Packer, L. (1968). Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics*, *125*(1), pp.189-198.

Heinemeyer, A., & Fitter, A. H. (2004). Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: growth responses of the host plant and its AM fungal partner. *Journal of Experimental Botany*, 55(396), 525-534.

Hirata, T., Ashida, Y. & Mori, J. (2002). A 37-kDa peroxidase secreted from liverworts in response to chemical stress. *Phytochemistry*, 55, 197-202.

Hiremath, P. C., Harini Kumar, K. M., & Patil, C. V. (1990). Density of vesicular-arbuscular mycorrhizat fungi in different crops grown under black soil. *Current trends in mycorrhizal research. Haryana Agricultural University, Hisar, India*, 20-21.

Hosseinifard, M., Stefaniak, S., Ghorbani Javid, M., Soltani, E., Wojtyla, Ł., & Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A review. *International Journal of Molecular Sciences*, 23(9), 5186.

Hristozkova, M., Geneva, M., Stancheva, I., Iliev, I., & Azcón-Aguilar, C. (2017). Symbiotic association between golden berry (*Physalis peruviana*) and arbuscular mycorrhizal fungi in heavy metal-contaminated soil. *Journal of Plant Protection Research*,57, 173-184.

Humphrey, H.B. (1906). The development of *Fossombronia longiseta*, *Australian Annals of Botany*, 20(77),83-108.

Hussain, S., Rao, M. J., Anjum, M. A., Ejaz, S., Zakir, I., Ali, M. A., ... & Ahmad, S. (2019). Oxidative stress and antioxidant defense in plants under drought conditions. *Plant abiotic stress tolerance: agronomic, molecular and biotechnological approaches*, 207-219.

Irshad, M. & Chaudhuri, P.S. (2002). Oxidant-antioxidant system: role and significance in human body. *Indian Journal of Experimental Biology*, 40, 1233-1239.

Jinger, D., Kaushal, R., Kumar, R., Paramesh, V., Verma, A., Shukla, M., ... & Kumawat, S. (2023). Degraded land rehabilitation through agroforestry in India: Achievements, current understanding and future prospectives. *Frontiers in Ecology and Evolution*, 11, 1088796.

Johnson, N. C., Gehring, C., & Jansa, J. (2016). Mycorrhizal mediation of soil: fertility, structure and carbon storage. Elsevier.

Joner, E.J. & Leyval, C. (2000) Bioavailability of heavy metals in the mycorrhizosphere, trace elements in the rhizosphere. CRC Press, 173–193.

Kachroo, P. (1954). MORPHOLOGY OF REBOULIACEAE. II On some species of *Mannia Corda, Asterella Beauv*. and *Plagiochasma* L. et L. *The Journal of the Hattori Botanical Laboratory*, 12, 34-52.

Kapoor, A. & Mukerji, K.G. (1987). Vesicular-arbuscular mycorrhizae pattern of modern research. *Frontiers in Applied Microbiology*. 2: 190-199.

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8), 982.

Kazadi, A. T., Ansey, B. K., & Muzulukwau, J. M. (2020). Density of arbuscular mycorrhizal fungi genus on three host plants (*Sorghum bicolor L., Plantago lanceolata L. and Eleusine coracana L.*) in a Plinthosol in Lubumbashi (Democratic Republic of Congo). *Global Scientific Journal*, *8*(7).

Khalid, M., Liu, X., ur Rahman, S., Rehman, A., Zhao, C., Li, X., ... & Hui, N. (2023). Responses of microbial communities in rhizo compartments of king grass to phytoremediation of cadmium-contaminated soil. *Science of The Total Environment*, 904, 167226.

Khaliq, A., Perveen, S., Alamer, K. H., Zia Ul Haq, M., Rafique, Z., Alsudays, I. M. & Attia, H. (2022). Arbuscular mycorrhizal fungi symbiosis to enhance plant–soil interaction. *Sustainability*, 14(13), 7840.

Khan, A. G. (1974). The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of Endogone spores in adjacent soils. *Microbiology*, 81(1), 7-14.

Khan, A. G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of trace Elements in Medicine and Biology*, 18(4), 355-364.

Klironomos, J. N., & Kendrick, B. (1995). Relationships among microarthropods, fungi their environment. *Plant and Soil*, 170, 183-197. 15.

Kloppholz, S., Kuhn, H., & Requena, N. (2011). A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Current biology*, 21(14), 1204-1209.

Kobae, Y., Ohtomo, R., Morimoto, S., Sato, D., Nakagawa, T., Oka, N. & Sato, S. (2019). Isolation of native arbuscular mycorrhizal fungi within young thalli of the liverwort *Marchantia paleacea. Plants*, 8(6), 142.

Kono, Y. (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of Biochemistry and Biophysics*, 186(1), pp.189-195.

Kormanik, P. P. (1982). Quantification of vesicular-arbuscular mycorrhizae in plant roots. *Methods and principles of mycorrhizal research*, 37-46. 18.

Kottke, I. & Nebel, M. (2005). The evolution of mycorrhiza-like associations in liverworts: an update. *New Phytologist*, 167(2), pp.330-334.

Kottke, I., Beiter, A., Weiss, M., Haug, I., Oberwinkler, F.& Nebel, M. (2003). Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. *Mycological Research*, 107(8), pp.957-968.

Kranner, I. (1998). Determination of glutathione, glutathione disulphide and two related enzymes, glutathione reductase and glucose-6-phosphate dehydrogenase, in fungal and plant cells. In *Mycorrhiza manual* (pp. 227-241). Berlin, Heidelberg: Springer Berlin Heidelberg.

Krings, M., Taylor, T.N., Hass, H., Kerp, H., Dotzler, N., & Hermsen, E.J. (2007). Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution and host responses. *NewPhytologist*, *174*(3),648-657.

Krishnan, R. & Murugan, K. (2013). Polyphenols from *Marchantia polymorpha* L. a bryophyta: a potential source as antioxidants. *World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS)*, *2*(6), 5182-5198.

Krzyzanski, H., Gutierre, M. A. M., & Carrenho, R. (2022). Arbuscular mycorrhizal and dark septate fungi are not common in roots of epiphytic pteridophytes of a transitional forest area in South Brazil. *Acta Botanica Brasilica*, *35*, 569-576.

Kumar, P. S., Sucheta, S., Deepa, V. S., Selvamani, P., & Latha, S. (2008). Antioxidant activity in some selected Indian medicinal plants. *African Journal of Biotechnology*, 7(12).165-173.

Kumar, A. and Purohit, S.S. (1997-98). Organic transport in plants. In "*Plant Physiology", Fundamentals and Applications*. Updesh Purohit (ed.), Agro Botanical Publications.

Kumar, R., Meena, R. K., Tak, Y., & Chachaiya, P. K. (2024). Fungi Boost Up Plant Growth by Improving Nutrient Uptake: A Review. *Environment and Ecology*, *42*(3B), 1364-1368.

Kumari, P. (2015). Studies on endophytic fungal association in bryophytes of some selected sites of Uttarakhad. Ph. D thesis, University of Delhi, Delhi.

Kumari, P., Bhatia, M., Giri, P. and Uniyal, P.L., 2024. Mycorrhizal association and its relation with pteridophytes. *Frontiers in Microbiology*, *15*, p.1406891.

Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, 15(1), 1-22.

Lara-Pérez, L. A., Valdés-Baizabal, M. D., Noa-Carrazana, J. C., Zulueta-Rodríguez, R., Lara-Capistrán, L., & Andrade-Torres, A. (2015). Mycorrhizal associations of ferns and lycopods of central Veracruz, Mexico. *Symbiosis*, 65, 85-92.

Lehnert, H., Serfling, A., Enders, M., Friedt, W., & Ordon, F. (2017). Genetics of mycorrhizal symbiosis in winter wheat (*Triticum aestivum*). *New Phytologist*, 215(2), 779-791.

Lewis, D. H. (1987). Evolutionary aspects of mutualistic associations between fungi and photosynthetic organisms. *Evolutionary biology of the Fungi*, 161-178.

Leyval, C., Turnau, K., & Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza*, 7, 139-153.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in enzymology* ,148, 350-382.

Liepiņa, L. (2012). Occurrence of fungal structures in bryophytes of the boreo-nemoral zone. *Environmental Experimental Biology*, *10*, 35-40.

Ligrone, R. **(**1988). Ultrastructure of a fungal endophyte in *Phaeoceros laevis* (L.) Prosk. (Anthoceratophyta). *Botanical Gazette*.149, 92-100.

Ligrone, R., & Lopes, C. **(**1989). Cytology and development of a mycorrhiza-like infection in the gametophyte of *Conocephalum conicum* (L.) Dum. (Marchantiales, Hepatophyta). *New Phytologist.* 111, 423-433.

Ligrone, R., Carafa, A., Bonfante, P., Bianciotto, V., & Duckett, J.G. (2007). Glomeromycotean associations in liverworts: A molecular, cytological and taxonomical survey. *American Journal of Botany*, 94, 1756 – 1777.

Ligrone, R., Pocock, K., & Duckett, J. G. (1993). A comparative ultrastructural study of endophytic basidiomycetes in the parasitic achlorophyllous hepatic *Cryptothallus mirabilis* and the closely allied photosynthetic species *Aneura pinguis* (Metzgeriales). *Canadian Journal of Botany*, *71*(5), 666-679.

Liu, Y., He, J., Shi, G., An, L., Öpik, M., & Feng, H. (2011). Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. *FEMS Microbiology Ecology*, 78(2), 355-365.

Lokhandwala, A., & Hoeksema, J. D. (2019). Priming by arbuscular mycorrhizal fungi of plant antioxidant enzyme production: a meta-analysis. *Annual Plant Reviews*, 2, 1069-1084.

Lovelock, C. E., & Robinson, S. A. (2002). Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function. *Plant, Cell & Environment*, 25(10), 1239-1250.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.

Lugo, M. A., González Maza, M. E., & Cabello, M. N. (2003). Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia*, 95(3), 407-415.

Lumini, E., Orgiazzi, A., Borriello, R., Bonfante, P., & Bianciotto, V. (2010). Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environmental Microbiology*, 12(8), 2165-2179.

Lutgen, E. R., Muir-Clairmont, D., Graham, J., & Rillig, M. C. (2003). Seasonality of arbuscular mycorrhizal hyphae and glomalin in western Montana grassland. *Plant and Soil*, 257, 71-83.

Magrou, J. (1927). Mycorrhiza Fungi and their role in plant growth. *Proceedings International Congress of Soil Science,* 111, 26-28.

Manoj, G.S. and Murugan, K. (2012). Phenolic profiles, antimicrobial and antioxidant potentiality of methanolic extract of a liverwort, *Plagiochila beddomei* Steph. *Indian Journal of Natural Products and Resources*, 3(2), pp.173-183.

Manoj, G.S., Krishnan, R. & Murugan, K. (2012). In vitro antioxidant potentiality and total phenolic content in selected bryophytes. *Asian Pacific Journal of Tropical Biomedical*, 2, pp.1-8.

Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and soil*, 159, 89-102.

Martin, F. M., & van Der Heijden, M. G. (2024). The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. *New Phytologist*, *242*(4), 1486-1506.

Mathew, M., Babu, T., & Mathew, A. (2023). Antioxidant capacity of selected bryophytes from Idukki district, Kerala. In *Proceedings of International Conference on Recent Advances in Biological Science* ,104.

McConaha, M. (1941). Ventral structures affecting capillarity in the Marchantiales. *American Journal of Botany*, 301-306.

Mejstrik, V. (1965). Study of the development of endotrophic mycorrhiza in the association of *Cladietum marisci.*

Mitra, D., Djebaili, R., Pellegrini, M., Mahakur, B., Sarker, A., Chaudhary, P. & Mohapatra, P. K. D. (2021). Arbuscular mycorrhizal symbiosis: plant growth improvement and induction of resistance under stressful conditions. *Journal of Plant Nutrition*, 44(13), 1993-2028.

Mizoguchi, T. (1992). Effects of inoculation of vesicular-arbuscular mycorrhizal fungi on growth and nutrient uptake of non-nodulated *Acacia* spp. seedlings in two soil water regimes. *Journal of the japanese forestry society*, 74(5), 409-419.

Mohandas, G. G., & Kumaraswamy, M. (2018). Antioxidant activities of terpenoids from *Thuidium tamariscellum* (C. Muell.) Bosch. and *Sande-Lac*. a Moss. *Pharmacognosy Journal*, 10(4).645-649.

Murthy, K.C., Vanitha, A., Rajesha, J., Swamy, M.M., Sowmya, P.R. & Ravishankar, G.A. (2005). In vivo antioxidant activity of carotenoids from *Dunaliella salina*—a green microalga. *Life Sciences*, 76(12), pp.1381-1390.

Muthukumar, T., & Prabha, K. (2013). Arbuscular mycorrhizal and septate endophyte fungal associations in lycophytes and ferns of south India. *Symbiosis*, 59, 15-33.

Muthukumar, T., & Udaiyan, K. (2000). Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. *Mycorrhiza, 9,* 297-313.

Muthukumar, T., & Udaiyan, K. (2002). Arbuscular mycorrhizas in cycads of southern India. *Mycorrhiza*, 12, 213-217.

Muthukumar, T., Sathiyaraj, G., Priyadharsini, P., Uma, E., & Sathiyadash, K. (2014). Arbuscular mycorrhizal and dark septate endophyte fungal associations in ferns and lycophytes of Palni Hills, Western Ghats, southern India. *Brazilian Journal of Botany*, 37, 561-581.

Muthuraja, R., Muthukumar, T., Sathiyadash, K., Uma, E., & Priyadharsini, P. (2014). Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal association

in lycophytes and ferns of the Kolli Hills, Eastern Ghats, Southern India. *American Fern Journal*, 104(2), 67-102.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, *22*(5), 867-880.

Nebel, M., Kreier, H.P., Preussing, M., Weiss, M. & Kottke, I. (2004). Symbiotic fungal associations of liverworts are the possible ancestors of mycorrhizae. *Frontiers in Basidiomycote Mycology*, 339-360.

Negi, J. S., Bisht, V. K., Singh, P., Rawat, M. S. M., & Joshi, G. P. (2013). Naturally occurring xanthones: chemistry and biology. *Journal of Applied Chemistry*, *2013*.

Nemec, B. **(**1899). Die Mykorrhizae iniger Lebermoose. *Berichteder Deutschen Botanischen Gesellschaf,*.17, 311 – 317.

Newman, E.I. & Redell, P. **(**1987). The distribution of mycorrhizas among families of vascular plants. New Phytologist. 106, 745-751.

Nicolson, T. H. (1967). Vesicular-arbuscular mycorrhiza—a universal plant symbiosis. *Science Progress (1933-)*, 561-581.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y.I., Neukermans, J., Marquez-Garcia, B, Queval, G. & Foyer, C.H. (2012). Glutathione in plants: an integrated overview. *Plant Cell &Environment*, 35(2),454-484.

O'keefe, D. M., & Sylvia, D. M. (1993). Seasonal dynamics of the association between sweet potato and vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza*, *3*, 115-122.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., & Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology*, 69(5), 2816-2824.

Okayama, Y. (2005). Oxidative stress in allergic and inflammatory skin diseases. *Current Drug Targets-Inflammation & Allergy*, 4(4), 517-519.

Osonubi, O., Mulongoy, K., Awotoye, O. O., Atayese, M. O., & Okali, D. U. U. (1991). Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. *Plant and soil*, 136, 131-143.

Oyedapo, O. O., Makinde, A. M., Ilesanmi, G. M., Abimbola, E. O., Akinwunmi, K. F., & Akinpelu, B. A. (2015). Biological activities (anti-inflammatory and antioxidant) of fractions and methanolic extract of Philonotis hastate (Duby Wijk & MargaDant). *African Journal of Traditional, Complementary and Alternative Medicines*, 12(4), 50-55.

Pandey, S., Rana, M., & Rawat, B. (2021). Antioxidant potential of polyphenol rich liverworts from the Western Himalayas. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 13(3), 485-492.

Paciolla, C., & Tommasi, F. (2003). The ascorbate system is made in two bryophytes: *Brachythecium velutinum* and *Marchantia polymorpha*. *Biologia Plantarum*, 47, 387-393.

Padamsee, M., Johansen, R. B., Stuckey, S. A., Williams, S. E., Hooker, J. E., Burns, B. R., & Bellgard, S. E. (2016). The arbuscular mycorrhizal fungi colonising roots and root nodules of New Zealand kauri *Agathis australis*. *Fungal Biology*, 120(5), 807-817.

Parke, J.L., & Linderman, R.G. (1980). Association of vesicular-arbuscular mycorrhizal fungiwiththemoss *Funaria hygrometrica*. *Canandian. Journal of Botany*,58,1898-1904.

Pérez, Y., & Schenck, N. C. (1990). A unique code for each species of VA mycorrhizal fungi. *Mycologia*, 82(2), 256-260.

Peterson, R.L., Massicotte, H.B., & Melville, L.H. (2004). Mycorrhyzas: anatomy and cellbiology. NRC Research Press, Ottawa, Quebec, Canada.

Peyronel, B. (1969). Terminology of mycorrhizae. *Mycologia*, *61*, 410-411.

Pocock, K., & Duckett, J.G., (1984). A comparative ultrastructural analysis of the fungalendophytes in *Cryptothallus mirabilis Malm*. and other British thalloid hepatics. *Journal of Bryology*, 13(2), 227-233.

Pressel, S., Bidartondo, M. I., Field, K. J., & Duckett, J. G. (2021). Advances in understanding of mycorrhizal-like associations in bryophytes. *Bryophyte Diversity and Evolution*, 43(1), 284-306.

Pressel, S., Ligrone, R., Duckett, J., & Davis, E. (2008). A novel ascomycetous endophyticassociation in the rhizoids of the leafy liverwort family, Schistochilaceae (Jungermanniidae, Hepaticopsida). *American Journal of Botany*,95,531-541.

Pütter, J. (1974). Peroxidases. In *Methods of enzymatic analysis* (pp. 685-690). Academic Press.

Qu, J., Xie, C., Guo, H., Yu, W. and Lou, H. (2007). Antifungal dibenzofuran bis (bibenzyl) is from the liverwort *Asterella angusta*. *Phytochemistry*, 68(13), pp.1767-1774.

Rabatin, S. C. (1980). The occurrence of the vesicular-arbuscular-mycorrhizal fungus Glomus tenuis with moss. *Mycologia*, 72(1), 191-195.

Ragazzi, E. & Veronese, G. (1973). Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. *Journal of Chromatography*, 77(2), 369-375.

Ragupathy, S., Mohankumar, V., & Mahadevan, A. (1990). Occurrence of vesicular—arbuscular mycorrhizae in tropical hydrophytes. *Aquatic Botany*, *36*(3), 287-291.

Rajan, S. S., & Murugan, K. (2010). Purification and kinetic characterization of the liverwort *Pallavicinia lyelli* (Hook.) S. Gray. cytosolic ascorbate peroxidase. *Plant Physiology and Biochemistry*, 48(9), 758-763.

Rao, A. C., & Reddy, A. R. (2008). Glutathione reductase: a putative redox regulatory system in plant cells. In *Sulfur assimilation and abiotic stress in plants* (pp. 111-147). Berlin, Heidelberg: Springer Berlin Heidelberg.

Rawat, K. K., Verma, P. K., & Alam, A. (2015). Nomenclatural updates in Kashyap's 'Liverwort flora of western Himalayas and Panjab Plains'. *Plant Science Today*, 2(4), 179-183.

Rayner, M.C. (1927). Mycorrhiza. An account of non-pathogenic infection by fungi in vascular plants and Bryophytes. *New Phytologist*, Reprint no. 15. London, UK.

Read, D. J. (1991). Mycorrhizas in ecosystems. *Experientia*, 47, 376-391.

Read, D.J., Duckett, J.G., Francis, R., Ligrone, R. & Russell, A. (2000). Symbiotic fungal associations in 'lower'land plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 355(1398), 815-831.

Redecker, D., Kodner, R., & Graham, L. E. (2000). Glomalean fungi from the Ordovician. *Science*, 289(5486), 1920-1921.

Reid, C. P. P., & Bowen, G. D. (1979). Effects of soil moisture on V/A mycorrhiza formation and root development in Medicago. In *The soil–root interface* (pp. 211-219). Academic Press.

Ridler, W.F. (1923). The fungus is present in *Pellia epiphylla* (L.) Corda. Ann. Bot. 36, 193-20

Rillig, M. C., & Field, C. B. (2003). Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO 2 as a function of soil depth. *Plant and Soil*, *254*, 383-391.

Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology letters*, *7*(8), 740-754.

Rimington, W.R., Pressel, S., Duckett, J.G. & Bidartondo, M.I. (2015). Fungal associations of basal vascular plants: reopening a closed book?. *New Phytologist*, 205(4), pp.1394-1398.

Rimington, W.R., Pressel, S., Duckett, J.G., Field, K.J., Read, D.J.& Bidartondo, M.I. (2018). Ancient plants with ancient fungi: liverworts associate with early diverging

arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B*, 285(1888), p.20181600.

Rimington, W.R., Duckett, J.G., Field, K.J., Bidartondo, M.I. &Pressel, S. (2020). The distribution and evolution of fungal symbioses in ancient lineages of land plants. *Mycorrhiza*, 30(1), pp.23-49.

Rosier, C. L., Hoye, A. T., & Rillig, M. C. (2006). Glomalin-related soil protein: assessment of current detection and quantification tools. *Soil Biology and Biochemistry*, 38(8), 2205-2211.

Rousk, K., Degboe, J., Michelsen, A., Bradley, R., & Bellenger, J. P. (2017). Molybdenum and phosphorus limitation of moss-associated nitrogen fixation in boreal ecosystems. *New Phytologist*, 214(1), 97-107.

Ruiz-Sánchez, M., Aroca, R., Muñoz, Y., Polón, R., & Ruiz-Lozano, J. M. (2010). The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. *Journal of Plant Physiology*, 167(11), 862-869.

Russell, J. E. (2002). Soil conditions and plants growth. Daya books.

Russell, J., & Bulman, S. (2005). The liverwort *Marchantia foliacea* forms a specialized symbiosis with arbuscular mycorrhizal fungi in the genus *Glomus. NewPhytologist*, 165(2), 567-579.

Saito, M. (2000). Symbiotic exchange of nutrients in arbuscular mycorrhizas: transport and transfer of phosphorus. In *Arbuscular mycorrhizas: physiology and function* (pp. 85-106). Springer, Dordrecht.

Samuel, S. S., & Veeramani, A. (2021). Advantages of arbuscular mycorrhizal fungi (AMF) production for the profitability of agriculture and biofertilizer industry. In *Mycorrhizal Fungi: Utilization in Agriculture and Forestry* (pp. 31-46). London, United Kingdom: IntechOpen.

Saroya, A. S., Singh, J., Saroya, A. S., & Singh, J. (2018). Medicinal Herbs Used in Herbal

Medicine for Neurological Disorders. *Pharmacotherapeutic Potential of Natural Products in Neurological Disorders*, 7-15.

Sathyarahini, K., Panneerselvam, P., & Ganeshamurthy, A. N. (2015). Studies on relationship between total glomalin and soil aggregates in perennial fruit crop orchards. *International Journal of Applied Pure Science & Agriculture*, 1(7), 47-52.

Schenck, N.C., & Perez, Y. (1990). Manual for Identification of Vesicular Arbuscular Mycorrhizal Fungi. (INVAM). University of Florida, Gainesville.

Schüßler, A. (2000). *Glomus claroideum* forms an arbuscular mycorrhiza-like symbiosis with the hornwort *Anthoceros punctatus*. *Mycorrhiza*, 10(1), 15-21.

Sedlak, J. & Lindsay, R.H. (1968). Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, *25*, 192-205.

Sharma, A. (2017). Studies on the diversity of Mycorrhizae associated with some bryophytes of Jammu Division. PhD Dissertation, University of Jammu, Jammu.

Sharma, A. & Langer, A. (2017). occurrence of mycorrhiza in a moss *encalypta vulgaris* hedw. *Journal of Plant Development Sciences*, 9(9), pp.881-883.

Sharma, A., Slathia, S., Gupta, D., Handa, N., Choudhary, S.P., Langer, A. & Bhardwaj, R., (2015). Antifungal and antioxidant profile of ethnomedicinally important liverworts (*Pellia endivaefolia* and *Plagiochasma appendiculatum*) used by indigenous tribes of district Reasi: Northwest Himalayas. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 85(2), pp.571-579.

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kumari, R., & Dhaka, N. (2013). Potential applications of antioxidants–A review. *Journal of pharmacy research*, *7*(9), 828-835.

Singh, L. P., Gill, S. S., & Tuteja, N. (2011). Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signaling &Behavior*, 6(2), 175-191.

Singh, M., Govindarajan, R., Nath, V., Rawat, A. K. S., & Mehrotra, S. (2006). Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *Journal of Ethnopharmacology*, 107(1), 67-72.

Siqueira, J. O., Hubbell, D. H., & Schenck, N. C. (1982). Spore germination and germ tube growth of a vesicular-arbuscular mycorrhizal fungus in vitro. *Mycologia*, 74(6), 952-959.

Smith, S.E., 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. *New Phytologis*t 114, pp.1-38.

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Academic press.

Smith, S. E., Facelli, E., Pope, S., & Andrew Smith, F. (2010). Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant and soil*, *326*, 3-20.

Smith, S. E., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual review of plant biology*, *62*, 227-250.

Souri, E., Amin, G., Farsam, H., Jalalizadeh, H., & Barezi, S. (2008). Screening of thirteen medicinal plant extracts for antioxidant activity. *Iranian Journal of Pharmaceutical Research*, *7*(2), 149-154.

Souri, Z., Karimi, N., Farooq, M. A., & Sandalio, L. M. (2020). Nitric oxide improves tolerance to arsenic stress in *Isatis cappadocica* desv. Shoots by enhancing antioxidant defenses. *Chemosphere*, *239*, 124523.

Spinedi, N., Storb, R., Aranda, E., Romani, F., Svriz, M., Varela, S. A., ... & Scervino, J. M. (2021). ROS-Scavenging Enzymes as an Antioxidant Response to High Concentration of Anthracene in the Liverwort *Marchantia polymorpha* L. *Plants*, 10(7), 1478.

Stahl, E. **(**1900). Der Sinnder Mycorhiz enbildung. Jahrb. Wiss. Bot. 34, 539-668.

Stahl, M. (1949). Die Mycorrhiza der Lebermoose mit besonderer Berücksichtigung der thallosen Formen. *Planta*, 37(1. H), 103-148.

Stockey, R. A., Rothwell, G. W., Addy, H. D., & Currah, R. S. (2001). Mycorrhizal association of the extinct conifer *Metasequoia milleri*. *Mycological Research*, *105*(2), 202-205.

Strullu-Derrien, C., Selosse, M. A., Kenrick, P., & Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist*, *220*(4), 1012-1030.

Sugiura, Y., Akiyama, R., Tanaka, S., Yano, K., Kameoka, H., Marui, S., ... & Saito, K. (2020). Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences*, *117*(41), 25779-25788.

Tanwir, M. & Langer, A. (2006). Liverworts of Ladakh, J&K State (North-West Himalaya). India, *Journal of Indian Botanical Society*, 85.

Tanwir, M. (2005). Studies on the diversity of hepatic flora of district Poonch (North-West Himalaya). PhD Dissertation, University of Jammu, Jammu.

Thakur, S., & Kapila, S. (2017). Seasonal changes in antioxidant enzymes, polyphenol oxidase enzyme, flavonoids and phenolic content in three leafy liverworts. *Lindbergia*, *40*(5), 39-44.

Tommerup, I. C. (1983). Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society*, *81*(2), 381-387.

Trappe, J. M. (2005). AB Frank and mycorrhizae: the challenge to evolutionary and ecologic theory. *Mycorrhiza*, *15*, 277-281.

Turnau, K., Anielska, T., & Jurkiewicz, A. (2005). Mycothallic/mycorrhizal symbiosis of chlorophyllous gametophytes and sporophytes of a fern, *Pellaea viridis* (Forsk.) Prantl (Pellaeaceae, Pteridales). *Mycorrhiza*, *15*, 121-128.

Upson, R., Read, D.J., & Newsham, K.K. (2007). Widespread association between the ericoid mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort in the maritime and sub-Antarctic. *New Phytologist*, 176, 460-471.

Van Der Heijden, M.G., Martin, F.M., Selosse, M.A. & Sanders, I.R. (2015). Mycorrhizal ecology and evolution: the past, the present and the future. *New Phytologist*, *205*(4), pp.1406-1423.

Varela, Z., Debén, S., Saxena, D.K., Aboal, J.R. & Fernández, J.Á. (2018). Levels of antioxidant compound glutathione in moss from industrial areas. *Atmosphere*, *9*(7), p.284.

Vasar, M., Davison, J., Sepp, S. K., Öpik, M., Moora, M., Koorem, K., ... & Zobel, M. (2021). Arbuscular mycorrhizal fungal communities in the soils of desert habitats. *Microorganisms*, *9*(2), 229.

Vats, S., & Alam, A. (2013). Antioxidant activity of *Barbula javanica* Doz. et Molk.: A relatively unexplored bryophyte. *Elixir Applied Botany*, *65*, 20103-20104.

Velikova, V., Yordanov, I., & Edreva, A. J. P. S. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, *151*(1), 59-66.

Veresoglou, S. D., Johnson, D., Mola, M., Yang, G., & Rillig, M. C. (2022). Evolutionary bet-hedging in arbuscular mycorrhiza-associating angiosperms. *New Phytologist*, *233*, 1984-1987.

Verma, M and Langer, A. (2011). Incidence of arbuscular mycorrhiza in some liverworts ofJammu& Kashmir, India. *Phytomorphology* 61:1-8.

Verma, M. and Langer, A. (2014). Studies on AM associations in *Marchantia nepalensis*L. et L. IOSR *Journal of Pharmacy and Biological Sciences*, *9*, 26-29.

Verma, M., Shabnam, S. K., & Kumar, A. (2024). Exploring the Influence of Arbuscular Mycorrhizal Symbology on the Antioxidant Potential of Liverwort Asterella multiflora: A Comprehensive Study on Rhizoid and Thallus Anatomy. *Int. J. Exp. Res. Rev*, *37*, 109-119.

Vlček, V., & Pohanka, M. (2020). Glomalin–an interesting protein part of the soil organic matter. *Soil and Water Research*, *15*(2), 67-74.

Vyas, D., Dubey, A., Soni, A., Mishra, M. K., Singh, P. K., & Gupta, R. K. (2008). Vesicular arbuscular mycorrhizal association in bryophytes isolated from Eastern and Western Himalayas. *Mycorrhiza News*, *19*, 16.

Wang, B., & Qiu, Y.L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza. 16,299–363.

Wang, L., Zhang, L., George, T. S., & Feng, G. (2023). A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. *New Phytologist*, *238*(2), 859-873.

Wasley, J., Robinson, S. A., Lovelock, C. E., & Popp, M. (2006). Some like it wet—biological characteristics underpinning tolerance of extreme water stress events in Antarctic bryophytes. *Functional plant biology*, *33*(5), 443-455.

Wright, S. F., & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and soil*, *198*, 97-107.

Wright, S. F., Upadhyaya, A., & Buyer, J. S. (1998). Comparison of N-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis. *Soil Biology and Biochemistry*, *30*(13), 1853-1857.

Wang, L., Zhang, L., George, T. S., & Feng, G. (2023). A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. *New Phytologist*, *238*(2), 859-873.

Wurzburger, N., & Brookshire, E. J. (2017). Experimental evidence shows that mycorrhizal nitrogen strategies affect soil carbon. Wiley online Library

Xiao, B., & Bowker, M. A. (2020). Moss-biocrusts strongly decrease soil surface albedo, altering land-surface energy balance in a dryland ecosystem. *Science of the Total Environment*, *741*, 140425.

Yadav, S., Srivastava, A., Biswas, S., Basu, S., Singh, S. K., & Mishra, Y. (2022). Seasonal changes in the antioxidative defence system of a liverwort *Dumortiera hirsuta*. *Journal of Plant Growth Regulation*, *41*(3), 1265-1275.

Yadav, S., Basu, S., Srivastava, A., Biswas, S., Mondal, R., Jha, V. K., ... & Mishra, Y. (2023). Bryophytes as Modern Model Plants: An Overview of Their Development, Contributions and Future Prospects. *Journal of Plant Growth Regulation*, 1-18.

Yang, S., Imran, P. & Ortas, I. (2023). Impact of mycorrhiza on plant nutrition and food security. *Journal of Plant Nutrition*, 1-26.

Yayintas, O. T., Sogut, O., Konyalioglu, S., Yilmaz, S. & Tepeli, B. (2017). Antioxidant activities and chemical compositon of different extracts of mosses gathered from Turkey. *AgroLife Scientific Journal*, 6(2), 205-213.

Yayintas, O. T. & Irkin, L. C. (2018). Bryophytes as hidden treasure. *Journal of Scientific Perspectives*, *2*(1), 71-82.

Yemm, E. W., & Willis, A. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical journal*, *57*(3), 508.

Zhan, F., Li, B., Jiang, M., Yue, X., He, Y., Xia, Y., & Wang, Y. (2018). Arbuscular mycorrhizal fungi enhance antioxidant defense in the leaves and the retention of heavy metals in the roots of maize. *Environmental Science and Pollution Research*, *25*, 24338-24347.

Zhang, Y., Guo, L. D., & Liu, R. J. (2004). Arbuscular mycorrhizal fungi associated with common pteridophytes in Dujiangyan, southwest China. *Mycorrhiza*, *14*, 25-30.

Zhu, X., Song, F., & Liu, S. (2011). Arbuscular mycorrhiza impacts drought stress of maize plants by lipid peroxidation, proline content and activity of antioxidant system. *Journal of Food Agriculture and Environoment, 9*(2), 583-587.

ANNEXURES

Annexure I

Conferences & workshops

1. Presented paper titiled "Studies on AM associations in two liverworts, *Plagiochasma appendiculatum and Reboulia hemispherica* of J&K" in International conference on sustainability: life on Earth 2021(ICS-LOE 2021) organized by Department of Botany and Zoology, School of Bioengineering and Biosciences,and Institute of Forest Productivity, Ranchi, Jharkhand at Lovely Professional University, Punjab.

2. Presented paper titled "Studies on AM associations in liverwort *Marchantia paleacea*. in the Two day "National Seminar on Plants and Fungi-Polyphasic Characterization and Bioprospection" on 25-26th March 2022, organized by Department of Botany, University of Jammu (J&K).

3. Presented paper titled "The enzyamatic and non-enzymatic profile of traditionally used medicinally important liverwort, *Plagiochasma appendiculatum*" in National conference on Emerging Trends in Genetic Engineering & Molecular Biology organized by center for Molecular Biology, Central University of Jammu (J&K) on 28th and 29th Sept., 2022.

4. Presented paper titled "Glomus forming arbuscular mycorrhizal association with the liverworts of Jammu & Kashmir, India''. in the Two day "2nd International conference on plant physiology and biotechnology" on 21-22rd April 2023, organized by Lovely Professional University.

5. Participated in Two-days National Workshop (22nd and 23rd Feb. 2018) on topic 'National Workshop on bryology' organized by Department of Botany, University of Jammu.

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

Certificate No. 238861

Certificate of Participation

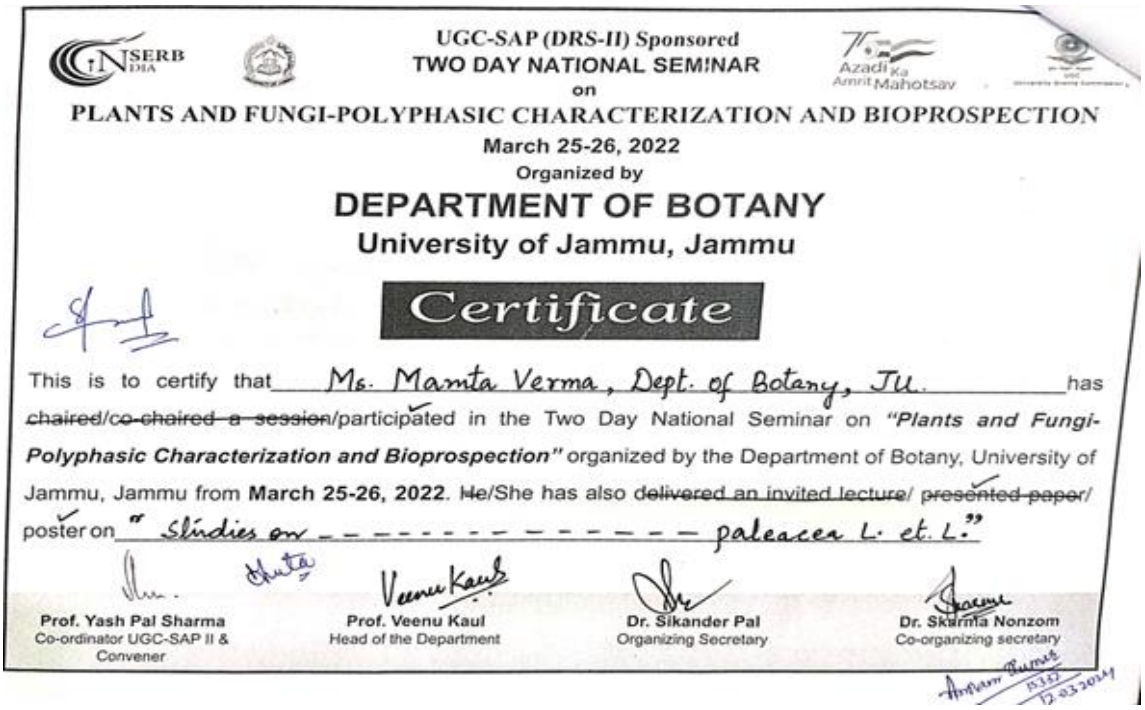
This is to certify that ***Prof./Dr./Mr./Ms. Mamta Verma*** *of* ***Lovely Professional University*** *has participated in* ***Poster Presentation*** *on the topic entitled* ***Studies on AM associations in two liverworts, Plagiochasma appendiculatum and Reboulia hemispherica of Jammu & Kashmir*** *in International Conference on* ***Sustainability: Life on Earth 2021 (ICS-LOE 2021)*** *held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand at Lovely Professional University, Punjab.*

Date of Issue: 18-02-2022
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Dr. Joydeep Dutta
Organizing Secretary, ICS-LOE, 2021

Dr. Neeta Raj Sharma
Convener, ICS-LOE, 2021



UGC-SAP (DRS-II) Sponsored
TWO DAY NATIONAL SEMINAR
on
PLANTS AND FUNGI-POLYPHASIC CHARACTERIZATION AND BIOPROSPECTION
March 25-26, 2022
Organized by
DEPARTMENT OF BOTANY
University of Jammu, Jammu

Certificate

This is to certify that Ms. Mamta Verma, Dept. of Botany, JU. has ~~chaired/co-chaired a session~~/participated in the Two Day National Seminar on ***"Plants and Fungi-Polyphasic Characterization and Bioprospection"*** organized by the Department of Botany, University of Jammu, Jammu from **March 25-26, 2022**. ~~He~~/She has also ~~delivered an invited lecture/ presented paper~~/ poster on " Studies on ---------- palearea L. et. L."

Prof. Yash Pal Sharma
Co-ordinator UGC-SAP II & Convener

Prof. Veenu Kaul
Head of the Department

Dr. Sikander Pal
Organizing Secretary

Dr. Sharmila Nonzom
Co-organizing secretary

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

SolResLab
Solutions for Research Laboratories

ICPPB
International Conference on Plant Physiology and Biotechnology

CERTIFICATE

This is to certify that Prof./Dr./Mr./Ms. MAMTA VERMA
of LOVELY PROFESSIONAL UNIVERSITY *has participated in Poster Presentation/Oral Presentation on the topic entitled.* GLOMUS FORMING ARBUSCULAR MYCORRHIZAL ASSOCIATION WITH LIVERWORTS OF JAMMU & KASHMIR, INDIA.
in 2nd International Conference on Plant Physiology and Biotechnology (ICPPB) held from 20-21 April 2023 organized by School of Bio-engineering and Biosciences, under the aegis of Lovely Professional University, Punjab.

Kumar
Dr. Umesh Goutam
Co-Convener
Dr. Neeta Raj Sharma
Convener
15332
17.03.2024

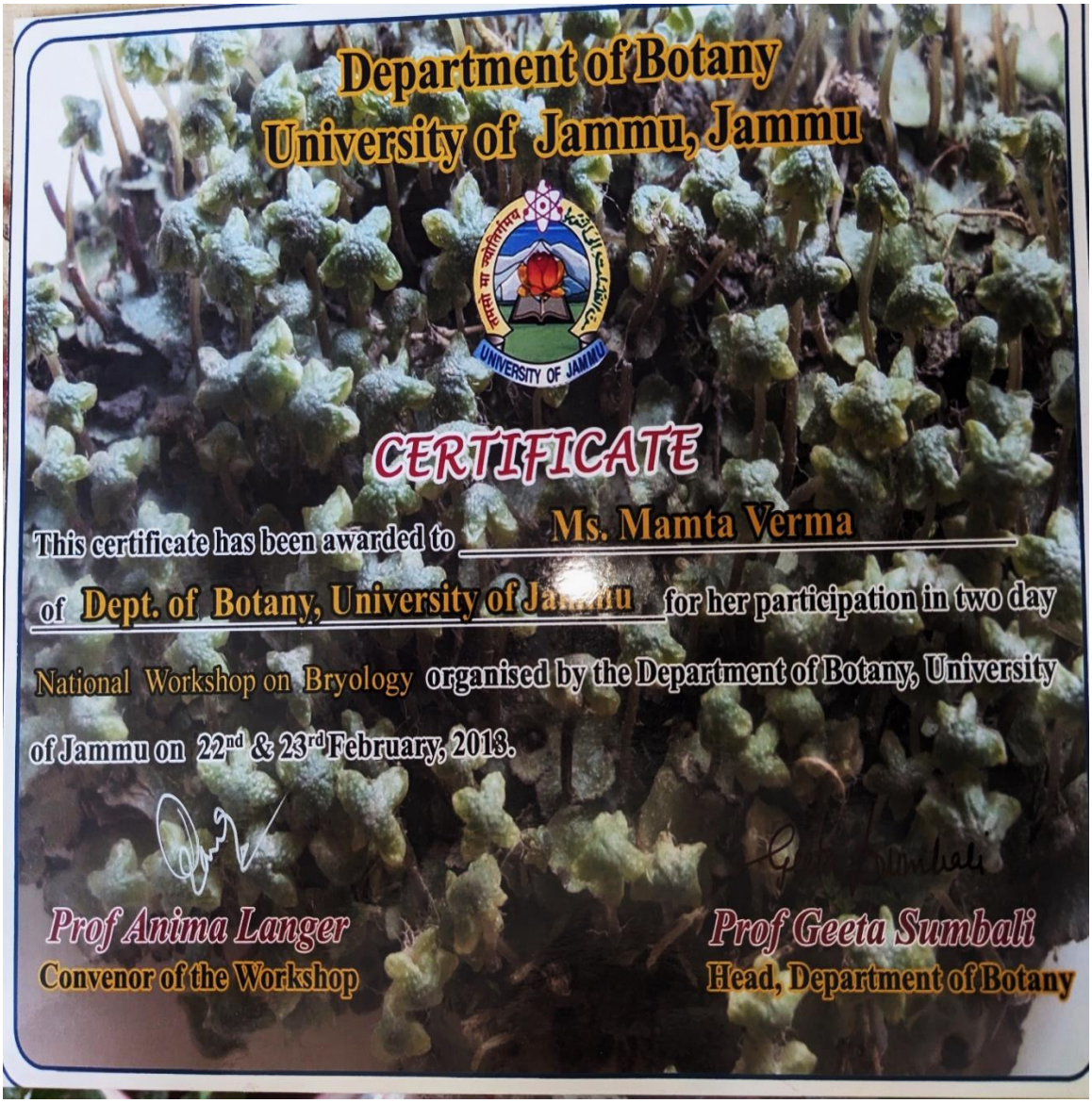
CENTRAL UNIVERSITY OF JAMMU

National Conference
on
Emerging Trends in Genetic Engineering & Molecular Biology
[ETGMB – 2022]

Certificate of Appreciation

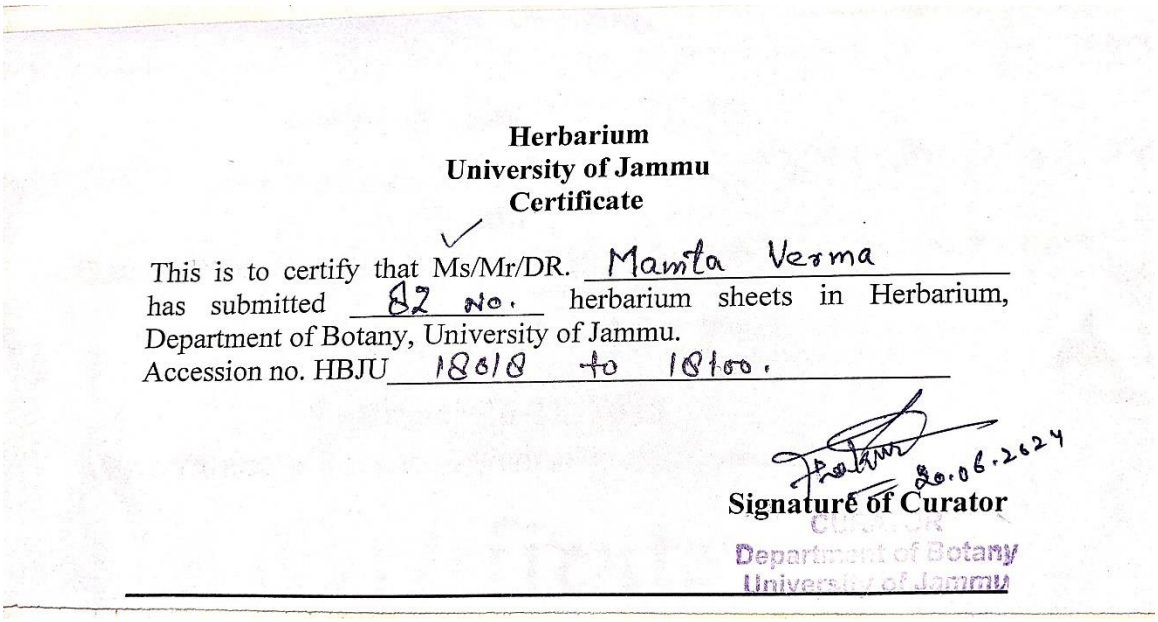
This is to certify that Prof./Dr./Mr./Ms. Mamta Verma, LPU presented a paper (Oral/Poster) on the topic titled The enzymatic and non enzymatic antioxidant profile of traditionally used medicinally important liverwort, Plagiochasma appendiculatum in National Conference organized by the Centre for Molecular Biology, Central University of Jammu on **"Emerging Trends in Genetic Engineering and Molecular Biology"** held on **28th-29th September, 2022**

Convener
Co-Convener cum Organizing Secretary
15332
12.09.22


Department of Botany
University of Jammu, Jammu
UNIVERSITY OF JAMMU
CERTIFICATE
This certificate has been awarded to Ms. Mamta Verma
of Dept. of Botany, University of Jammu for her participation in two day
National Workshop on Bryology organised by the Department of Botany, University
of Jammu on 22nd & 23rd February, 2018.
Prof Anima Langer
Convenor of the Workshop
Prof Geeta Sumbali
Head, Department of Botany

Annexure II

Herbarium Certificate:



Herbarium
University of Jammu
Certificate

This is to certify that Ms/Mr/DR. Mamta Verma
has submitted 82 no. herbarium sheets in Herbarium, Department of Botany, University of Jammu.
Accession no. HBJU 18018 to 18100.

20.06.2024

Signature of Curator

Curator
Department of Botany
University of Jammu

Annexure III

Publications

1. Published Paper titled "The Antioxidant Profile of Two Traditionally Used Medicinal Liverworts, *Marchantia Paleacea* and *Conocephalum Conicum* Growing in District Kishtwar, Jammu and Kashmir" in UGC Journal "Asian Journal of Organic and Medicinal Chemistry". ISSN:2456-8937.

Asian Journal of Organic & Medicinal Chemistry
Vol. 7 No. 2 (April - June, Special Issue - V 2022)
ISSN Online: 2456-8937
UGC CARE APPROVED JOURNAL

The Antioxidant Profile of Two Traditionally Used Medicinal Liverworts, *Marchantia Paleacea* and *Conocephalum Conicum* Growing in District Kishtwar, Jammu and Kashmir

Mamta Verma[1], Parul Parihar[1] and Nitan Katoch[2]
[1]Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University
[2]Regional Institute of Management and Technology University

ABSTRACT
The present work is an effort to assess the potential of the antioxidant for two ethnobotanically important liverworts say., *Marchantia paleacea* and *Conocephalum conicum*.The antioxidant system comprising of enzymatic and non-enzymatic antioxidants have been evaluated. The values derived are close enough to the higher plants. For the *Marchantia paleacea*, the content of superoxide dismutase and soluble sugar are higher than *Conocephalum conicum*, whereas, the ascorbate activity and Guaiacol peroxidase for *Conocephalum conicum* are higher than *Marchantia paleacea*. All the other parameters including glycine and MDA content are high in the case of *Conocephalum conicum*. However, parameters like total glutathione, chlorophyll a, proline, Chlorophyll b are high in case of *Marchantia paleacea*.

Keywords: *Marchantia paleacea, Conocephalum conicum,* Antioxidant

INTRODUCTION
Many disorders are treated using medicinal plants since they have proved to have pharmacological activity [1]. Medical herbalism is becoming more and more popular because it is cheap, doesn't have side effects, and doesn't lead to drug resistance as often as standard synthetic medicines.

It is known that the bryophytes, which include liverworts, hornworts, and mosses, contain a number of secondary metabolites with a variety of bioactivities. These tiny, inconspicuous, non-vascular plants have been shown to have antibacterial, antioxidative, cytotoxic, and other properties [2]. It is known that bryophytes keep a wide range of secondary metabolites because they are always fighting off biotic and abiotic stressors.

The bryophytes have been one of the first terrestrial plants in the history of evolution. They have made a substantial addition to our perception of plant growth, physiology, stress-induced responses, and phylogenetics [3]. Additionally, their well-developed conduction systems, absence of significant cuticle or epidermis, and ability to absorb metals make them effective bioindicators, biomonitoring agents, and metal absorbers [4]. Although numerous compounds along with the secondary metabolites were identified from various liverworts [5], the conventional use of the species is based on the idea of signatures and is practiced among the indigenous communities to date, highlighting the importance of bryophytes in ethnomedicine. The bryophytes are employed in smaller amounts for the various needs of humans as compared to angiosperms, gymnosperms, and pteridophytes.

In India, approx. 22 bryophyte species with documented ethnobotanical applications are present, including one in the Nicobar Islands [6]. People from the tribe named Gaddi of the Kangra valley, state of Himachal Pradesh use a native species called "Patharshali,", later found to be *P. appendiculatum*, to treat boils and blisters [7].

In North American, Chinese, Indian and European traditional medicine, a variety of chemical compounds were identified from the bryophytes and used to treat burns, tonsillitis, bronchitis, skin conditions, and illnesses of the skin [8]. Additionally, they have been shown to have cardiotoxic, cytotoxic, antitumor, antifungal, and antibacterial effects [9]. Most of the biological activity of bryophytes comes from chemical substances, especially secondary metabolites [10].

The body produces ROS, the species of reactive oxygen resulting from a variety of internal and external reactions, and antioxidants work to stop the damage the radicals do to healthy cells. Numerous studies have reported on the therapeutic potential of natural antioxidants. A lot of research [11,12,13] has been done on dietary sources of antioxidants, and it has been shown that certain foods have health benefits.

In the current years, the antioxidant titers of pteridophytes and angiosperms were evaluated [14,15,16]. However, very less data is present at present from the other plant groups say, bryophytes. Therefore, the recent study was basically designed to calculate the activity of antioxidants like (catalase, ascorbate peroxide, superoxide dismutase, Glutathione reductase, Guaiacol peroxidase), the non-enzymatic antioxidants (total

Paper titled "Exploring the influence of arbuscular mycorrhizal symbology on the antioxidant potential of liverwort *Asterella multiflora*: A comprehensive study on rhizoid and thallus" published in scopus indexed journal "International journal of experimental research and review" E-ISSN 2455-4855.

DOI: https://doi.org/10.52756/ijerr.2024.v37spl.009

Int. J. Exp. Res. Rev., Vol. 37: 109-119 (2024)

Original Article | Peer Reviewed | Open Access

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in

ISSN 2455-4855

Exploring the Influence of Arbuscular Mycorrhizal Symbology on the Antioxidant Potential of Liverwort *Asterella multiflora*: A Comprehensive Study on Rhizoid and Thallus Anatomy

Mamta Verma[1], Shabnam[1], Sandeep Kotwal[2] and Anupam Kumar[3]*

[1]Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144402, Punjab, India; [2]Department of Botany, Government Degree College Doda-182202 affiliated to University of Jammu, Jammu and Kashmir, India; [3]Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144402, Punjab, India

E-mail/Orcid Id:

MV, vermamamta52@gmail.com, https://orcid.org/0009-0006-8251-6896; S, shabnam.28725@lpu.co.in, https://orcid.org/0000-0003-2949-3518; SK, s.kotwal@yahoo.com, https://orcid.org/0000-0002-7995-5437; AK, anupam.kumar167@gmail.com, https://orcid.org/0000-0002-6608-3860

Article History:
Received: 12th Oct., 2023
Accepted: 12th Mar., 2024
Published: 30th Mar., 2024

Keywords:
Asterella multiflora, Antioxidants, Arbuscular Mycorrhiza, Liverworts

How to cite this Article:
Mamta Verma, Shabnam, Sandeep Kotwal and Anupam Kumar (2024). Exploring the Influence of Arbuscular Mycorrhizal Symbology on the Antioxidant Potential of Liverwort *Asterella multiflora*: A Comprehensive Study on Rhizoid and Thallus Anatomy. *International Journal of Experimental Research and Review*, 37(Spl.), 109-119.
DOI:
https://doi.org/10.52756/ijerr.2024.v37spl.009

Abstract: Arbuscular mycorrhizal (AM) symbiosis is a vital ecological interaction between plants and fungi that enhances nutrient uptake and plant resilience. While extensively studied in vascular plants, AM symbiosis in liverworts remains relatively unexplored. Six populations of *Asterella multiflora* were collected throughout the year to scan for arbuscular mycorrhizal colonization and enzymatic antioxidant activity. Percent mycorrhizal colonization was measured in smooth as well as tuberculated rhizoids. Anatomical detail of the thallus was also observed. Arbuscules were observed in the cells of the storage zone of the thallus. Enzymatic antioxidant activities, i.e., superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT), ascorbate peroxidase activity (APOX) and glutathione reductase activity (GR) were calculated. Maximum enzyme activity was observed in ascorbate peroxidase, which calculated 1.92-2.09 UA/mg protein, while catalase showed minimum activity of 0.01 UA/mg protein. A positive correlation was observed between enzyme activities and percent mycorrhizal colonization. This study delves into AM symbiosis in liverworts, focusing on *Asterella multiflora* and investigates the impact of AM colonization on enzymatic antioxidant activities, *i.e.*, superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT) ascorbate peroxidase activity (APOX) and glutathione reductase activity (GR). Antioxidants play a crucial role in stress tolerance and plant health, making them central to understanding the implications of symbiotic relationships.

Introduction

Bryophytes comprise three major groups: hepatics (liverworts), hornworts and mosses. They are an extraordinary group among embryophytes and have high species diversity that is only next to angiosperms (Christenhusz and Byng, 2016). They are very small in size and lack true roots and a vascular system. In the life cycle of bryophytes, the gametophyte is dominant and free-living, while the sporophyte partially or wholly depends on the gametophyte for water and nutrition supply. The gametophytes of bryophytes show a thalloid organization or differentiate into root-like rhizoids, stem-like colloids and leaf-like phylloid structures. The bryophytes are very interesting miniature plants that lie between green algae and vascular plants and, thus, are considered to arise soon before the origin of land plants (Bowman et al., 2016). Bryophytes play a significant role in the ecosystem in many aspects, viz., water retention, ecological indicators, soil improvement and nutrient cycling (Rousk et al., 2017; Glime, 2020; Xiao and Bowker, 2020). They are the economically important amphibians of the plant kingdom. Furthermore, their simplicity and evolutionary significance make them

*Corresponding Author: anupam.kumar167@gmail.com