"PHYSIOLOGICAL STUDIES OF SOME MOSSES AND THEIR APPLICATION ON SEED GERMINATION OF SEMI-ARID CROPS."

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SUBMITTED BY

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July, 2019

Certificate of the Co-Guide

CERTIFIED that the work incorporated in the thesis **"Physiological studies of some mosses and their application on seed germination of semi-arid crops**" submitted by Mr. Wadavkar Dadasaheb Shivaji was carried out by the candidate under my supervision / guidance. Such material has been obtained from other sources has been duly acknowledged in the thesis.

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Dr. Shashikant. J. Chavan

Date: 30/ 06 /2019 (Research Guide)

Declaration by the Candidate

I declare that the thesis entitled "Physiological **studies of some mosses and their application on seed germination of semi-arid crops**" submitted by me for the degree of Doctor of Philosophy is the record of work carried out by me during the period from 01/07/2014 to 01/07/2019 under the guidance of Dr. Shashikant. J. Chavan and has not formed the basis for the award of any degree, diploma, associate-ship, fellowship, titles in this or any other University or other institution of Higher learning.

I further declare that the material obtained from other sources has been duly acknowledged in the thesis.

Mr. Dadasaheb S. Wadavkar

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INDEX

List of Table

List of Figures

List of Plates

ABBREVIATIONS

	Abbreviations
aq.	Aqueous extract
ATP	Adenosine Triphospahate
AEC	Anion Exchange Capacity
AAS	Atomic Absorption Spetrophotometer
Ach	Acetylcholine
α	Alpha
β	Beta
Ca	Calcium
OC	Organic carbon
cm	Centimetre (s)
Chl	Chlorophyll (s)
ChE	Cholinesterase
n	Chromosome number
et al.	Co- workers
Conc.	Concentrated
Cu	Copper
DNSA	3,5-dinitrosalicylic acid
2,4-D	2, 4 Dichlorophenoxy acetic acid
DCPIP	2,6 Dichlorophenol Indophenol
dSm-1	Deci semen per meter

^{o}C	Degree Celsius
DNA	Deoxyribose nucleic acid
DTPA	Diethylenetriaminepentaacetic acid
DMSO	Dimethyl Sulphoxide
D.W.	Distilled Water
DW	Distilled Water
dS / m	Deciseimens per meter
dry. wt.	Dry Weight
E	East
EC	Electrical conductivity
E.C.	Enzyme code
Fig.	Figure (s)
F. W.	Fresh weight
ft.	Feet
G –ve	Gram-negative
G +ve	Gram-positive
>	Greater than
hrs	Hour (s)
pH	Hydrogen ion concentration
H_2O_2	Hydrogen peroxide
IAA	Indole Acetic Acid
Fe	Iron
KD	Kilo Dalton

Kg	Kilogram (s)
Kg/ha	Kilograms per hectare
Km	Kilometer
<	Less than
lit.	Liter
Mg	Magnesium
Mn	Manganese
m RNA	messenger RNA
m	metre (s)
µg	Microgram (s)
µg	micrograms
µl	microlitre
ml	Mililitre (s)
mM	Milimolar (s)
mg	milligram (s)
mm	Millimetre (s)
min	Minute (s)
M	Molar
NEEDA	N (l-Napthyl) ethylene diamide hydrochloride
nm	Nanometre (s)
n	Mole nanomoles
n	Number of chromosomes
NAD	Nicotinamide adenine dinucleotide

NADH	Nicotinamide adenine dinucleotide reduced
NR	Nitrate Reductase
NiR	Nitrite Reductase
N	Nitrogen
N	Normal
N	North
No.	Number
OD	Optical Density
OC	Organic Carbon
ppm	Parts per million
g-1	Per gram
%	Percentage
P	Phosphorus
K	Potassium
ROS	Reactive oxygen species
rpm	Revolutions per minute
Sec	Seconds
Sp., Spp.	Species
±	Standard deviation
S.D.	Standard deviation
Sq. Km.	Square Kilometers
Sr.	Serial
Std.	Standard

t- RNA	Transfer RNA
UV	Ultraviolet
viz.	Videlicet = namely
wt.	Weight
w/v	Weight / volume
Zn	Zinc

CHAPTER I

INTRODUCTION

1. Introduction:

The term Bryophyte is derived from the Greek word 'Bryon' meaning a moss. Mosses are considered to have the largest number of species among green plants, next to angiosperms. They have a long history of evolution compared to flowering plants, with fossil record dated back 440 million years ago in the Silurian period. They are the simplest and most primitive land plants. They do not have well developed conductive tissue system. They were through to be of little economic value. Mosses belong to the group of bryophyta which never produces flowers and seeds. Most mosses are small cryptogramic, non-vascular, land plants but some may be as large as 35 cm tall while some semi-aquatic. Mosses do not have veins to transport water and food; instead all parts of the plant absorb water and nutrients. Mosses have rhizoidal system instead of roots, which are branched threads. While the rhizoids can absorb water, they are mainly useful for support and to anchor the plants.

The life cycle of mosses has two stages namely gametophyte and sporophyte stage. Gametophyte stage is dominant. Gametophyte forms the green leafy structure known as protonema. Mosses reproduce sexually but also they depend on water for fertilization. Male gamete (spermatozoid) and female gamete (egg cell) both are produced on the gametophyte in antheridia and archegonia respectively. The next generation, the sporophyte or spore-bearing structure is grown on gametophyte. Sporophyte is typically a capsule developed on the apical region of a stalk called the seta. The mature sporophyte dries out and releases the spores which germinate into protonema as a gametophyte stage.

Mosses are an advanced class of bryophytes with about including 900 genera, 17,000 species, 3 subclasses, 4 orders and 89 families about the world (Richardson, 1981; Vitt, 1984). Crosby *et al.* (1999) reported mosses lists 12,800 species in 901 genera, including 42 synonymous genera. Bryopsida (Mosses) are the largest group of leafy forms, with over 21 orders, 117 families, 650 genera and 14,000 species (Buck and Goffinet, 2000). Geffert *et al.* (2013) reported more than 100000 distribution records for over 400 different geographical units and standardized species taxonomy using the TROPICOS database of the Missouri Botanical Garden.

India is one of the 17 mega biodiversity countries in the world and one of the hot spots of biodiversity. The large area and a variety of phytoclimatic conditions contribute to the great diversity of the Indian flora (Singh, 1997; 2001). In case of bryophytes and especially mosses, rich diversity is found in different regions of India. According to records about 2000 species of mosses, 816 species of liverworts and 34 species of hornworts are occurring in India. The plants are distributed in Eastern and Western Himalayas, South India, Rajasthan, Gujarat, Punjab, Central India, Andaman and Nicobar Islands. Mosses are richly distributed in the Indian subcontinent and it has almost species diversification. Mosses belong to class Bryophyta, which previously also included liverworts and hornworts. Now liverworts and hornworts have separate divisions. There are ten to twelve thousand taxa of moss exist under the Bryophyta (Vitt, 1984).

In case of bryophytes, especially mosses show rich diversity in different regions of India. Several bryologists of India have assessed the moss flora of different bryological regions from time to time, such as Gangulee (1978-80) published "Mosses of Eastern India and Adjacent regions" in eight fascicles which included 990 species. Chopra (1975) observed nearly 2,000 species belonging to 329 genera under 56 families. Lal (2005) published a checklist of Indian mosses and listed 1623 taxa of mosses from India. Alam (2015) recently compiled of the moss flora of India revealed the occurrence of total 1578 species of mosses which belong to 21 orders, under 66 families and 328 genera. After that, no valid attempt has been made to provide a complete wealth of mosses in India with their current status.

In Maharashtra also, the Bryo-floristic take a look at mainly mosses are very fragmentary. The earlier morphological and anatomical work carried on mosses by Birdwood (1886, 1887 and 1897), Sedgwick (1910, 1911, 1913), Blatter (1909), Dixon (1909), Dabhade (1988) and Magdum *et al*., (2017) etc. was meagre. Bryologists like Dabhade (1998) described 87 species from mosses of Khandala and Mahabaleshwar. Chaudhary *et al*., (2008) described 100 mosses from North Konkan of Maharashtra especially from Western Ghats. Hile (2011) described 17 species of mosses from Kasara Ghat. Recently, Magdum *et al*., (2017) checked mosses of Western Ghats of Maharashtra. It consists of 128 species of mosses, 59 genera, belonging to 11 orders and 26 families.

All workers have insisted that enough focus has not been given to study the moss flora in Maharashtra. They have so far been ignored for many reason especially their relatively less economic value. But recent studies have proven that they could be the resource of many organic

and inorganic solutes, enzymes, secondary metabolites, biochemical and pharmaceutical compounds.

The present investigation the findings reported gives broad idea about the working of important physiological processes like photosynthesis mineral nutrition, biochemical analysis and secondary metabolites. In these mosses high potential of organic and inorganic elements are important factors which determining the response of seed germination and seedling growth of semi - arid region crops like cereals, pulses, legumes, spices and vegetable.

The present study undertaken in a less explored area of the Maharashtra, among the hotspots of biodiversity, which reveals its remarkable bryological potential. The present study gives ecological adaptability and the occurrence of rare and threatened moss species which needs conservation. The need is for the study related to the many physiological adaptations and processes, organic potential for germination, pharmacological and other relevant medicinal aspects of mosses. The mosses can be ecologically important, as they help in altering soil pH, absorbing carbon regulation, nutrient cycling, colonizing barren surfaces, creating soils and reducing soil erosion. It often plays important role of elements in the local water cycle, absorbing and holding of moisture which benefit other plants. The majority of mosses is not tolerant to pollution and often disappears from contaminated areas, which shows sensitivity towards good indicators of clean air and water quality.

Moreover, mosses are studied for its habitat, taxonomical and anatomical characters. An output of work on mosses is further extended to autecological point of view from Maharashtra. The selected locality shows heavy rainfall, higher altitude, extreme humidity, humus rich, more or less acidic and alkaline soil. Physico-chemical properties of rhizosphere soil for pH, electric conductivity and soil constituents have also been observed from various localities. The soil analysis is reported for nitrogen, phosphorus, potassium and organic carbon.

Biochemical analysis of primary metabolites like total carbohydrates, reducing sugar, starch, total soluble proteins free amino acids and free proline in mosses has been carried out alongwith constituents like photosynthetic pigments and mineral nutrients. Secondary metabolites like total polyphenols and tannins have been studied for its antioxidant and defense mechanism. The anti-oxidative enzymes have also been attempted. Along with these parameters, the probable aspects of its growth potential are being found out. The effect of mosses by using

their aqueous extracts is studied seed germination, seedling growth of some cereals, pulses, legumes, spices and vegetable seeds of semi-arid region crops.

The overview of this research is presented here in the form of thesis. The thesis divided into five chapters. The first chapter is the introduction which is emphasizing present mosses scenario and the work done so far in different parts of the world. There is data for studies in various areas for better understanding of physiological studies and the strategies adapted. The information clearly justifies the need of present work.

The more elaborative review and literature presented in chapter second. the details has been given on various taxonomy, physiology, biochemistry, enzymology, photosynthesis, secondary metabolites, nutrients, ecological, medicinal, horticulture, allelopathy, cytology as well as genetic aspects looking above available information the need of more research in moss physiology can be clearly visualized.

The methodology followed in present investigation is presented in details in chapter third. It includes basic frame work and design of the research work undertaken different technique and methodologies is applied also have been taken help of other laboratories as per the need of the experimentation for clear and objective understanding.

The findings of present investigations are provided in relevant logical manner and the same are discussed in light of relevant literature and this forms the content of chapter fourth i.e. result and discussion. In the last chapter of the thesis the significance findings are briefly summarized in the form of summary and conclusion. The literature sited in the thesis is nearly shown in references part at the end of thesis.

In the past few years, rapid progress has been made to isolate various plant based therapeutic compounds. Mosses being rich source of a variety of secondary metabolites like polyphenols, tannins etc. could be a promising source of the bioactive compounds with immense therapeutic potential. Mosses are rich source of a variety of antioxidant enzymes, find out its key role protection against herbivorous and elimination of ROS.

Furthermore, the use of mosses demonstrates mineral nutrients potential of seed for germination and seedling growth of semi-arid region crop plant.

Objectives:

1. Identification and selection of some moss species from different localities.

2. To perform autecological studies of selected moss species.
3. To determine the physiological characters of moss species.
4. To find out effect of aqueous moss extracts on seed germination of semi-arid crops.

CHAPTER II

REVIEW OF LITERATURE

2. Review of Literature:

2.1. Geological History of Mosses:

The fossil record of moss is sparse, due to their soft-walled and fragile nature. Unambiguous moss fossils have been recovered from as early as the Permian of Antarctica and Russia, and a case is put forwards for Carboniferous mosses (Thomas, 1972). It has further been claimed that tube-like fossils from the Silurian are the macerated remains of moss calyptrae (Kodner and Graham, 2001). Recent research shows that ancient moss could explain why the Ordovician ice ages occurred? When the ancestors of today's moss started to spread on land 470 million years ago, they absorbed CO_2 from the atmosphere and extracted minerals by secreting organic acids, which dissolved with the rocks and mosses, were grown on it. These chemically altered rocks in turn reacted with the atmospheric CO_2 and formed new carbonate rocks in the ocean through the weathering of calcium and magnesium ions from silicate rocks. (www.wikipedia.org.).

2.2. Indian status of Mosses:

The floristic study of the mosses, shows rich distribution in the Indian subcontinent, but received little attention throughout the past probably due to non-availability of literature. Even though the hepaticae received attention of Indian bryologists since Kashyap (1914) and other researchers on Indian mosses began only after one or two decade. However, contribution of scientists from abroad is continued even during the early half of this century.

The assets of this work include almost all previous work and reports from these regions of India, like, Dixon (1909 a) described the species *Brachymenium turgidum* collected by L. J. Sedgewick from Lonavala in the Western Ghats. It was followed by the publication of a very valuable account of the mosses from the Western Ghats collected by G. B. Savery and sent to him by L. J. Sedgewick from various parts of the southern region of Bombay Presidency. Dixon (1909b) this included two new species *viz.*, *Pterobryopsis maxwelli* Cardot and Dixon and *P. kavarensis* Cardot and Dixon. Dixon and Varde (1927) described a large number of new species and proposed two new genera *viz.*, *Foreauella* and *Trigonodictyon.* Blatter (1929) listed the mosses collected from the Bombay Presidency. Bruehl (1931) published a census of Indian mosses in which he enumerated 2471 species including about 1500 species of the Indian continent.

Deb (1955) reported 35 species of mosses belonging to 28 genera and 17 families, from Manipur with a note on their habitats. Chopra (1960) listed 158 species of mosses belonging to 68 genera under 25 families from Nainital. Foreau (1961) compiled the result of the survey on the Moss Flora of Palni hills and enumerated 424 species. The genus *Alonia* was recorded for the first time from India by Wadhwa and Vohra (1963) from Garhwal with a detailed account of *A. rigida*. Foreau (1964) reported *Bryum coronatum* Schwaegr. from Idukki district of Kerala and *B. curyphyllum* Dixon and P. Varde, *Philonotis subrigida* var. *adpressa* Cardot and P. Varde and *Physcomytrium insigne* Dixon and P. Varde from Kerala but without mentioning the precise localities. Srivastava (1966) listed 137 species of mosses belonging to 70 genera and 27 families from Kumaun. Raghavan and Wadhawa (1968) reported that 28 species of mosses belonging to 21 genera and 16 families from Agumbe-Hulical ranges in the Shimoga district of Karnataka state included two new records to India and Southern India. Vohra (1970) observed that 165 species of mosses from Western Himalayas, of these three species were new to India and five were new to Western Himalayas.

Chopra (1975) published his monumental work *Taxonomy of Indian Mosses* which includes nearly 2000 species belonging to 329 genera under 56 families. He also included the collections from the neighboring regions such as Pakistan, Nepal, Bhutan and Western and South-eastern Tibet as most of the species extend to other regions also. Vohra (1978) described a new species, *Lescuraea darjeelingensis* and a new variety, *Haplocladium microphyllum* ssp. *capillatum* var. *bhutanicum*. Chopra and Kumar (1981) published a well illustrated taxonomic account of 65 mosses of Western Himalayas and adjacent plains. Gangulee (1985) in his "*Handbook of Indian Mosses"* enumerated 100 species including acrocarpous and pleurocarpous mosses with colour plates. Ochyra (1998) discussed the identity of two species of *Hygroamblystegium* endemic to northern India.

Chaudhary and Deora (2001) provided an illustrated account of 25 species of mosses belonging to 18 genera and 8 families from Mt. Abu. Among them 23 species occur in Western Himalaya, 19 in Eastern Himalaya and 15 in South India. Daniel and Daniel (2003) added *Fissidens grifithii* to Indian bryoflora from Kanyakumari district of Tamil Nadu. He in the same year described six species *viz., Fissidens kalimpongensis* Gangulee, *F. leptopelma* Dixon, *Leptolejeunea sikkimensis* Udar and U. S. Awasthi, L. jhimalayensis Pande and Misra, *Radula madagascariensis* Gottsche and *Leucobryum juniperoideum* (Brid.) C. Muell. as new record to

Peninsular India. Nair *et al*. (2004) recorded *Bryum tuberosum* Mohamed and Damanhuri, which was known earlier from the Peninsular Malaysia, as a new record for India from Uduppi of Karnataka State. Singh and Nath (2004) added an epiphytic liverwort *Frullania rotundistipula* Steph. from Khasi hills, Meghalaya to the Indian bryoflora. Singh and Singh (2004) added *Lejeunea flava* (Swartz) Nees to the bryoflora of Western Himalaya, a species already known from Eastern Himalaya to Indian.

Saxena and Gangwar (2005) observed that taxonomical study of *Dicranum scoparium* Hedw. from Kumaon hills. Saxena *et al.* (2006) studied distribution of some Mosses in Nainital, Almora and Pithoragarh District of Kumaon Region, India. Saxena *et al.* (2007) studies taxonomy of moss *Isopterygium elegans* (Brid.) from Kumaon hills. Saxena and Arfeen (2009) noted that taxonomy and distribution status of moss *Racomitrium crispulum* in Kumaon hill of Western Himalaya. Saxena *et al.* (2010) reported of the moss *Rhynchostegiella divaricatifolia* (Renauld & Cardot) Broth. from Western Himalayan region of India. The presence of 24 species of mosses which belong to 12 genera falling under 7 families. Pottiaceae, Bryaceae, Plagiothiaceae and Fissidentaceae are the dominant moss families of Gujarat (Gujar *et al.,* 2016).

Besides, this recently several valuable checklists published are checklist of Indian mosses and listed 1623 taxa of mosses from India (Lal, 2005). Diversity of Bryophytes in Eraviculam National Park, Kerala (South India) by Madhusoodan *et al.* (2007), Mosses of the southern Western Ghats by Daniels and Daniel (2007), Checklist of the Bryophytes of Kerala by Manju *et al.* (2008), Contribution to the bryophyte flora of India: the Aralam Wildlife Sanctuary in the Western Ghats by Manju *et al.* (2009 a), Contribution to the bryophyte flora of India: Agasthyamalai Biosphere Reserve in Western Ghats by Manju *et al.* (2009 b), Moss Flora of Palni Hills (Tamil Nadu) India- A Checklist by Alam *et al.* (2011), A checklist of the mosses of Karnataka, India by Frahm *et al.* (2013), A contribution to the bryoflora of the Western Ghats in Karnataka State by Schwarz and Frahm (2013), An updated checklist of bryophytes of Karnataka by Schwarz (2013).

2.3. Maharashtra status of Mosses:

Mosses of Maharashtra have been accrued from numerous localities by enthusiastic botanists like Sedgwick (1910, 1911 and 1913) reported that different localities in Western Ghats such as Purandar, Poona, Mahabaleshwar, Lonavala, Khandala, Trimbakeshwar. They were published in three different papers on the mosses of Western Ghats. Dixon (1910 -1921) studied

the mosses of Sahayadris Mountain or Western Ghats Mountains listed several new genera and species like *Merceyopsis* sp., *Hyophilopsis* sp., *Bryosedgewickia kirtikari* Card. et. Dix. *etc.* Mohamed (1986) reported 42 species of mosses from Mahabaleswar, Pune and Khandala in north Western Ghats. Dabhade (1998) reported that 87 species of mosses of Khandala and Mahabaleshwar. Chaudhary *et al*. (2008) listed the 100 mosses from North Konkan of Maharashtra especially from Western Ghats.

Recently, Hile (2011) reported that 17 species of mosses from Kasara Ghat, Maharashtra and 8 terricolous mosses from 5 families, collected different locations of area. Soman (2016) reported five different species of epiphytic mosses have so far been encountered growing on various arboreal species in Mumbai area viz. *Macromitrium sulcatum* (Hook.) Brid, *Octoblepharum albidum* Hedw., *Calymperes thwaitessii* (Beisch.) Fleisch, *Erpodium magnifera* C. Muell. and *Stereohyllum tavoyense* (Hook.) Jaeg. Recently Magdum *et al*. (2017) compiled the checklist of mosses of Western Ghats of Maharashtra including 128 species of mosses, belonging to 11 orders; 26 families and 59 genera.

2.4. Physiology of Mosses:

Only few years in the past, the chemistry of mosses was meagre. Current studies at the biology of mosses and progress is analytical techniques has ended in a deeper expertise about the chemical components of mosses, despite the fact that our understanding of their biochemical techniques, in particular biosynthetic pathways, as compared to vascular flora, is still rather poor. So, this chapter aimed to give it in tandem with new trends in the physiology of mosses.

a. Germination:

Few reports on germination studies of mosses are available like Vallane (1966; 1971) studied germination of spores of *Ceratodon purpureus* and Olesen and Mogensen (1978) observed the ultrastructure and histochemistry on germination of mosses *Ceratodon purpureus*, *Funaria hygrometrica*, *Macromitrium sulcatum* and *Polytrichum commune*, emphasizing the analysis in electron microscopy. Nehira (1983) described and detailed the types of development of protonema of mosses and liverworts. Wiklund and Rydin (2004) reported that two mosses species regarding physiological features to spore establishment and Zhao *et al.* (2004) observed the characteristics of spore germination in *Lindenbergia brachyptera*. Maciel-Silva *et al.* (2009) studied that the effects of water availability and light on spore germination of the moss

Octoblepharum albidum. The influence of light and nutrients on the different germination phases of *Bryum argenteum* spores was studied by Silva *et al.* (2010).

b. Mineral Nutrients:

Shacklette (1965) determined the mineral contents of 29 species of bryophytes and found that the concentrations of Al, Ba, Cr, Cu, Fe, Ga, Ni, Pb, Ag, V and Zn were higher in bryophytes than those in angiosperms. The heavy metal ions the retention efficiency order is $Cu^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Mn^{2+}$ as shown for *Hylocomium splendens* by (Rohling and Tyler, 1970). Pakarinen and Tolonen (1977) reported major nutrients (N, P, K, Ca, Mg, Na, and Fe) were analysed in 38 Sphagnum and water samples taken mainly from bogs and poor fens. The contents of K, Mg and Ca in mosses were significantly higher in minerotrophic fens than in ombrotrophic bog sites. Wojtun (1993) studied mineral content in *Sphagnum* mosses from ombrotrophic bogs of South Western Poland: Pattern in species and elements. Aulio (1980) observed that nutrient accumulation in *Sphagnum* mosses. Multivariate summarization of the mineral element composition of 13 species from an ombrotrophic raised bog. Aulio (1982) it was observed nutrient accumulation in *Sphagnum* mosses intra- and interspecific variation in four species from ombrotrophic and minerotrophic habitats.

Saxena *et. al.* (2005) detected chemical constituents like Copper, Nickel and Iron from corticalous mosses from Mahabaleshwar in Western Ghats. Saxena and Arfeen (2009) evaluate antioxidant, photosynthetic and productivity of moss *Racomitrium crispulum* (Hook. f. et Wils.) under various phytotoxic concentration of metals Cu and Cd for different days. Exogenous supplied Cu and Cd to *R. crispulum* significantly give stress on oxidative enzymes as well as on photosynthesis. Kaur *et al.* (2010) determined the effect of some heavy metals of Cu, Zn, Fe, Hg, Cd, Co and Ni regarding their toxic effect has been studied in seven taxa of mosses like *R. roseum*, *P. elongata*, *A. pallidum*, *T. recognitum*, *F. taxifolius*, *B. kannounense* and *F. hygrometrica.* It is found that Zn, Cu, Fe are better tolerated than Hg, Cd, Ni, Zn is considered to be an essential element as in higher plants. Saxena and Arfeen (2010) studied element concentration in *Racomitrium crispulum* was in the order of Zn > Pb > Cu > Cd in summer, Zn> Cu > Pb > Cd in winter season and Zn > Cu > Pb > Cd in monsoon which reflect atmospheric trace elemental load.

Lou *et al.* (2013) found that the content of the heavy metals Pb, Cr and Cu in the moss *Haplocladium microphyllum* correlated with the concentrations in the medium. Iron (Fe), on the other hand, increased in a similar manner until the concentration in the medium reached 400 mg L^{-1} as Fe^{++} below that level the iron facilitated uptake of other nutrient ions. The absorption capacity for these metals follows the order Fe > Cr > Cu > Pb. Like most things in nutrient relationships, the amount matters. At low concentrations, both lead and copper ions, as with iron, promote the absorptive capacity of other nutrient elements. At high concentrations the same metals decrease uptake of other nutrient elements. Chromium is an exception, inhibiting absorption capacity of the nutrients P, K, Ca, S, Fe and Cu even when the Cr concentrations are low.

So above studied literature the role of mosses in nutrient uptake within ecosystems is generally ignored because of their small stature.

c. Photosynthesis:

Chlorophyll is a green compound found in leaves and green stems of plant and it is also very important green pigment found in better plants and mosses also. Qualitative and quantitative analysis regarding pigments of mosses during different growth stages i.e. gametophyte, sporophyte and germinating spores was carried out by Taylor *et al*. (1972). Karunen and Ihantola (1977 a) found that the amount of total chlorophyll was singnificantly higher in the sporophyte than in the gametophyte and during germination the amount of carotenoids per chloroplast was decreased. Karunen and Ihantola (1977 b) carried out qualitative and quantitative analysis of pigments in *Polytrichum commune*. Alpert (1984) observed that chlorophyll content in mosses through extraction in DMSO. Deora and Chaudhary (1991) reported that chlorophyll content in 16 species of mosses and four species of liverworts and concluded that bryophytes exhibit low chlorophyll concentration and high a:b ratios in high solar irradiances. The chlorophyll content in number of Indian bryophytes that ranged from 0.402 ± 0.052 to 2.002 ± 0.700 mg g-1 dry mass for chlorophyll a and 0.265 ± 0.067 to 1.634 ± 0.070 mg g-1 dry mass for chlorophyll b.

Robinson *et al.* (2000) observed that tolerance of desiccation was examined in three species of moss; the physiological tolerance to desiccation was measured using chlorophyll fluorescence in plugs of moss during natural drying in the laboratory. Marschall and Proctor (2004) reported that the data for 39 mosses and 16 liverworts for ratios of chlorophyll and

total carotenoids and light saturation of photosynthetic electron flow or photosynthetic CO_2 uptake in relation to the postulate that bryophyte cells in general show shade-plant characteristics. Fisher (2006) reported that accumulation of chlorophyll in *Thuidium gratum* and *Hyophila involuta* (forest moss species) was higher than that of the derived savanna mosses, *Archidium ohioense.* This could be due to the fact that these forest mosses possess leaves in which chlorocysts are sandwitched between several layers of large empty non-chlorophyllous hyalocysts, as a result of which the photosynthetic cells are protected from photo-oxidation.

Devmalkar *et al*. (2014) studied the amount of chlorophyll concentration content in the six species of the classes Anthocerotae, Hepaticae and Musci from the different localities of Purandar fort, Maharashtra. Negi (2016) have argued that highest chlorophyll a was detected in *Brachythecium plumosum* (4.45 mg/g FW) and lowest in *Entodon plicatus* (1.22 mg/g FW). Maximum chlorophyll b was found in *Ditrichum heteromallum* (1.85 mg/g FW) and minimum in *Pogonatum microstomum* (0.36 mg/g FW). Aroyehun *et al*. (2016) reported that the extraction of chlorophylls from selected forest mosses (*Hyophila involuta* and *Thuidium gratum*) and derived Savanna moss (*Archidium obioense*) using dimethyl sulphoxide (DMSO) and 80% acetone.

d. Enzymology:

The preliminary report for the presence of certain common representatives of the assimilating enzymes in mosses. The t-RNA- nucleotidyl transferase enzyme characteristics, cytokinin effect moss protonema; *Ceratodon purpureus.* The activity in protonema has been found of an enzyme which on the basis of preliminary characteristics has been classified as tRNA-nucleotidyl transferase (EC 2.7.7.25) responsible for the synthesis of the terminal nucleotide sequence pCpCpA of the 3'- end of all active t-RNA molecules. As a result of cytokinin (6-LI 2-isopentenylaminopurine) treatment an increase in enzyme activity occurs in the protonema after 1-3 hours of hormone action (Schneider and Szweykowska, 1975).

Dhindsa and Motowe (1981) reported the response of bryophytes to drought by studying SOD and catalase activity and lipid peroxidation in *Tortula ruralis*, a drought tolerant and a drought sensitive moss *Cratoneuron filicinum*. They reported that in *T. ruralis* activities of SOD and catalase were increased during slow drying while the level of lipid peroxidation was declined and exactly opposite results were observed during subsequent rehydration. However, in *C. filicium* the activites of SOD and catalase declined during drying as well as during subseqent rehydration. There was rapid increase in lipid

peroxidation during rehydration in both mosses. Dhindsa (1991) observed little change in *Tortula ruralis* as the activity of malate deydrogenase increase in the activities of glutathione reductase and glutathione S-transferase was largely prevented while effect on glutathione peroxidase was much smaller. The oxidized glutathione (GSSG) percentage of total glutathione is increased.

Gupta *et al*. (2001) reported the neurotransmitter acetylcholine (ACh) is present in plants including bryophytes. The first biochemical evidence for ACh hydrolysis by enzyme cholinesterase (ChE) in bryophytes is presented. For 39 species belonging to 16 families and 30 species belonging to 13 families showed ChE activity of the bryophytes tested, in which *Anoectangium bicolor* showed the highest ChE activity. Turner *et al*. (2001) reported that phosphatase activities and environmental features were characterized for 12 terrestrial and aquatic mosses in upland northern England, along with four species sampled from subarctic Sweden. Kaur *et al.* (2010) In seven moss taxa, protease showed a relatively greater specific activity while α-amylase showed a relatively lesser specific activity and reported four tested enzymes given sequential order as Protease > Polyphenol oxidase > α-amylase > β-amylase.

Krywult *et al.* (2013) investigated nitrate reductase activity in green tissues of *Brachythecium rutabulum* (Hedw.) Schimp. and *Atrichum undulatum* (Hedw.). Kapila *et al.* (2014) reported the seasonal variations in storage compounds and enzyme activities related to these storage compounds in three species of the family Marchantiaceae: *Marchantia palmata, M. nepalensis* and *Dumortiera hirsuta*. *Dumortiera hirsuta* growing near water streams or hydric habitat shows higher carbohydrate as well as protein content and exhibits low seasonal changes as compared to *M. palmata* and *M. nepalensis* which grows in mesic conditions. In all the species the activity of α-amylase, β-amylase and invertase were decreasing towards the end of the primary growth season due to carbohydrate accumulation in their thalli in this period.

2.5. Biochemistry:

In contrast to leafy liverworts, the mosses have a simple soluble carbohydrate pool consisting of sucrose (Smirnoff, 1992). Whereas, Melick and Seppelt (1994) showed the total soluble carbohydrate levels higher in *G. antarctici* collected from wetter sites and suggests that the shorter growing season experienced by plants from drier sites, results in reduced production of carbohydrates.

In leafy liverwort was analyzed for carbohydrates by Suleiman *et al*. (1979) and Marschall *et al*. (1998) investigated that carbohydrate composition and invertase activity of the leafy liverwort *Porella platyphylla*. Robinson *et al*. (1999) studies the levels of soluble carbohydrates were also measured in these samples following desiccation and these indicate the presence of stachyose, an oligosaccharide known to be important in desiccation tolerance in seeds, in *B*. *pseudotriquetrum*. Both gross morphology and carbohydrate content are likely to contribute to differences in desiccation tolerance of the moss species. Zuniga-Gonzalez *et al*. (2016) reported that soluble carbohydrate content variation in *Sanionia uncinat*a and *Polytrichastrum alpinu*m, two Antarctic mosses with contrasting desiccation capacities.

Dhindsa and Bewely (1977) studied protein synthesis status in drought tolerant and drought sensitive mosses and found that protein was stable during desiccation and subsequent rehydration in selected plant species. Davey (1999) recorded similar observation in the protein content of hydric mosses as compared to that of drier habitat Antarctic mosses. The continuous flushing of nutrients in hydric habitats may be one of the reasons of higher protein content in these plans. Kaur *et al*. (2010 a) investigated amount of chlorophyll content, proteins, carbohydrates and RNA has been studied in ten taxa of Mosses. Kaur *et al*. (2010 b) reported amount of chlorophyll content, proteins, carbohydrates and RNA has been studied in ten taxa of hepaticae and *Anthoceros erectus.* Kapila *et al.* (2014) recorded the higher protein content observed that protein content of *D. hirsute* was found to be higher than for the two species of *Marchantia* which is suggesting that the liverworts growing along water streams and in more shaded areas contain higher protein content than the liverworts that growing on wet soil in mesic conditions.

Almost no report has been found with respect to concentration of different amino acids except in few regarding the quantitative estimation of amino acids in which mosses *Tortula princeps*, *Rhynchostegium* sp. *Platyhypendium ripariodoides*, *Homalothecium* sp and *Camptothecium* sp. contain 0.51, 0.96, 1.10, 0.44 and 0.64 mg /g $^{-1}$ dry tissue respectively (Margaris, 1974). Simola (1975) observed that effect of several protein, amino acids and some inorganic nitrogen sources on the growth of *Sphagnum nemoreum.* The amino acid accumulation and growth of *Sphagnum* under different levels of N deposition reported by Nordin and Gunnarsson (2000). Krab *et al*. (2008) reported that amino acid uptake among wide-ranging moss species may contribute to their strong position in higher-latitude ecosystems. Sawant

(2010) reported the amount of free amino acids is higher in case of *T. hypophylla,* which is followed by *A. subtilis* and rather similar amounts in case of *A. wallichiana*. In *C. cavernarum* amino acids content is respectively 1.28 and 1.51 mg /g^{-1} dry tissue and finally the lowest concentration of these constituents is observed in case of *P. intermedium.*

Liu *et al*. (2001) reported that accumulation of free proline in two species of mosses, *Plagiomnium acutum* (Lindb.) T. Kop. and *Thuidium cymbifolium* (Doz. et Molk.) Doz. et Molk. under high temperature stress . Panda (2003) reported that as heavy metals induce water-deficit conditions, an increase in proline accumulation may help in osmoprotection. The osmoprotectant was accumulated uniformly in moss *Taxithellium* sp. under heavy metal treatment, with a maximum in chromium treatment (0.001nm). Baron *et al*. (2009) observed that response to water deficit of the moss *Racomitrium crispipilum* (Taylor) A. Jaeger, a bryophyte typical of open sites and expose to water shortage, measurements of proline contents. Sawant (2010) observed that content of proline in *T. hypophylla* contains the highest amount of proline (11.29 mg g^{-1} dry tissue) while about half and fairly similar amount is present in case of *A. subtilis* and *A. wallichiana*. and the lowest amount in *P. intermedium* (3.93 mg g^{-1} dry tissue).

2.6. Secondary metabolites:

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs. Xie and Lou (2009) investigated the secondary metabolites in Bryophytes. Adebiyi *et al*. (2012) reported that phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins and steroids in varying quantities in the two moss plants but there was absence of phenol in *Barbula indica.* These results suggest that the two moss plants can be veritable and potential source of useful drugs in treatment of ailments.

Klavina *et al*. (2018) reported that the total concentration of polyphenols and carbohydrates, the amount of dry residue and the radical scavenging activity were determined for a preliminary evaluation of the chemical composition of moss extracts. Kadam (2016) observed that the polyphenols and anti-microbial activity was assessed from the bryophytes viz.

Anthoceros erectus, Asterella angusta, Cyathodium tuberosum, Plagiochasma articulata and Targiona hypophylla grown in Malavli area of Lonavala. Wadavkar *et al.* (2017) observed that. polyphenol and tannin were higher in *Steeriophyllum anceps* and *Brachymenium turgidum* while case of *Hypnum reflexum* those were minimum. Klavina *et al*. (2018) observed that seasonal changes in chemical composition and composition of secondary metabolites in four moss species (*Sphagnum fallax*, *Sphagnum magellanicum*, *Polytrichum juniperinum*, and *Pleurozium schreberi*). Concentration of main groups of chemical constituents as well as antioxidant activity were determined and related to climate parameters (temperature, precipitation etc.) during two vegetation seasons. Dependence of both primary and secondary metabolism on climatic conditions was confirmed. Seasonal changes of 57 secondary metabolites reflected species-specific features. The pattern of seasonal changes of major groups of moss secondary metabolites was determined, allowing selection of the best timing for bioprospecting studies as well as for use as possible indicators of environmental stress.

2.7. Allelopathy:

Reports of allelopathic studies of mosses only a few reports are available. Gavrillova (1970) reported that aqueous extracts of *Polytrichum commune* and *Sphagnum* sp. inhibited the growth of *Pinus* and *Picea* seedlings, but stimulated the growth of *Larix* seedlings and *Plagiomnium integram* were found most effective where the seed germination was completely checked. The effect of aqueous and methanolic extract of moss and liverwort on seed germination and seedling growth was recorded. The inhibition in germination rate was observed at high concentration of methanol and aqueous extract in the both seeds of Mung bean and Bengal gram, however, highly diluted aqueous extracts showed an increase in the germination and promoted the growth in both crop species by (Bhadauriya *et al.,* 2016).

Bryophyte extracts exhibit dual effects on seedling germination depending on the species. It was shown that extract of the liverwort *Porella platyphylla* inhibits the growth of radice seedlings, whereas the extract of *Brachythecium rutabulum* promotes the growth of radice seedlings by Frahm, *et al.*(2012). Chavan *et al*. (2014) also reported that biochemical analysis and germination effect of mosses on different seeds. The allelopathic effects of liverworts on seed germination and seedling growth of a weed *Bidens pilosa*. The liverwort species selected for the study were *Plagiochasma appendiculatum, Targionia indica, Conocephalum conicum*

and *Dumortiera hirsuta*. In control experiments seed germination was 100 percent, where as the liverwort extracts in both the solvents exhibited different degrees of inhibitory effect on seed germination of the weed under study (Thakur *et al.,* 2015).

2.8. Medicinal Importance:

Wu (1977) reported that use of *Rhodobryum giganteum* to cure angina, because it contains volatile oils, lactones and amino acids. Indirectly, bryophytes provide special conditions for ethno medicinal production. Ding (1982) observed that 40 bryophyte species have been used as crude drugs by the Chinese people. Some of these species are hepatics *Frullania tamarisci Reboulia hemisphaerica, Conocephalum conicum, Marchantia polymorha* and mosses *Sphagnum* spp*., Weissia controversa, Funaria hygrometrica, Bryum argenteum, Rhodobryum roseum, Climacium dendroides* and *Polytrichum commune.* Among these species *Sphagnum teres* is very popular to apply for eye diseases. *Rhodobryum giganteum* and *R. roseum* are widely used within China for cardiovascular disease and nervous prostrations. *Polytrichum commune* is used as an antipyretic, diuretic and hemostatic and *Haplocaldium microphyllum* is applied for tonsillitis, bronchitis, timpanists and cystitis.

Kumar *et al.* (2000) reported the use of *Plagiochasma* for treating skin diseases. The fresh mature thalli with female receptacle is made into paste for the treatment of burns, boils, blisters on the body and also skin eruptions caused by hot sunlight in the summer. Singh *et al.* (2000) investigated that *C. polyanthus, D. albicans, P. juniperinu,* etc. may be used for curing deadly cancer while *M. ploymorpha, M. stellata, D. taxifolium, D.* albicans, *P. commune*, *W. denudata* for the tumor suppression and *Sphagnum* spp. is used since ancient times in the form of bandages. Asakawa (2007) studied biologically active used for scents, pungency, bitterness and display a quite extraordinary array of bioactivities and medicinal properties.

Shirsat *et al.* (2008) also studied ethno-medicinal uses of some common Bryophytes and Pteridophytes used by tribals of Melghat. Remesh and Nair (2009) studied the ethnobryological notes from Western Ghats, India. Singh *et al.* (2011) studied that *Plagiochasma appendiculatum*, *Conocephalum conicum*, *Bryum argenteum* and *Mnium marginatum* for the treatment of burn, cuts, wounds, and pores and skin disorders. Lubaina *et al.* (2014) reported the 35 medicinal bryophytes belonging to 3 groups were used to treat various diseases like liver, cardiac ailments, skin allergies, inflammation, diarrhea and sterility. In the present review we focused on

therapeutic uses of mosses in detail that will widely open the door for the use of different mosses in plant biotechnology and to meet the demand of novel drug discovery.

2.9. Horticultural Importance:

Mosses are used in horticulture as soil additives, ornamental material for cultivation and for beautification of gardens. Peat is one of the most important soil conditioners and is commonly used in agriculture throughout the world. Mosses are used as ornamental plants in gardens, to give beauty and an ancient look to gardens by clothing tree trunks, rocks and stone. In horticulture, position of mosses is almost incomparable in any other existing bryophyte industry (Nelson and Carpenter, 1965). Peat mosses have played a foremost task in horticulture for centuries (Perin, 1962; Arzeni, 1963; Adderley, 1965 and Sambo *et al.,* 2008). Even if their application as part of the background beautification has conventionally been used by Asian, they have frequently been utilized as soil additives and bedding for hot house crops, potted decorative plants, bonsai and seedling beds (Dhanda, 1984; Yoshimura and Halford 1957; Cox and Westing, 1963; Bland, 1971 ; Sjors, 1980 and Tan 2003).

2.10. Pharmacology:

Sakai, *et al.* (1988) reported the antitumor principles in Mosses. Dey and Mukherjee (2015) have been investigated bryophytes are used pharmacologically for active biomolecules. Several constituents with therapeutic potential have been isolated, characterized and investigated for antibacterial, antifungal, antiviral, antioxidative, anti-inflammatory and anticancerous efficacy. Chandra *et al.* (2017) showed that bryophytes are used to cure hepatic disorders, skin diseases, cardiovascular diseases, used as antipyretic, antimicrobial, wound healing and many more other ailments by different tribal communities of Africa, America, Europe, Poland, Argentina, Australia, New Zealand, Turkey, Japan, Taiwan, Pakistan, China, Nepal and different parts of South, North and Eastern India.

2.11. Antimicrobial activity:

According to Sabovljevic *et al.* (2006) investigated that extract obtained from *Bryum argenteum* have been proved to be active against all bacteria and fungi. Singh *et al*. (2007) evaluate the antimicrobial activity of 15 Indian mosses. The antibacterial activity of ethanolic extracts was investigated against five bacterial strains. The antibacterial ethanolic extracts of *Sphagnum junghuhnianum, Barbula javanica, Barbula arcuata, Brachythecium populeum,*

Brachythecium rutabulum, Mnium marginatum and *Entodon rubicundus* were found to be most active against all the organisms.

Yayintas and Yapici (2009) determine the *in vitro* antimicrobial activity of ethanol extracts of *Brachythecium campestre* and *Eurhynchium pulchellum* . The antimicrobial activity of the E1 and E2 was evaluated according to the disc diffusion method against 10 bacteria. Alam *et al*. (2012) reported antibacterial activity of the ethanolic and methanolic extracts from the moss *Entodon nepalensis* Mizush. was observed against three bacterial species and the maximum antagonistic effect showed against tested *Escherichia coli* followed by *Salmonella typhimurium* and least against *Bacillus subtilis*. Osman *et al*. (2010) observed that antimicrobial activity of the essential oils of mosses *Tortula muralis* Hedw., *Homalothecium lutescens* (Hedw.) H. Rob., *Hypnum cupressiforme* Hedw., and *Pohlia nutans* (Hedw.) Lindb.) from Turkey.

2.12. Cytology:

Wylie (1957) studied chromosome numbers of mosses and Khanna (1960) reported the cytological studies carried out on the Himalayan acrocarpic mosses of chromosome number for one family, 16 genera and 49 species for the first time. Gangulee and Chatterjee (1962) reported that cytology of the mosses occurring in Eastern India.

Sharma (1962) investigated chromosome number of sixteen species and one variety. Ramsay (1969) studied the gametophyte mitoses from 26 of the species. Karyotype comparisons are made within the genera *Polytrichum* and *Dicranum* and the value of such comparisons is stressed. Where possible gametophytic mitotic observations have been correlated with sporophytic meiosis from the same gatherings. Wigh (1973) reported the accessory chromosomes in four mosses belonging to the family Brachytheciaceae.

Bryan (1973) investigated the chromosomes in forty-six species from sixteen families of mosses and observed chromosome morphology, staining characteristics and behavior in meiosis or mitosis. The lowest numbers yet found are reported for *Dicranum fuscescens*, n=8, and *Timmia bavarica*, n=8+1 m. For the latter species the study was made from mitotic configurations, and offers evidence that m-chromosomes may be normal members of the gametophytic set. These investigations reveal for the first time m-chromosomes in *Dicranoweisia cirrata, Pottia lanceolata, Timmia bavarica, Orthotrichum pumilum, Homalothecium lutescens, Brachythecium velutinum, Rhyncostegium murale, Rhyncostegiella pumila*, and *Hypnum cupressiforme*. Although the genera *Bartramia* and *Plagiopus* previously

have been characterized by different numbers of chromosomes, n=8 and n=7 respectively, plants of *Plagiopus oederi* from Carinthia and Moravia were found, however, to have n=8. One species, *Isopterygium seligeri* and one variety, *Orthotrichum anomalum* var. *saxatile*, have not been studied previously.

Verma and Kumar (1980) reported the chromosome numbers in the genus *Gymnostomiella, G. vernicosa n*=13, and in the three members of Bartramiaceae, *Anacolia sinensis n*=6, *Philonotis angustata n*=12 and *P. revoluta n*=6 are reported for the first time. The counts for *Physcomitrium cyathicarpum n*=52, *P. repandum n*=52, *Bartramia hallerana n*=9, *B. subpellucida n*=6 and*Philonotis falcata n*=12 are at variance with earlier reports. Intraspecific polyploidy is recorded in *Philonotis turnerana*with *n*=6 12. Quadrivalents are observed in *Philonotis angustata*. A dimorphic bivalent, rare in monoecious bryophytes, is observed in *Bartramia hallerana*. Unlike that of many moss species, the smallest member of the set is heteromorphic in *Bartramidula bartramioides*, *Philonotis calcarea*, *P. revoluta* and *P. turnerana*.

Przywara and Bowers (1992) reported the chromosome numbers for five liverwort and twenty moss species. chromosome numbers in *Porella platyphylloidea* (Schwein.) Lindb. (n = 8) and *Polytrichum pallidisetum* Funck (n = 14) are given here for the first time. New counts are reported for *Anomodon attenuatus* (Hedw.) Hueb. (n = 10) and *Atrichum undulatum* (Hedw.) P. Beauv. var. oerstedianum (C. Muell.) Crum (n = 7). *Bazzania trilobata* (L.) S. Gray (n = 9 + m), *Plagiochila asplenioides* (L.) Dum. (n = 8), *Neckera pennata* Hedw. (n = 10), and *Hylocomium splendens* (Hedw.) Schimp. (n = 11) have not been studied karyologically from North America. Selvan and Kumar (2000) the cytological studies are made in seven moss taxa. The chromosome numbers of *Atrichum longifolium* Card. *Et* Dix. (n = 7), *Enthostodon pilifer* Mitt. (n = 26) and *Pohlia camptotrachela* (Ren. *et* Card.) Broth. (n = 11) are reported for the first time.

Recently Bolaji *et al.* (2017) reported that chromosome numbers in *Hyophila crenulata* n = 4; *Thuidium gratum* n = 12; *Barbula lambarenensis* n=3; *Stereophyllum nitense* n = 9 and *Bryum coronatum* n = 10. It could be concluded that the details of the morphological and anatomical descriptions as well as the chromosome numbers being reported for the first time.

2.13. Genetics:

The genetics of bryophytes was reviewed by Allen in (1935, 1945). Lewis (1961) studied that genetics of bryophytes. Cove *et al.* (1991) reported that development of the haploid gametophyte stage of *Physcomitrella patens* presents excellent opportunities for the detailed study of plant morphogenesis at the cellular level. The filamentous protonema undergoes a number of developmental transitions that can be observed directly in living material using time-lapse video microscopy and that can be manipulated both by treatment with phytohormones and by environmental stimuli. Mutants affecting these processes can be isolated and can be analysed using conventional as well as parasexual methods.

Molecular biological techniques are now being established since these will be essential if the mechanisms that bring about morphogenetic processes are to be understood at the molecular level. A technique for genetic transformation has been devised and is being exploited to attempt to tag developmentally-relevant genes using maize transposons. Changes in gene activity associated with developmental transitions and in response to treatment with phytohormones and environmental stimuli are being studied using c-DNA library subtraction techniques. Heterologous genes involved in the control of transcription and of the cell cycle are being used to probe the *P. patens* genome to identify possible homologues involved in developmental regulation. Reski (1998) studied that molecular genetics techniques have been applied mainly to *Physcomitrella patens* (Hedw.) B.S.G.,where efficient protocols for transformation of nuclear DNA have been established and several nuclear, chloroplast and mitochondrial genes have been analysed.

CHAPTER III

MATERIAL AND METHODS

3. Material and Methods:

3.1 Habitat analysis:

3.1 .1 Study area:

Maharashtra occupies the western and central part of the country and has a protracted shoreline stretching nearly 720 Kilometers alongside the Arabian Sea. The Sahyadri mountain ranges provides a physical backbone to the nation at the west, whilst the Satpuda hills alongside the north and Bhamragad - Chiroli - Gaikhuri stages at the east serve as its herbal borders. The state is covered by North West of Gujarat, north to the Madhya Pradesh, Chhattisgarh to the east, the south east to Andhra Pradesh, Karnataka to the south and south west to Goa. Maharashtra state has a geographical place of 3,07,713 sq. Km and is bounded with the aid of North latitude 15° 40' and 22°00' and East longitudes 72°30' and 80°30' It is the third largest state in terms of area in the country.

Physiographically this state may be divided into three natural divisions - the coastal strip (the Konkan), the Sahyadri or the Western Ghat and the plateau. The Konkan consists undulating low lands. North Konkan has the vast hinterlands. The Western Ghats running almost parallel to the sea coast. The average height of Sahyadri is 1,200 meters. The slopes of the Sahyadri gently descending towards the east and south-east. Tapi, Godavari, Bhima and Krishna are the main rivers of the state. Maharashtra receives its rainfall mainly from south-west monsoon. The rainfall in state varies considerably. There is heavy rainfall in the coastal region, scanty rains in rain shadow areas in the central part and moderate rains in eastern parts of the state.

The soil status of Maharashtra is residual, derived from the underlying basalts. Inside the semidry plateau, the regur (black-cotton soil) is clayey, rich in iron and moisture-retentive, even though negative in nitrogen and organic count. While re-deposited along the river valleys, the kali soils are deeper and heavier, better perfect for Rabi plants. Farther away, with a higher aggregate of lime, the moorland soils form an appropriate Kharif sector. The higher plateau areas have pather soils, which comprise greater gravel.

Maharashtra has typical monsoon climate, with hot, rainy and cold weather seasons. However, dew, frost and hail also occur sometimes, depending upon the seasonal weather. The winter in January and February is followed by summer between March and May and the monsoon season between June and September. Summers are extreme with March, April and May as the hottest months. During April and May thunderstorms are common all over the state. Temperature varies between 22 °C and 39 °C during this season. Rainfall starts normally in the first week of June. July is the wettest month in Maharashtra, while August also gets substantial rain. Monsoon starts its retreat with the coming of September to the state. Winter season is a cool, dry spell, with clear skies gentle breeze; pleasant weather prevails from November to February. But the eastern part of Maharashtra sometimes receives some rainfall. Temperature varies between 12 °C and 34 °C during this season. Rainfall in Maharashtra differs from region to region.

Khandala: It is famous cousin town of Lonawala lies just 5 Km away. Although is comparatively small, equally blessed with natural beauty and bounty. Deep valley on one side and high hills on the other side divide Khandala and Lonawala. It is located at 17° 42' 0" N, 73° 50’ 0" E.

Lonawala: It lies at 18°44’59"N, 73°25'2"E and 5 Km, apart on the Western slopes of Sahyadris, straddling the Mumbai-Pune highway at an altitude of 625 m. Temperatures vary from 12 °C in winter to around 36 °C at the height of summer. The annual rainfall averages 450 cms.

Lohagad: It is one of the hill forts located 18.7102° N, 73.4759° E of Maharashtra state in India and situated close to the hill station Lonavala and 52 Km Northwest of Pune, It is located at about 2000 - 3000 ft above sea level. The average temperature is 26.3 °C and average rainfall is 534 mm.

Tamhini Ghat: It is a mountain passage located 18° 26′ 57.62″ N, 73° 25′ 21.79″ E between Mulshi and Tamhini in Maharashtra, India. Placed on the crest of the Western Ghat mountain ranges and has a maximum elevation of 2,627.59 ft.

Lavasa: It is a private, planned city being built near Pune located 18° 24′ 19.01″ N, 73° 30′ 22.57″ E. Temperature ranges from a minimum of 6 º C to a maximum of 36 º C. in summer.

Sinhagad: It is located roughly 30 Km Southwest of the Pune city. Previously called 'Kondana' was also strategically located at the center of a string of other forts such as Rajgad, Purandar and Torna. The fort is located at 18°21'56"N 73°45'20"E, in Sahyadri Mountains on a deserted cliff of Bhuleshwar range at a height of 1350 m above the sea level.

Purandar: It is located at 18°16'50"N, 73°58'27"E stands 4,472 ft. above the sea level 1,387 m. It actually consists of two forts Purandar and Vajragad. The relative humidity and moisture is normally high during rainy season which favours growth of mosses.

Raireshwar: It is placed at 18° 3′ 0″ N 73° 44′ 0″E. It is situated in Bhor Taluka near Pune, India, 82 Km away. It is situated in between various hills and forts such as Kenjalgad. It has an average elevation of 1000 meters. The annual rainfall averages 643 cms.

Dhom Dam (Wai): It is located at 17.94°N 73.88°E, approximately 95 km south of the city of Pune and 37.9 Km distance from Satara. It is surrounded by the mountainous region of the Sahyadris. It has an average elevation of 718 meters. The average annual rainfall is 5761 mm.

Pratapgad: It is a large fort located 17.9335° N, 73.5806° E in Satara district of the Western Indian state of Maharashtra .It is located 15 Km from Poladpur and 23 Km West of Mahabaleshwar, a popular hill station in the area. The fort stands 1,080 m. above Sea level.

Mahabaleshwar: It is located at 17°55'N 73°40'E. It has an average elevation of 4,439 ft. It is located about 120 Km Southwest of Pune, and 285 Km from Bombay and a vast plateau measuring 150 Km and bound by valleys on all sides. It reaches a height of 1,438 m at its highest peak above Sea level known as 'Wilson Point'

Kaas Plateau (Satara): It is also known as the Kaas Pathar or Kas Sadas, is a plateau situated 25 Km West from Satara city in Maharashtra. It is geographically located at 17°42'0"N, 73°50'E and situated at an altitude of 1200 m. It is a biodiversity hotspot known for various types of seasonal wild flowers blooming in the months of August and September.

Aundh (Satara): It also a Village in Khatav Taluka in Satara District of Maharashtra State, India. It belongs to Desh or Paschim Maharashtra region. It belongs to Pune Division. It is located 45 Km towards East from District head quarters Satara. It is located at 17° 33' 0"N, 74° 20' 0"E.

Koynanagar: It is located at 17° 24′ 0″ N, 73° 45′ 36″ E. It is a Taluka place of Satara district in Maharashtra, India. It has an average elevation of 746 meters. The average precipitation is 2447 mm.

3.2. Surveying, Collection and Identification of Mosses:

3.2.1 Surveying and Collection of Mosses:

Systematic collection of mosses was carried out by conducting frequent field visits in different seasons and from different localities covering almost 14 regions of Maharashtra state during 2014-2018. Mosses were collected from different localities like Khandala, Lonawala, Lohagad, Tamhini ghat, Lavasa, Sinhagad, Purandar, Raireshwar, Dhom Dam (Wai), Pratapgad, Mahabaleshwar, Kaas Plateau, Aundh (Satara) and Koynanager from Maharashtra, India. The collected specimens were put in polythene covers with field numbers. The field data were recorded in the field book such as field number, habitat, habit, locality, date, altitude, type of vegetation, significant characters, associated plants etc., Before evaluation, material has been cleaned along with dried to room temperature for 24 hrs after that this dried up plant material was useful for practical performance.

3.2.2 Identification of Mosses:

Fresh materials were subjected to study whenever possible for identification. External morphological features were studied under a stereo dissection microscope and

internal features by a digital microscope. Size of leaves, capsules, cells apex margin and spore were measured using micrometry. Identification of the specimens was done by referring authentic literatures and also in consultation with experts. The specimens were also identified by western circle botanical survey of India, Pune.

3.2.3 Descriptive key for identification of mosses:

A comprehensive key to the families of mosses is given and also keys have been provided for all the genera in each family for easy identification. A key is purely dichotomous indented type based on reliable characters. The genera arranged in sequential manner. (Dabhade, 1998)

Key to genera

1. Leaves distichons, with vaginant lamina---------- *Fissidens* Hedw.

   Leaves tri or polystichous, absence of vaginant lamina ---------- 2.

2. Lamina generally unistratose or multilayered chlorocysts and
   leucocysts absent---------- 3.
3. Lamellae present on the inner face of the leaf capsule cylindrical,Peristome
   teeth 32---------- *Pogonatum* P. Beauv.
   Capsule angular, Peristome teeth 64 ---------- *Polytrichum* Var.

   Lamellae absent ---------- 4.

4. Basal cells either not hyaline or if hyaline without the
   marginal green cells ---------- 5.
5. Acrocarpous mosses (and some of the isobryales) ---------- 6.
6. Leaf cell prosenchymatous (ends pointed) ---------- 7.
   Leaf cell parenchymatous (ends pointed) ---------- 8.
7. Leaf cells elongated rhombic or rhombic ---------- G - I
   Leaf cells narrowly linear in the upper half of the leaf ---------- G - II
8. Cell wide hexagonal, elongated hexagonal or rectangular---------- G - III
   Cell small, quadrate, rounded hexagonal or rectangular
   but never distinctly prosenchymatous ---------- 9.

9. Primary stem either creeping or if erect with
   distinct and defined alar cells ---------- G - IV
   Stem always erect, alar cells never defined ---------- 10.
10. Cells smooth ---------- G - V
    Cells papillose or mamillose ---------- G - VI
11. Alar cell absent or indistinct ---------- 12.
    Alar cell distinctly developed ---------- 14.
12. Leaf cell prosenchymatous, linear ---------- G - VII
    Leaf cell both prosenchymatous and elongated
    rhombic or parenchymatous ---------- 13.
13. Leaf cell elongated rhombic,rarely short rhombic---------- G - IX
    Leaf cell parenchymatous ---------- G - VIII
14. Leaf cells more or less isodiametric ---------- G - X
    Leaf are not isodiametric ---------- 15.
15. Never present, single or double and at least
    1 /4 the length of the leaf---------- G -X

Group - I

Acrocarpous mosses. Leaf cell prosenchymatous, but either elongated rhombic or or sometimes elongate rectangular in the upper half.

1. Plant small, branching sparingly by innovation below the perichaetium. capsule spherical, dehiscing irregularly or the wall decaying ,spore a few (8-12) in each capsule,100-200 μ in diameter
2. Above combination of characters lacking
   Spore numerous and smaller ---------- 2.
3. Neither the plants and innovations catenulate.
   Leaves obovate-oblong to oblong, lanceolate or broadly
   spathulate, cells wide rhombic, rectangular or polygonal--------- 3.
4. Peritome single ---------- 4.
   Peritome double---------- 5.
5. Capsule erect ---------- 6.

Capsule distinctly inclined to pendulous ---------- 7.

6. Leaves not appearing longer for the size of the
   plant; oblong, elliptic to spathulate; inner
   peristome with a basal membrane ----------*Brachymenium* Schwaegr.
   Leaf cell narrow ---------- *Pohila* Hedw.
   Leaf cell widely rhombic to hexagonal---------- 8.
7. Capsule pyriform or spherical, erect or oblique,
   Operculum epicaranoid ---------- 9.

   Capsule ovoid, shortly cylindric, pendulous, operculum
   Shortly convex or conical, often beaked or mamillose,
   Peristome metacranoid ---------- *Bryum* Hedw.
8. Seta thick capsule either inclined or oblique or if straight and symmetrical narrow- mounted, Peristome usully perfect ---------- *Funaria* Hedw.

Group - II

Acrocarpous mosses. Leaf cell prosenchymatous but narrowly linear in the upper half.

1. Alar cells not differentiated ---------- 2.
   Leaf cells thin- walled ---------- *Pohila* Hedw.
2. Alar cells frequently strongly differentiated

   Leaf often complanate, uni or binerved or nerveless--------- *Hypnum* F. E. Tripp.

Group - III

Acrocarpous mosses. Leaf cell prosenchymatous, wide hexagonal or rectangular

1. Leaf cells regularly hexagonal or rhomboidal hexagonal---------- 2.
2. Leaf cells smooth ---------- 3.
3. Leaf margin not bordered ---------- 4.
4. Rhizoid smooth ---------- 5.
5. Calyptras smaller,cucullate and covering only a part of
   capsule,margin entire---------- 6.
6. Leaves wider and shorter;seta not thin,casule narrow

mouthed; peritome perfect ---------- *Funaria* Hedw.

Leaves narrower and longer; seta very thin, capsule erect, symmetrical

Wide mouthed; peritome imperfect ---------- *Funaria* Subg. *Entosthodon* (Schwaegr.)

Group - IV

Acrocarpous mosses. Cell small quadrate rounded hexagonal or rectangular but never prosenchymatous, either the primary stem creeping or erect and then with distinctly defined.

1. Primary stem creeping with erect branches---------- *Macromitrium* Brid.

Group - V

Acrocarpous mosses. Cell small quadrate rounded hexagonal or rectangular but never prosenchymatous, primary stem always erect; alar cells never defined.

1. Leaf margin incurved with spathulate in shape ---------- *Hyophila* Brid.
2. Leaf with broad, obtuse tips, basal cells not very lax--------- *Barbula* Hedw.

Group - VI

Acrocarpous mosses. Cell small quadrate rounded hexagonal or rectangular but never prosenchymatous, cells papillose or mamillose instead of being smooth.

1. Leaves not bordered, leaf base not sheathing, distal part not spreading ------ 2.
2. Basal cells like parallel organs, leaves translucent -- *Hydrogonium* (C. Muell.) Jaeg.

Group - X

Pleurocarpous mosses with distinctly developed alar cells. Leaf cells isodiametric or rarely elongate rhombic.

1. Primary stem creeping, secondary stem woody, erect or pendulous, never single in the mid leaf or below the apex------ *Trachypodiopsis* (Mitt.) Fleisch.
2. Primary stem creeping, secondary stem woody, pendulous, never absent or very short---------- *Pterobryopsis* Fleisch.
3. Primary stem creeping, secondary stem erect, Never single or double and then at least one –fourth length of the leaf ---------- *Stereophyllum* Mitt.

List of collected moss species from study area:

1. *Fissidens crenulatus* Mitt.
2. *Pogonatum* Sps.
3. *Polytrichum commune* Var.
4. *Brachymenium turgidum* Broth.
5. *Pohlia nutans* (Hedw.) Lindb.
6. *Bryum argenteum* Hedw.
7. *Bryum coronatum* Schwaegr
8. *Bryum ghatense* Broth. et Dix.
9. *Bryum pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey. & Scherb.
10. *Funaria hygrometrica* Hedw.
11. *Funaria nutans* Mitt.
12. *Hypnum reflexum* F. E. Tripp.
13. *Macromitrium sulcatum* Brid.
14. *Hyophila involuta* (Hook.) Jaeg
15. *Barbula unguiculata* Hedw.
16. *Hydrogonium arcuatum* (Griff.) Wijk & Margad.
17. *Trachypodiopsis blanda* (Mitt.) Fleisch.
18. *Pterobryopsis walkeri* (Broth.) Broth.
19. *Steeriophyllum anceps* (Bosch et Lac.) Broth.

3.2.4 List of dominant moss species selected for physiological study:

1. *Fissidens crenulatus* Mitt.
2. *Brachymenium turgidum* Broth.
3. *Bryum coronatum* Schwaegr
4. *Funaria hygrometrica* Hedw.
5. *Hypnum reflexum* F. E. Tripp.
6. *Macromitrium sulcatum* Brid.
7. *Hyophila involuta* (Hook.) Jaeg.
8. *Barbula unguiculata* Hedw.
9. *Trachypodiopsis blanda* (Mitt.) Fleisch.
10. *Steeriophyllum anceps* (Bosch et. Lac.) Broth.

3.2.5 Taxonomical characters:

1. *Fissidens crenulatus* Mitt.

Plants small with reddish brown, stem up to 6 mm high. Leaves oblong –lanceolate, 1.5 mm long and 3 mm wide. Leaf broadest at base and acute towards the apex. Nerve light brown. Leaf cells with single, mamillose papilla, irregularly hexagonal, leaf apex margin more or less crenulate. Mid-leaf and leaf base margin bordered by 1-3 rows of elongated, cartilaginous cells. Leaf base rounded at the place of attachment. Seta apical, orange brown, erect, near about 5 mm long. Spores circular, light brown, smooth.

2. *Brachymenium turgidum* Broth.

Plant forming cushions with innovations at apex, growing on branches of trees. Plants erect, 1-1.5 cm height, resolute and caespitose. Leaves narrow, sub erect, twisted when dry, widely spreading and slightly falcate, ovate, lanceolate or ligulate. Leaf margin extremely narrow border. Leaf cell pellucid, elongated hexagonal and basal cells elongated to sub-rectangular. Nerve excurrent. Apices differ with allied species by the turgid, Su-Pendulous capsules.

3. *Bryum coronatum* Schwaegr.

Dioecious, tuft of slender dull, yellowish-green plants growing on sandy soil. Stems erect, 1 cm in height. Leaves contorted when dry, ovate, concave and lanceolate. Capsule cylindrical or oblong, pear shaped, pendulous, 3 mm high with distinct neck, Operculum slightly pointed, epitome teeth papillose, transversely barred, yellowish orange, spores light brown, globose or oval, smooth margin.

4. *Funaria hygrometrica* Hedw.

Plants loosely or closely tufted, 1-1.5 cm high. Leaves yellowish green, upper leaf larger, imbricate concave, lanceolate or wider oblique, shortly pointed, lower leaf small nerves ceasing at apex, laminar cells elongate, hexagonal to rectangular, polygonal to rhombic, long at base, shorter towards the apical region, little narrower, margin entire. Basal cells large, rectangular to sub-rectangular, polygonal and long , set a long, flexure, reddish, strangely twisted and hygroscopic when dry, brownish with yellow

tinge, deep red or orange, with a side mouth, capsule symmetrical, inclined, pyriforrn, oblique, gibbous at backstage, furrowed, wide mouthed when dehiscing, sulcate when dry, 3 mm long, 1.5 -2 mm broad. Peristome complete, oblique, 5 mm long and broad at base, inner ones or equal length with the outer, processes shorter than the teeth. Operculum convex. Spores rounded, brown, rounded. Capsule mouth asymmetrical red-brown wide, orange to yellow mouthed capsule is very easily recognized from other species.

5. *Hypnum reflexum* F. E. Tripp.

Plant stems 2 – 2.5 cm long, greenish brown, secondary branches narrow, erect, irregularly branched, procumbent, flatly leafy. Leaf cells rhomboidal, linear, apical cells papillose, cells ending projecting. Operculum projecting or short prostrates peristome double, 32, exostome teeth straight and cross band at the end. Spores brown. Plants dull green, prostrate, creeping on bark of tree.

6. *Macromitrium sulcatum* Brid.

Red-brown plants with golden – yellow tip densely tufted, robust, glossy and bright. Branches 5 cm long, tomentose. Leaves all alike, crowded, erect, strongly contorted, crispate when dry erect and spreading when moist, 4-5 mm long, acuminate, transversely undulate, acuminate. Leaf margin erect, irregularly denticulate towards the apex. Leaf cells smooth, rounded, incrassate, elongate in point,; leaf basal cells narrowly rectangular, broad, pale, walls delicate, strongly thick-walled. A small group of lax, rhomboidal, smooth cells present at the base of leaf by the side of nerve, well differentiated from adjacent. Perichatial leaves sharply acuminate with more uniformly elongate areolation. Seta erect, soon becoming lateral, red, smooth walled, 10-12 mm long. Capsule exerted, ovoid, small-mouthed, strongly sulcate towards base, peristome double, calyptras naked, deeply laminate. Spores light to dark brown, globose to sub-globose. Marked characters of species are epiphytic moss growing on branches of trees with sulcate nature of capsule and papillose cells in the leaves.

7. *Hyophila involuta* (Hook) Jaeg.

Plants dark green with tuft of rhizoids at base. Stem 1 – 1.5 cm high. Leaves long, spathulate, falcate lamina, Leaf margin wavy, entire or serrate to crenulate to upper side and mostly involutes when dry. Nerve percurrent, casing below the 2-3 cells of apex. Leaf cells small, quadrate. Seta erects, elongate, 2 – 2.5 cm high. Capsule 4-5 mm high, erect, cylindrical or needle like, lid conical, beak oblique. Spores light brown, rounded. Plants commonly grow on sandy soil and compound walls.

8. *Barbula unguiculata* Hedw.

Plants small to large, slender to rather robust, 5–40 mm high, green to brownish green, in soft, loose tufts. Stems erect, usually irregularly branched, often radiculose at base. Leaves contorted or crisped when dry, erect-spreading when moist, ovate-lanceolate to narrow-ligulate, obtusely pointed to apiculate; margins entire, plane above, recurved in lower half; costa stout, shortly excurrent as a mucro; upper leaf cells quadrate to hexagonal, rather thin-walled, obscure, multi-papillose, with several, small, C-shaped papillae; basal cells differentiated, short-rectangular, somewhat thick-walled, smooth, rarely papillose, hyaline to green, Gemmae absent. Setae 10–15 mm long, reddish brown; capsules erect, cylindrical; peristome teeth filiform, twisted, densely papillose; opercula conic-rostrate, with an erect beak.

9. *Trachypodiopsis blanda* (Mitt.) Fleisch.

Plants very slender, greenish brown, densely tufted. Primary stem creeping, filiform, secondary stem pendulous, pinnate or bipinnately branched, leaves closely oppressed, when dry spreading, erect to erecto-patent. Apex acuminate with serrate margin. Sporophyte with oval capsule, seta 2 mm long, spores spherical to oval. Plants are epiphytic on branches of trees.

10. *Steeriophyllum anceps* (Bosch et. Lac.) Broth.

Plant medium, robust, green, Main tem creeping, erect, 1 or 2 branched. Leaves imbricate, erectopatant, elliptic-lanceolate, concave, acute apex and margin dentate at tip. Costa single, leaf cells rhomboid. Sporophyte formed on main stem, capsule erect or

lightly nodding, ovate, cylindrical. Long operculum conic short roseate. Spores small, yellow and smooth.

3.3 Soil analysis:

3.3.1 Collection of rhizosphere soil samples:

Soil samples from fields were brought to laboratory and spread on a paper. Stones, gravels and other coarse residues were removed. Larger pieces of soil were broken by hand. The air dried soil samples were crushed in mortar and pestle and sieved through 2 mm nylon sieve. These samples were used further for analysis of various physical and chemical characters.

3.3.2 Physical characteristics of rhizosphere soil:

1. pH:

One gram of soil sample collected was homogenized in mortar with pestle in 25 ml D.W. and from the filtrate pH was determined using Elico Digital pH meter.

2. Electrical Conductivity (EC) :

From the above sample, electrical conductivity was measured with the help of Conductivity Bridge (Elico Model cm. 827).

3.3.3 Chemical characteristics of rhizosphere soil:

The element analysis was made at Soil Testing Laboratory, Krushi Vidnyan Kendra, Shardanagar and Post-Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati (Dist. Pune). Available Nitrogen (N) are estimated by method of Bremner (1960); Phosphorus (P) by Chapman and Pratt (1961); Potassium (K) by Toth *et.al.* (1948) and Organic carbon percentage (C %) by Walkley-Black (1934).

1. Total Nitrogen:

For estimation of the available N content of the soil, alkaline permanganate method of Bremner (1960) was followed. Oven dried, powdered 0.5 g of soil sample was taken in Kjeldahl flask with a pinch of micro salt (200 g K_2SO_4+ 5g $CuSO_4$, dehydrated) and to it 5 ml H_2SO_4 (1:1) was added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion a faint yellow solution was obtained which was cooled to room temperature, transferred to volumetric flask and

diluted to 100 ml with distilled water.

In very clean Nessler's tube, 1 ml of soil extract and different concentrations of standard ammonium sulphate solution (0.236 g Ammonium sulphate dissolved in water and few drops of H_2SO_4 are added and the volume is made 1000 ml. This solution contains 0.05 mg of nitrogen per ml) are taken. In control tube 1 ml distilled water was taken. To this, 1 drop 8% KH_2SO_4 is added and volume is made 35 ml with distilled water. To this, 15 ml Nessler's reagent is added (Reagent A : 7 g KI + 10 g $HgCl_2$ in 40 ml distilled water, Reagent B : 10 g NaOH in 50 ml water, A and B are to be mixed in proportion of 4:5 at the time of estimation). After 15 minutes, the absorbance of the chromophore developed is read at 520 nm using ELICO spectrophotometer. Nitrogen value is calculated from the standard curve. Values are expressed as Kg/ha dry matter.

2. Phosphorous:

For estimation of P^{5+} the method of Chapman and Pratt (1961) was followed. Here, phosphorus reacts with Vanadate-Molybdate reagent to give yellow colour complex. By estimating calorimetrically the intensity of the colour developed and by comparing it with the colour intensity of known standards, phosphorus content was estimated.

To 1 ml of acid digest in a test tube, 2 ml of 2 N HNO_3 was added with 1 ml Vanadate-Molybdate reagent (1. 25 g of ammonium molybdate in 500 ml 1 N HNO_3 + 25 g of ammonium vanadate in 500 ml distilled water mixed in equal volumes) and volume was made to 10 ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes, color intensity developed was measured at 420 nm spectrophotometrically using a reaction blank containing no phosphorus.

Calibration curve of standard phosphorus was prepared from standard phosphorus solution (0.110 g KH_2PO_4 per liter - 0.025 mg P^{5+}/ ml) taking different concentrations (0.025, 0.05, 0.1, 0.2, 0.4 mg P); other steps being essentially similar to the one described above. With the help of standard curve, amount of phosphorus in the soil was calculated. Values are expressed as Kg/ha dry matter.

3. Potassium:

The soil sample was acid digested following the method of Toth *et.al.* (1948) in concentrated nitric acid and perchloric acid. The acid digest served as sample. Potassium

is estimated according to standard flame photometric process employing ELICO Flame-photometer. Absorbance of flame color intensity was measured on Flame photometer with specific color filter for Potassium.

For standardization, various concentrations of K^+ were prepared ranging from 1 - 10 ppm by diluting stock solution of KCl (100 ppm) from galvanometer readings. Potassium was estimated using calibration curve of known concentration (K^+). Values are expressed as Kg/ha dry matter.

4. Organic Carbon :

The standard wet chemistry technique for the sample extraction involving the rapid dichromate oxidation of organic matter was employed as per Walkley-Black (1934). In this procedure, potassium dichromate ($K_2Cr_2O_7$) and conc. H_2SO_4 were added to 1.0 g of the soil sample. The solution is swirled and allowed to cool. Prior to adding water to halt the reaction, Orthophosphate, H_3PO_4 is added to the digestive mixture to eliminate interferences from the ferric ($Fe^{3+)}$ iron that may be present in the sample.

One gram of the prepared soil sample was taken in a 500 ml conical flask. To this, 10 ml of 0.1667 M $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 containing Ag_2SO_4 was added, mixed thoroughly and finally allowed the reaction to complete for 30 minutes. The Reaction mixture was diluted with 200 ml with distilled water and 10 ml of H_3PO_4 and then to this 10 ml of NaF solution and 2 ml of diphenylamine indicator was added. The final solution was titrated with standard 0.5M $FeSO_4$ solution till a brilliant green color was obtained. Simultaneously, a blank without sample was also run.

The percentage of organic carbon is calculated using following formula

$$\text{Organic Carbon} = 3\ (S - T) / S$$

Where:

S = ml of $FeSO_4$ solution required for blank.

T = ml of $FeSO_4$ solution required for soil sample.

3.4 Physiological parameters of mosses:

3.4.1 Mineral nutrients:

Ten moss species were analyzed for macro and micro nutrients like N, P, K and Ca, Mg, Fe, Mn, Zn, Cu, respectively at the Maharashtra Rajya Draksha Bagaitdar Sangh, Manjari Pune.

Analysis of various inorganic macronutrients like Nitrogen, Phosphorus, Potassium, Magnesium and Calcium. Microelements like Iron, Manganese, Copper, and Zinc from mosses were carefully separated and subjected to drying separately in oven at temperature 60°C. Oven dried plant material served as sample for analyzing various mineral nutrients. Methods for analysis of mineral nutrients are as follows:

1. Macronutrients:

A. Total Nitrogen:

For estimation of the available N content of the soil, alkaline permanganate method of Bremner (1960) was followed. Oven dried, powdered 0.5 g of moss sample was taken in Kjeldahl flask with a pinch of micro salt (200g K_2SO_4+ 5g $CuSO_4$, dehydrated) and to it 5 ml H_2SO_4 (1:1) was added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion a faint yellow solution was obtained which was cooled to room temperature, transferred to volumetric flask and diluted to 100 ml with distilled water.

In very clean Nessler's tube, 1 ml of moss extract and different concentrations of standard ammonium sulphate solution (0.236 g Ammonium sulphate dissolved in water and few drops of H_2SO_4 are added and the volume is made 1000 ml. This solution contains 0.05 mg of nitrogen per ml) are taken. In control tube 1 ml distilled water was taken. To this, 1 drop 8% KH_2SO_4 is added and volume is made 35 ml with distilled water. To this, 15 ml Nessler's reagent is added (Reagent A: 7 g KI + 10 g $HgCl_2$ in 40 ml distilled water, Reagent B: 10 g NaOH in 50 ml water, A and B are to be mixed in proportion of 4:5 at the time of estimation). After 15 minutes, the absorbance of the chromophore developed is read at 520 nm using ELICO spectrophotometer. Nitrogen value is calculated from the standard curve. Values are expressed as mg/g dry matter.

B. Phosphorous:

For estimation of P the method of Chapman and Pratt (1961) was followed. Here, phosphorus reacts with Vanadate-Molybdate reagent to give yellow colour complex. By estimating calorimetrically the intensity of the colour developed and by comparing it with the colour intensity of known standards, phosphorus content was estimated.

To 1 ml of acid digest in a test tube, 2 ml of 2 N HNO_3 was added with 1 ml Vanadate-Molybdate reagent (1.25 g of ammonium molybdate in 500 ml 1 N HNO_3 + 25 g of ammonium vanadate in 500 ml distilled water mixed in equal volumes) and volume was made to 10 ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes, color intensity developed was measured at 420 nm spectrophotometrically using a reaction blank containing no phosphorus.

Calibration curve of standard phosphorus was prepared from standard phosphorus solution (O.110 g KH_2PO_4 per liter- 0.025 mg P^{5+}/ ml) taking different concentrations (0.025, 0.05, 0.1, 0.2, 0.4 mg P); other steps being essentially similar to the one described above. With the help of standard curve, amount of phosphorus in the sample was calculated. Values are expressed as mg/g dry matter.

C. Potassium:

The moss sample was acid digested following the method of Toth *et.al.* (1948) in concentrated nitric acid and perchloric acid. The acid digest served as sample. Potassium is estimated according to standard flame photometric process employing ELICO Flame-photometer. Absorbance of flame color intensity was measured on Flame photometer with specific color filter for Potassium.

For standardization, various concentrations of K^+ were prepared ranging from 1 - 10 ppm by diluting stock solution of KCl (100 ppm) from galvanometer readings. Potassium was estimated using calibration curve of known concentration (K^+). Values are expressed as mg/g dry matter.

D. Calcium:

Calcium was also estimated from the sample solution flame photometrically using the same procedure of Toth *et al.,* (1948) to get acid digest. The acid digest served as sample.

For Standardization, various concentrations of Ca^{+} were prepared ranging from 50-200 ppm by diluting stock solution of $CaCO_3$ (100 ppm). From galvanometer readings, calcium was estimated using calibration curve of known concentration (Ca^{+}). Values are expressed as mg/g dry matter.

E. Magnesium :

Magnesium was estimated following the method of Drosdoff and Nearpass (1948). To 5 ml of acid digest in a 50 ml volumetric flask, following reagents were added in a sequential order and mixed thoroughly: 1ml hydroxylamine (5% w/v), 5 ml starch compensating solution (equal volume of freshly prepared 2 % starch solution and compensating solution : 3.7 g calcium chloride, 0.6 g of trisodium phosphate all dissolved in distilled water containing 10 ml conc. HCL and then volume made to 1 lit.), 1 ml Thiazole yellow (0.1% aqueous) and 5 ml 2.5 N NaOH. Volume was made to 50 ml with distilled water and after 30 minutes colour intensity was measured at 525 nm spectrophotometrically.

Reagent blank was prepared in the same manner as above except sample was replaced by distilled water. A standard curve for Mg^{+} was also prepared with the help of different concentrations of Mg^{+} from stock solution of Mg (100 ppm) and with this Mg^{+} content in the sample was calculated. Values are expressed as mg/g dry matter.

2. Micronutrients :

Iron, Manganese, Zinc, Copper:

DTPA (Diethylene Triamine Penta Acetic acid) extraction method was followed (Lindsay and Norvell, 1978) by using AAS for above mentioned micronutrients analysis.

About 5 gram of sample was taken in extraction flask. To this, 40 ml of DTPA was added using calibrated dispenser and then sample was shaken on shaker table at 200 rpm for 2 hr. After this, samples were filtered using Whatman Filter Paper No.1 and finally filtrate was used for analysis on Atomic Absorption Spectrophotometer (Perkin - Elmer - 3030). Results are expressed as mg /g dry matter.

3.4.2 Photosynthetic pigments:

A. Estimation of Chlorophylls:

The estimation was done as per Arnon (1949) method. Take 0.5 gm fresh green plant material. Then cleaned with distilled water and it allowed airing dry. The plant materials were homogenized in 10 ml of 80% chilled acetone with the help of the mortar and pestle in dark. A pinch of $MgCO_3$ powder was added to neutralize the acids release during extraction. The extract was filtered through Whatman No.1 filter paper using the Buchner's funnel under suction. Final volume of the filtrate was made to 100 ml. with 80% acetone in conical flask. The filtrate was transferred in the conical flask and wrapped with black carbon paper to prevent the Photo-oxidation of the pigments. Read absorbance at 645 and 663 nm wavelengths with spectrophotometer with 80% acetone as a blank.

Chlorophylls (mg 100 g''' fresh tissue) were calculated using the following formulae:

Chlorophyll a = $12.7 \times A_{663} - 2.69 \times A_{545} = X$

Chlorophyll b = $22.9 \times A_{645} - 4.68 \times A_{663} = Y$

Chlorophyll a+ b = $(A_{663} \times 22.2) + (A_{645} \times 8.02) = Z$

Chlorophyll a / b / a + b

$$\text{mg 100 g fresh tissue} = \frac{X / Y / Z \times \text{Vol. of extract (ml)} \times 100}{1000 \times \text{Wt. of plant material (g)}}$$

B. Estimation of Carotenoids:

The estimation was done as per Kirk and Allen (1965) method. Fresh green plant sample 0.5 g of was weighed. Then cleaned with distilled water and it allowed airing dry. The plant materials were homogenized in 10 ml of 80% chilled acetone with the help of the mortar and pestle in dark. A pinch of $MgCO_3$ powder was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper using the Buchner's funnel under suction. Final volume of the filtrate was made to 100 ml. with 80% acetone in conical flask. The filtrate was transferred in the conical flask and wrapped with black carbon paper to prevent the photo-oxidation of the

pigments. Read absorbance at 480 nm wavelengths with spectrophotometer with 80% acetone as a blank.

Total carotenoids were estimated using formula of Liaaen-Jensen and Jensen (1971).Values of carotenoids is expressed as mg 100 g^{-1} fresh tissue.

Carotenoids = $A_{480} + 0.114 \times A_{663} - 0.636 \times A_{645}$

3.4.3 Enzymes:

A. Estimation of Catalase (E.C. 1.11.1.6):

The enzyme preparation made for the assay of peroxidase was also used for the assay of catalase. Assay of the enzyme was done following the method described by Herbert (1955). The assay mixture contained 5 ml distilled water, 1 ml 0.2 % H_2O_2 and 1 ml enzyme, then incubate 15 min. The reaction was terminated by adding 5 ml 10 % H_2SO_4 , titrated against 0.05 N $KMnO_4$. End point was colorless to just pink. The difference between the titrated reading of control set (without enzyme) and reaction mixture (with enzyme) provides the amount of H_2O_2 which was decomposed by enzyme action. The activity of the enzyme is expressed as mg H_2O_2 liberated min^{-1} g^{-1} dry tissue.

B. Estimation of Peroxidase (E.C. 1.11.1.7) :

Peroxidase was determined from moss species following Maehly (1954) method. Enzyme was extracted by homogenizing the plant material (0.5g) in 10 ml chilled phosphate buffer. It was then filtered through moist cheese or muslin cloth and the filtrate so obtained was centrifuged at 10,000 rpm for 10 min and the supernatant was used as an enzyme source. Enzyme assay mixture contained 4.5 ml phosphate buffer (pH 7, 0.1 ml), 1 ml Guiacol (20 mm) and 1 ml enzyme. The reaction was started by addition of 0.5 ml H_2O_2 (10 mm). Change in the optical density due to oxidation of guiacol was recorded per minute at 470 nm. Double Beam UV-Vis Spectrophotometer. Enzyme activity is expressed as Δ O D. min^{-1} g^{-1}

C. Estimation of Polyphenol oxidase (E.C. 1.14.18.1):

The estimation of polyphenol oxidase was assayed as per the method of Sato and Hasegawa (1976). Take material 0.5 gm was extracted in 10 ml 0.1 M potassium

phosphate buffer (pH 6.5) and filtered through muslin cloth. Filtrate was centrifuged at 10,000 rpm for 10 min. Supernatant used as enzyme source. A mixture of 0.2 ml enzyme sample, 0.3 ml 0.01M catechol, 2.5 ml 0.1M Potassium phosphate buffer was incubated at 25^0C. Read the absorbance at 490 nm spectrophotometrically. The difference between two readings served as enzyme activity. Polyphenol oxidase activity is expressed as Δ OD. min. $^{-1}$ g^{-1}

D. Estimation of Nitrate reductase (E.C. 1.6.6.1) :

The estimation was done as per Jaworski (1967) method. Take a weight of 0.5g fresh green plant material. The plant materials cut into small pieces, was suspended in 5ml mixture of 0.1 M phosphate buffer (pH 7.0). Then take fresh plant material cut into small pieces incubated in 5ml reaction mixture containing 0.1 M KNO_3, 5 % n-propanol, and 0.05 % Triton-X-100 in dark for 60 min. After 60 min., nitrate reductase was measured by treating 0.4 ml of incubation mixture with 0.3 ml of each of 1% sulphanil amide and 0.02 % NEEDA (N-1-Napthyl-ehylenedimine hydrochloride) for 20 min. The samples diluted 2. 2 ml distilled water. Read absorbance at 540 nm wavelengths with spectrophotometrically standard curve was prepared different concentration of $NaNo_2$.The enzyme activity is expressed as mg NO_3 consumed nmole h $^{-1}$ g^{-1} dry tissue.

E. Estimation of Nitrite reductase (E.C. 1.6.6.4):

The estimation was done as per Rama Rao *et al*., (1983) method. Take a Weight of 0.5gm fresh green plant material. The plant materials cut into small pieces, was suspended in 5ml mixture of 0.1 ml phosphate buffer (pH 7.0). Then take fresh plant material cut into small pieces incubated in 5ml reaction mixture containing 0.1 M KNo_3, 5 % n-propanol, 0.05 % Triton-X-100 in dark for 60 min. After 60 min., nitrate reductase was measured by treating 0.4 ml of incubation mixture with 0.3ml of each of 1% sulphanil amide and 0.02% NEEDA (N-1-Napthyl-ehylenedimine hydrochloride) for 20 min. The samples diluted 2.2 ml distilled water. Read absorbance at 540 nm wavelengths with spectrophotometrically standard curve was prepared different concentration of $NaNo_2$.The enzyme activity is expressed as mg NO_2 consumed nmole h $^{-1}$ g^{-1} dry tissue.

3. 5 Biochemical analysis:

3.5.1 Total carbohydrates:

The total carbohydrates were determined by the phenol-sulphuric acid method (Dubois *et al*., 1956). For this study, 0.5 g of moss sample was used. It was put in test tube and boiled. The material hydrolyzed by keeping it in boiling water bath for 3 hrs with 5 ml of 2.5 N HCL and cooled at room temperature. Neutralized it with solid Sodium Carbonate (Na_2CO_3) until effervesces seen. Made the volume 100 ml in 2.5 N HCL and centrifuged. Then collect supernatant and take 0.5 and 1 ml of the aliquant for analysis. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard of the series of test tube. Made the volume 1 ml in each test tube by distilled water. Add 4 ml anthrone reagent in each test tube. Heat for 20 minutes in boiling water bath then cool rapidly and observed the green to dark green colour. Read the absorbance at 630 nm. Calculated amount of total carbohydrates present the sample solution using standard graph. Results are expressed as mg /gm dry tissue.

3.5.2 Starch:

The estimation of starch was done by Holligan and Lewis (1974) method. Take, 0.5 g of plant material were homogenized in 10 ml of 80% hot ethanol with the help of the mortar and pestle to remove sugars. Centrifuged at 7000 rpm for 10 minutes and then retain the wash residue repeatedly with 80% hot ethanol till the washing does not give colour with anthrone reagent then dry residue over water bath. Residue added 5ml water and 6.5 ml of 52% Perchloric acid. Re-extract centrifuge for 20 minutes and save supernatant. Repeat the re-extraction using fresh Perchloric acid. Then Centrifuge and pool the supernatant and make up to 100 ml volume. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard of the series of test tube. Make a volume 1 ml. with distilled water. Add 4 ml anthrone reagent to each test tube. Heat for 8 minutes in boiling water bath. Cool rapidly and read the intensity green to dark green colour at 630 nm. Calculated amount of total starch present the sample solution using standard graph.

3.5.3 Reducing sugar:

Estimation of reducing sugar was done by dinitrosalicylic acid method. Take, 0.5 g of plant material were homogenized in 10 ml of 0.05 M acetate buffer with the help of

the mortar and pestle. Centrifuge at 15000 rpm for 15 minutes. Supernatant were collected and used for estimation reducing sugars. 0.1 ml filtrates were taken in test tubes. Different concentrations of standard glucose were taken in other test tubes. In each test tube requisite amount of distilled water was added to make final volume 1 ml. In case of blank 1 ml distilled water was taken instead of filtrate or standard glucose, then each tubes, add 1 ml of 0.05 M acetate buffer (pH 4.8) and mix. Add 3 ml DNSA reagent to all the test tubes and mix well. Place the tubes in boiling water for 5 minutes. Cool the tubes to room temperature and measure the absorbance readings were recorded on a double beam spectrophotometer at 540 nm.The amount of reducing sugars was estimated with the help of calibration curve of standard glucose and the values were expressed as mg/g dry tissue.

3.5.4 Soluble proteins:

The proteins present in the mosses estimated by Lowery's method (1951) of protein estimation. For protein estimation, the 10 types of different mosses are used. Collected plant material cleaned and dried then used for practical work. Weight accurate 0.5 gm of dried material homogenized with mortal and pestle in 10 ml phosphate buffer. Centrifuged it and collect supernatant and stored in ice bath. Taking 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml standard proteins in different test tubes and 0.1, ml of plant extract in to separate test tubes. Adjusted final volume 1 ml with the help of distilled water. Prepared blank with 1 ml distilled water. Added 05 ml reagent C (i.e. reagent A 49 ml with reagent B 1 ml) including blank and allow standing for 10 min. then added 0.5 ml Folin Ciocalteu's reagent D and shacked. Incubate at room temperature (DARK) for 10 minutes. Read absorbance at 660 nm by spectrophotometrically. Amount of protein calculated mg/g^{-1} dry wt. using standard protein graph.

3.5.5 Free amino acids:

Free amino acids have been estimated by method of Moore and Stein (1948) Take, 0.5 gm of plant material was homogenized in 10 ml of 80% ethanol with the help of the mortar and pestle. Centrifuged at 15000 rpm for 15 minutes. The residue was re-extracting with 10 ml of 80% ethanol and centrifuged. Supernatant were collected and used for quantitative estimation of total free amino acids. Pipette out 0.2, 0.4, 0.6, 0.8 and

1.0 ml of working standard of the series of test tube. 1 ml of ninhydrin reagent was added 0.5 ml of extract in each test tube. Make a volume 2 ml. with distilled water. Tubes heated in a boiling water bath for 20 minutes. Add each test tube in 5 ml diluents solvent (equal vol. of water and n - propanol) added the contents were mixed well and after 15 min. absorbance developed purple colour. Read the absorbance at 570 nm. Calculated total free amino acids present the sample solution using standard graph.

3.5.6 Free proline:

Free Proline content was determined according to the method (Bates *et al.,* 1973). Take a 0. 5 gm. of plant material was homogenized in 10 ml sulphosalicylic acid (3.0 %) and then filtered through Whatman filter paper No. 1. 2 ml of these filtered was reacted with 2 ml glacial acetic acid and 2 ml Ninhydrin reagent (1.25 gm. of Ninhydrin in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M Orthophosphoric) in a test tube for 1 hour at 100 °C in boiling water bath. Similar procedure was also followed for using standard Proline solution (1 mg/µl Proline) after boiling the reaction was terminated by transferring in the test tube immediately to ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously with test tube for 15 -20 second. Reaction mixture was then brought to room temperature and absorbance measured at 520 nm spectrophotometrically using toluene blank. Proline concentration was calculated from calibration curve and final volumes were expressed as mg / g dry tissue.

3.6 Secondary metabolites:

3.6.1 Total polyphenols :

Total Polyphenols were determined according to Folin and Denis (1915). 0.5 gm. of plant material was crushed in 10 ml ethanol using mortar and pestle. The material was centrifuged at 10,000 rpm for 20 min then collect the supernatant then residue was taken at five times by same process. The supernatant was evaporated over water bath to dryness. After that residue was dissolved by adding 5 ml distilled water filter it and use for reaction mixture. 0.1 ml plant sample was taken .Made 3 ml volume with distilled water then 0.5 ml Folin *&* Ciocalteu's was added. Wait for 3 min then 20 % Na_2Co_3 was added. The mixture was boiling for 1 min and O D. was taken at 650 nm and final volumes were expressed as mg / g dry tissue.

3.6.2 Tannins:

The tannin content in dried moss plant material was estimated spectrophotometrically by Folin-Denis method. Take, a 0.5 gm plant material was hydrolyzed with 70 ml D.W. in 250 ml. volumetric flask by keeping it in boiling water bath for 30 min. The content was cooled and centrifuged at 10,000 rpm for 20 min. and supernatant was collected in 100 ml. volumetric flask. Final volume was made up 100 ml using distilled water and this served as source of tannins. 1 ml of sample extract, 5ml of Folin - Denis reagent. 10 ml of 20% Na_2Co_3 was added and volume was made 100 ml. distilled water. Read the absorbance at 700 nm and final volumes were expressed as mg / g dry tissue.

3.7 Effect of aqueous moss extracts on seed germination and seedling growth of semi-arid region crops:

3.7.1 Preparation of aqueous extracts:

One gram of dried moss material was homogenized in mortar and pestle in 25 ml. distilled water and made final volume 100 ml. The filtrate was used for treatments of semi-arid crop seeds.

3.7.2 Aqueous extract bioassay:

Prepared aqueous extracts from 10 moss species and gave treatments to semi-arid crop seeds of ten dicot and monocot plants were soaked in 100 ml aqueous extracts of test 8 hrs. For control, the seeds were soaked in distilled water. Lots of twenty five seeds treatments were placed on germination papers in 9 cm diameter Petri plates. Plates were incubated in a growth room at 25 ± 28 °C for 5 days. After 5 days of incubation, germination percentage, root and shoot length was recorded. Their percentage frequency of occurrence was calculated by applying the following formula:

$$\text{Seed Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds}} \times 100$$

List of semi-arid region crop seeds:

Crops	Common name	Botanical name	Variety
Cereals	Jowar Bajara Wheat	*Sorghum vulgare L.* *Pennisetum glaucum* L. *Triticum aestivum L.*	Maldandi Kaveri Lokvan
Pulses	Red gram Chick Pea	*Cajanus cajan L.* *Cicer arientinum L.*	Gavran Tur Desi Yellow gram
Legumes	Ground-nut Green gram	*Arachis hypogaea L.* *Phaseolus aureus Roxb.*	Western -44 Gavran Mug
Spices	Onion Mustard	*Allium cepa L.* *Brassica juncea L.*	Garvi Rai mustard
Vegetables	Tomato	*Solanum lycopericum L.*	Pusa

3.8 Statistical analysis:

The data obtained in various experiments was subjected to statistical analysis. For this, expertise was obtained from Post Graduate Department of Statistics, Tuljaram Chaturchand College, Baramati. Also the primary data were analyzed using Past 3 software and the values presented in tables and figures are statistically processed wherever necessary.

CHAPTER IV

RESULTS AND DISCUSSION

4. Results and Discussion:

4.1 Habitat study:

The 10 mosses are collected from 14 localities of Maharashtra such as *Barbula unguiculata* Hedw., *Hypnum reflexum* F. E. Tripp., *Steeriophyllum anceps* (Bosch et. Lac.) Broth, *Fissidens crenulatus* Mitt, *Trachypodiopsis blanda* (Mitt.) Fleisch, *Funaria hygrometrica* Hedw, *Hyophila involuta* (Hook.) Jaeg., *Brachymenium turgidum* Broth., *Bryum coronatum* Schwaegr and *Macromitrium sulcatum* Brid. from 09 families during the period of July 2015 to October 2018. All the 10 species were observed during the rainy season and summer season. Most of species grow on rainy season due to high moisture percentage in atmosphere. Mosses are sensitive to natural fluctuations in humidity. They have capacity absorb water from fog, mist, and dew for their life protect.

The mosses grow on different habitats such as bark of trees, plastered and un-plastered house walls, blocks, rock surfaces and sand soil. There are two terrestrial species *Hyophila involuta* (Hook) Jaeg. and *Bryum coronatum* Schwaegr. which grow on solid substratum and holsters of soil. Six epiphytic species such as *Hypnum reflexum* F. E. Tripp., *Steeriophyllum anceps* (Bosch et. Lac.), *Fissidens crenulatus* Mitt. Broth, *Trachypodiopsis blanda* (Mitt.) Fleisch., *Brachymenium turgidum* Broth. and *Macromitrium sulcatum* Brid. occur on portion of bark or trees. Two lithophytic species *Barbula unguiculata* Hedw. and *Funaria hygrometrica* Hedw. grow on basalt and latirus rocks (Table 1) .

4. 1. 1 Mosses from the different localities of Maharashtra.

The present investigation shows 10 species from 14 localities of Maharashtra (Table 2). *Barbula unguiculata* occurs in Purandar, Rayereshwar, Lonawala and Dhom dam (Wai). *Hypnum reflexum* is found in Mahabaleshwar and Tamhini ghat. *Steeriophyllum anceps* occurs in Purandar, Sinhagad, Kaas Plateau and Mahabaleshwar. *Fissidens crenulatus* found only in the localities of Lonawala. *Trachypodiopsis blanda* also occurs in Rayereshwar and Mahabaleshwar area. *Funaria hygrometrica* commonly occurs in most of the localities were Purandar, Rayereshwar, Lonawala, Khandala, Mahabaleshwar, Lawasa, Lohagad and Tamhini ghat. *Hyophila involuta* frequently occurs in Pratapgad, Purandar, Rayereshwar, Sinhagad, Lonawala, Khandala, Lawasa, Wai - Dhom dam and Koynanagar.

Brachymenium turgidum occurs in Pratapgad, Rayereshwar, Sinhagad, Mahabaleshwar, Lawasa and Lohagad. *Bryum coronatum* species occurs more frequently as compared to other species, it is seen almost all the localities except Purandar, Sinhagad, Kaas Plateau and Wai - Dhom dam. *Macromitrium sulcatum* rarely found in studied area it is only accrued in Kaas Plateau. According to Dabhade (1998) described 87 species from mosses of Khandala and Mahabaleshwar.

4. 1. 2 Localities with their autecological characters:

The localities with their autecological characters (Table 3) like altitude ranges from 550 meters to 1,438 meters. There are 8 localities in the studied area in which higher altitude 1000 meters above sea level are Lohgad, Tamhini ghat, Sinhagad, Purandar, Raireshwar, Pratapgad, Mahabaleshwar, Kaas Plateau. Whereas, other 6 localities in studied area altitude is below 1000 meters. The averages annual rainfall ranges from 534 to 6498 mm. Most of the localities average annual rainfall is more than 2000 mm. They are Tamhini ghat, Lawasa and Dhom dam. The averages humidity varied from ranges 30 % to 78.5 %. The maximum 78.5 % humidity found at Khandala and minimum 30 % at Aundh (Satara). The above discussion suggests that the distribution of mosses is governed by the altitude, average rainfall and humidity.

Table 1: List of mosses collected from the different habitats of Maharashtra

Sr. No.	Order	Family	Species	habitats
1	Fissidentales	Fissidenthceae	*Fissidens crenulatus* Mitt.	Epiphytic (on bark of tree trunk)
2	Bryales	Bryaceae	*Brachymenium turgidum* Broth.	Epiphytic (on trees)
3	Bryales	Bryaceae	*Bryum coronatum* Schwaegr.	Terrestrial (on sandy soil)
4	Funariales	Funariaceae	*Funaria hygrometrica* Hedw.	Lithophytic (on rock)
5	Hypnales	Hypnaceae	*Hypnum reflexum* F. E. Tripp.	Epiphytic (on trees)
6	Hypnales	Thuidiaceae	*Steeriophyllum anceps* (Bosch et. Lac.) Broth	Epiphytic (on tree branches)
7	Orthotrichales	Orthotrichaceae	*Macromitrium sulcatum* Brid.	Epiphytic (on bark of trees)
8	Pottiales	Pottiaceae	*Hyophila involuta* (Hook) Jaeg.	Terrestrial (on sandy soil)
9	Pottiales	Pottiaceae	*Barbula unguiculata* Hedw.	Lithophytic (on rock)
10	Isobryales	Trachypodaceae	*Trachypodiopsis blanda* (Mitt.) Fleisch.	Epiphytic (on trees branches)

Table 2 : List of mosses from the different localities of Maharashtra.

Sr. no.	Name of species	Localities													
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**
1	*Barbula unguiculata* Hedw.	-	+	-	-	-	-	+	+	+	-	-	-	-	-
2	*Hypnum reflexum* F. E. Tripp.	-	-	-	+	-	-	-	-	-	-	+	-	-	-
3	*Steeriophyllum anceps* (Bosch et. Lac.) Broth	-	-	-	-	-	+	+	-	-	-	+	+	-	-
4	*Fissidens crenulatus* Mitt.	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	-	-	-	-	-	-	-	+	-	-	+	-	-	-
6	*Funaria hygrometrica* Hedw.	+	+	+	+	+	-	+	+	-	-	+	-	-	-
7	*Hyophila involuta* (Hook) Jaeg.	+	+	-	-	+	+	+	+	+	+	-	-	-	+
8	*Brachymenium turgidum* Broth.	-	-	+	-	+	+	-	+	-	+	+	-	-	-
9	*Bryum coronatum* Schwaegr.	+	+	+	+	+	-	-	+	-	+	+	-	+	+
10	*Macromitrium sulcatum* Brid.	-	-	-	-	-	-	-	-	-	-	-	+	-	-

Present (+), Absent (-)

Localities:

A. Khandala, B. Lonawala, C. Lohagad, D. Tamhini ghat, E. Lavasa, F. Sinhagad G. Purandar, H. Raireshwar, I. Dhom Dam (Wai), J. Pratapgad, K. Mahabaleshwar, L. Kaas Plateau, M. Aundh (Satara) and N. Koynanager.

Table 3 : Localities with their autecological characters.

Sr. No.	Locality	Altitude (meters)	Averages rainfall mm.	Humidity %
1	Khandala	550	1050	78.5
2	Lonawala	625	450	75
3	Lohagad	1,033	534	52
4	Tamhini ghat	1,075	6498	40
5	Lawasa	630	2858	44
6	Sinhagad	1,350	722	68
7	Purandar	1,387	722	39
8	Raireshwar	1000	643	64
9	Dhom Dam (Wai)	718	5761	31
10	Pratapgad	1,080	587	41
11	Mahabaleshwar	1,438	5761	75
12	Kaas Plateau	1,200	2000	32
13	Aundh(Satara)	626	543	30
14	Koynanagar	746	2447	53

Table 4: Soil pH and its interpretation (Winston, 1968).

Range	Denomination
> 4.0	Very strongly acidic
4.0 > 5.5	Strongly acidic
5.5 > 6.0	Medium acidic
6.0 > 6.5	Slightly acidic
6.5 > 7.0	Very slightly acidic
7.0 > 7.5	Very slightly alkaline
7.5 > 8.0	Slightly alkaline
8.0 > 8.5	Medium alkaline
8.5 > l0.0	Strongly alkaline
> 10	Very strongly alkaline

4.2 Soil analysis:

4.2.1 Physical characteristics of rhizosphere soil:

1. pH:

Physico - chemical characteristics of rhizosphere soil of mosses showed that pH ranges from 5.77 to 7.35. The pH value of rhizosphere soil with *Bryum coronatum* Schwaegr., *Hypnum reflexum* F. E. Tripp., *Brachymenium turgidum* Broth. and *Barbula unguiculata* Hedw. was very slightly alkaline (7.0 > 7.5). In *Steeriophyllum anceps* (Bosch et. Lac.) Broth and *Funaria hygrometrica* Hedw. pH is Medium acidic (5.5 > 6.0). The pH is slightly acidic (6.0 > 6.5) in *Hyophila involuta* (Hook)Jaeg. and *Fissidens crenulatus* Mitt. However the *Trachypodiopsis blanda* (Mitt.) Fleisch. and *Macromitrium sulcatum* Brid. has pH very slightly acidic (6.5 > 7.0). Therefore most of the mosses restricted to acidic soils and few are restricted to alkaline in nature. Soil pH refers to a soil acidity or alkalinity and is the measure of hydrogen ions (H+) concentration. The rhizosphere soils of mosses are grouped in to various soil natures by changing their pH range interpretation is shown in Table 4 (Winston, 1968).

Ikenberry (1936) found that *Bryum argenteum* and *Funaria hygrometrica* were more restricted to alkaline soils. They found the optimum growth of *F. hygrometrica* on slightly alkaline substratum with pH value of 8.0. Hoffman (1966) observed that in nature *F. hygrometrica* inhabited soils with pH ranging between 5.4 and 8.4 while in laboratory under experimental conditions, it showed profuse growth on media of low (pH 5.0) as well as high pH (12.0). Dietert (1979) found optimal growth of this species on soils with pH 8.0 to 8.5. Proctor (1981) also considered *F. hygrometrica* and *B. argenteum* as indicators of neutral or alkaline soils. During the present study, *F. hygrometrica* showed normal growth on almost neutral soil (pH 6.9).

The above discussion suggests that the distribution of mosses is not governed by the pH alone but also by the mineral status of the substrata along with their environmental factors.

2. Electrical conductivity (EC):

Soil EC analysis of rhizosphere soil of mosses recorded in Table 5. It was found that EC was ranging from 0.15 dS m^{-1} to 7.64 dS m^{-1}.

Electrical conductivity (EC) is a measurement of the dissolved material in an aqueous solution, which relates to the ability of the material to conduct electrical current through it. EC is measured in units called deciseimens per meter (e.g. dS m^{-1}) and higher dissolved material in a water or soil sample, the higher EC will be in that material.

EC of soil indicates the cation exchange capacity, porosity and salinity of soil. In normal soils, Ca and Mg are principally absorbed on soil particles. The soil properties like dispersion of particles, infiltration, permeability, structure, stability of aggregate etc. are sensitive to exchangeable cations mainly Ca. If EC of saturated soil solution is 4 dS m^{-1} or more than the soil is considered as saline.

Our results showed that rhizosphere soils of only three mosses are saline in nature. Thus there is stress on plants growing on three mosses only i.e. *Steeriophyllum anceps*, *Macromitrium sulcatum* and *Barbula unguiculata*.

4.2.2 Chemical characteristics of rhizosphere soil:

The soil was collected from all the localities during rainy season for biomonitoring of nutrients. Soils are natural unconsolidated materials on the surface of the earth and are composed of solid, liquid, and gases. They have organic as well as inorganic matter, which are intimately mixed together by natural processes. By this mixing and transforming, they are aggregated into a porous body. The pores accommodate air and water. Thus, there are four major components of soil i.e. mineral matter, organic matter, water and air. The soil has evolved through pedogenic processes as a dynamic and a three-dimensional body. Soils have attained the capacity of supporting various ecosystems on the Earth. They provide anchorage, nutrients, water, air and warmth to the plants and also protect them from toxins. Soils have crucial ecological functions. It is a transformer of energy, it is a recycler of materials, it is a purifier of water and above all, it functions as an ecosystem component (Khan T. O., 2013).

For the present study, rhizosphere soil samples were collected from 14 localities like Khandala, Lonawala, Lohagad, Tamhini ghat, Lavasa, Sinhagad, Purandar, Raireshwar, Dhom Dam (Wai), Pratapgad, Mahabaleshwar, Kaas Plateau, Aundh (Satara), and Koynanagar from Maharashtra India. The rhizosphere soil nutrient content in various habitats like terrestrial, epiphytic and lithophytic shown in Table 6, the results indicated that rhizosphere soil nutrients contents, including nitrogen, phosphorus, potassium and organic carbon. The nitrogen content rhizosphere soil of mosses ranges from 111.14 – 335.55 %. In terrestrial moss species nitrogen content is maximum in *Bryum coronatum* and minimum in *Hyophila involuta.* The nitrogen content of epiphytic mosses is higher in *Steeriophyllum anceps* and less in *Hypnum reflexum* and nitrogen content of lithophytic mosses is higher in *Funaria hygrometrica* and lower in *Barbula unguiculata*. The phosphorus content rhizosphere soil of mosses ranges from 1.29 – 15.03 %. In terrestrial moss species phosphorus content maximum in *Hyophila involuta* and minimum in *Bryum coronatum.* The phosphorus content of epiphytic mosses is higher in *Steeriophyllum anceps* and less in *Fissidens crenulatus* and phosphorus content of lithophytic mosses is higher in *Barbula unguiculata* and less in *Funaria hygrometrica*.

The potassium content rhizosphere soil of mosses ranges from 114.10 – 548.00 %. In terrestrial moss species potassium content maximum in *Hyophila involuta* and minimum in *Bryum coronatum.* The potassium content of epiphytic mosses is higher in *Hypnum reflexum* and less in *Macromitrium sulcatum* and potassium content of lithophytic mosses is higher in *Funaria hygrometrica* and less in *Barbula unguiculata*. The organic carbon content rhizosphere soil of mosses ranges from 0.23 - 3.46 %. In terrestrial moss species organic carbon content is maximum in *Bryum coronatum* and minimum in *Hyophila involuta.* The organic carbon content of epiphytic mosses is higher in *Fissidens crenulatus* and less in *Trachypodiopsis blanda* and organic carbon content of lithophytic mosses is higher in *Funaria hygrometrica* and less in *Barbula unguiculata*. It was concluded that the rhizosphere soil of mosses are rich inorganic matter with amount of available nitrogen, phosphorus and potassium for favourable growth of mosses.

Table 5: pH and EC analysis of rhizosphere soil of mosses.

Habitat	Moss species		pH	EC dS m^{-1}
Terrestrial	1	*Hyophila involuta* (Hook)Jaeg.	6.3	1.00
	2	*Bryum coronatum* Schwaegr.	7.01	0.53
Epiphytic	1	*Hypnum reflexum* F. E. Tripp.	7.1	0.53
	2	*Steeriophyllum anceps* (Bosch et. Lac.) Broth.	5.94	6.10
	3	*Fissidens crenulatus* Mitt.	6.20	0.17
	4	*Trachypodiopsis blanda* (Mitt.) Fleisch.	6.52	1.80
	5	*Brachymenium turgidum* Broth.	7.35	0.89
	6	*Macromitrium sulcatum* Brid.	6.98	5.93
Lithophytic	1	*Barbula unguiculata* Hedw.	7.01	7.64
	2	*Funaria hygrometrica* Hedw.	5.96	0.15

Table 6: Nutrient contents of rhizosphere soil of mosses.

Habitat	Moss species		N %	P %	K %	Organic Carbon %
Terrestrial	1	*Hyophila involuta* (Hook) Jaeg.	149.00	14.20	473.00	0.62
	2	*Bryum coronatum* Schwaegr.	335.55	1.39	347.02	3.37
Epiphytic	1	*Hypnum reflexum* F. E. Tripp.	111.14	2.41	548.00	2.80
	2	*Steeriophyllum anceps* (Bosch et. Lac.) Broth	183.1	14.4	498	0.76
	3	*Fissidens crenulatus* Mitt.	171.36	1.29	527.74	3.46
	4	*Trachypodiopsis blanda* (Mitt.) Fleisch.	162.6	13.62	296	0.26
	5	*Brachymenium turgidum* Broth.	133.1	12.2	457	0.87
	6	*Macromitrium sulcatum* Brid.	122.82	14.1	114.27	1.83
Lithophytic	1	*Barbula unguiculata* Hedw.	155.4	15.03	207	0.23
	2	*Funaria hygrometrica* Hedw.	196.43	2.87	501.76	3.31

4.3 Physiological parameters of mosses:

4.3.1 Mineral nutrients:

There are 17 elements which are considered essential for plants to complete their life cycle. These essential nutrients are divided into two categories; macronutrients and micronutrients. Macronutrients include carbon (C), hydrogen (H),oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). Micronutrients are zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (CI) and nickel (Ni) (Epstein, 1972).

1. Macronutrient:

A. Total Nitrogen(N) :

Nitrogen is one of the widely distributed elements in nature. The highest amount is present in a fixed form in the earth's crust in rocks and sediments. Dry plant material contains about 20 - 40 mg/g Nitrogen. Nitrogen is an indispensable elementary constituent of numerous organic compounds of general importance viz. amino acids, proteins, nucleic acids. In plants nitrogen is continuously being converted from the inorganic to organic form. Absorbed nitrogen is rapidly converted into amino acids and amides. It is a constituent of protein, enzymes and chlorophyll. It is involved in all processes associated with protoplasm, enzymatic reactions and photosynthesis and thus forms an important constituent of plant. A number of reports indicate that the uptake of both nitrogen-forms NO_3^+ and NH_4^+ is temperature dependent (Clarkson and Warner, 1979).

Nitrogen content of various mosses is mentioned in Table 7. It is evident from the results that nitrogen content is maximum in *Hyophila involuta i.e*. 23.7 mg/g dry wt. and *Bryum coronatum* (0.41 mg/g dry wt.) have less amount of nitrogen. Rastorfer (1974) has reported that the concentration of nitrogen in different Alaskan Artic mosses ranges from 9-15 mg/g dry wt. The elemental compositions of N have been determined from mosses 4-58 mg/g by (Zaccone *et al.,* 2007). Kļaviņa (2018) has reported that the concentration of N in mosses ranges from 4-20 mg/g. It is evident from the results that, as compared to optimum level of nitrogen in different mosses are low.

B. Phosphorus (P):

The optimum concentration of phosphorus is 2 mg/g dry wt. (Stout, 1961 and Epstein, 1972). The export of energy out of the chloroplast requires inorganic phosphate (Walker, 1980). A primary effect of phosphate on autotrophic growth is thus the provision of chemical energy produced in the chloroplast. As numerous metabolic processes directly or indirectly depend on this energy supply, inadequate phosphate nutrition may affect various processes including protein synthesis and the synthesis of nucleic acids. The rate of phosphate uptake declines rapidly with increasing pH.

It is clear that the concentration of phosphorus, one of the important macronutrient ranges from 0.8 - 3.3 mg/g dry wt. (Table 7). In the present investigation it is evident that the maximum amount of P was found in *Trachypodiopsis blanda* (3.3 mg/g dry wt.) and minimum amount in *Brachymenium turgidum (*0.8 mg/g dry wt.) as compared to remaining mosses. phosphorus content of mosses from Alaskan Artic region was in the range of 1.8 - 2.3 mg/g dry wt. (Rastorfer, 1974). It is evident that, from the present results as compared to this optimum level phosphorus, the level of phosphorus is relatively very high in all mosses.

C. Potassium (K):

Potassium is an important cation with respect to its physiological and biochemical functions. The K^+ sensitive processes are water uptake and retention, phosphorylation, phloem transport and diffusion of CO_2 to mesophyll. The processes are closely related to the K^+ nutritional status and ultimately also to the control of growth and crop production

It is investigated from the present results (Table 7) that the maximum amount of potassium was found in the *Macromitrium sulcatum* (6.5 mg/g) while lowest in *Fissidens crenulatus* (2.5 mg/g) among all mosses. Rastorfer (1974) has reported 5.4 - 8.8 mg/g potassium in the mosses. According to Sawant (2010) concentration of K in the hornwort i.e. *A. subtilis* is (1.7 mg/g) while maximum amount is found in the liverwort *A. wallichiana* (21.3 mg/g).

D. Calcium (Ca):

Calcium is the fifth most abundant metallic element in the Earth's crust and particularly abundant in areas with limestone. Higher plants often contain Ca in appreciable amounts and generally in the order of about 5- 30 mg g^{-1} dry matter. Ca is of the fundamental importance for membrane permeability and the maintenance of cell integrity. Ca is an activator of several enzyme systems in protein synthesis and carbohydrate transfer.

The concentration of Ca in mosses mentioned in table 7, it is clear that, the concentration of Ca is highest among all nutrients studied mosses. It was reported that the level of Ca is usually more than the other macroelements viz. N, P, K, S, and Mg in mosses. From the present results, it is evident that the concentration on Ca in mosses ranges from 10- 26.5 mg/g dry wt. Maximum amount of Ca is found in *Macromitrium sulcatum* and minimum amount in *Bryum coronatum* as compared to remaining mosses. Rastorfer (1974) has reported 1.5-10.2 mg/g Ca in mosses. Brown and Buck (1979) reported that Ca concentration was elevated in bryophytes from calcareous substrata especially as passive extra cellular binding by exchange is probably responsible for most Ca binding

E. Magnesium (Mg):

Magnesium is a mobile element and it is found both in bound as well as free form (Gilbert, 1957). One major role of Mg^{2+} is as a cofactor in almost all enzymes activating phosphorylation process. Thus it affects the protein synthesis because it stabilizes the ribosomal particles in the configuration necessary for protein synthesis. Mg is associated with inorganic anions and organic acid anions such as malate and citrate; it is also associated with in diffusible anions including oxalate and pectate. Though, Mg participates in a number of physiological and enzymatic reactions, its requirement for the plant growth is relatively low. An average value of magnesium for optimal growth is about 2 mg/g on the basis of dry weight (Epstein, 1972), while according to Mengel and Krikby (1982) the magnesium level in many plants is about 5 mg/g of dry weight.

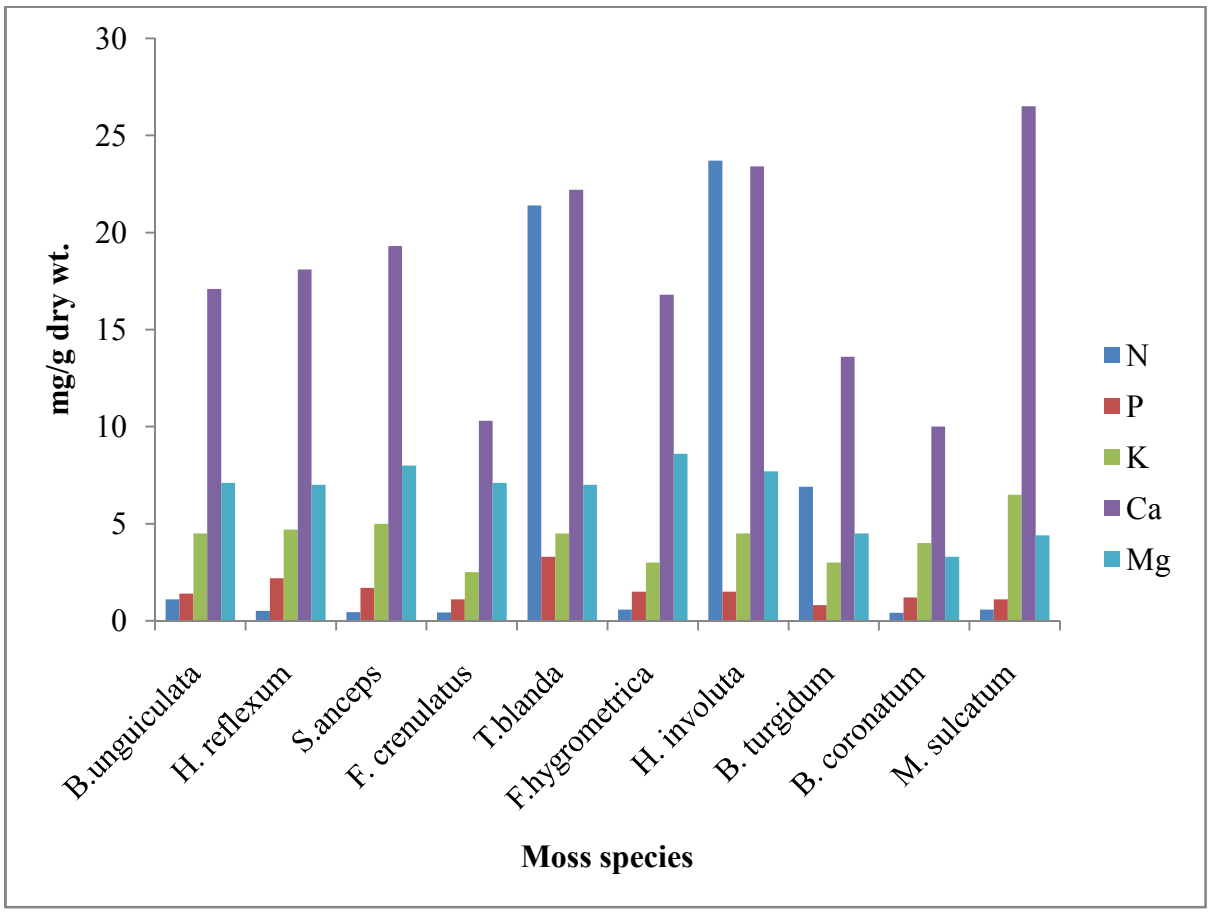
The present result (Table 7) in some mosses reveals that, the level of Mg is lowest among macronutrients studied. It ranges from 3.3 - 8.6 mg/g dry wt. with the highest concentration in *Funaria hygrometrica* (8.6 mg/g dry wt.), while its concentration is low in case *Bryum coronatum*. The level of Mg in the mosses studied higher than the optimal levels of Mg given by Mengel (1982) and Epstein (1972) for higher plants. Rastorfer (1974) has recorded the concentration of Mg in mosses from Artic region which ranges from 1.3- 4.5 mg/g.

Table 7: Contents of macronutrients (mg/g dry wt.) in mosses.

Sr. No.	Name of species	N	P	K	Ca	Mg
1	*Barbula unguiculata* Hedw.	1.1	1.4	4.5	17.1	7.1
2	*Hypnum reflexum* F. E. Tripp.	0.51	2.2	4.7	18.1	7.0
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth.	0.45	1.7	5.0	19.3	8.0
4	*Fissidens crenulatus* Mitt.	0.43	1.1	2.5	10.3	7.1
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	21.4	3.3	4.5	22.2	7.0
6	*Funaria hygrometrica* Hedw.	0.58	1.5	3.0	16.8	8.6
7	*Hyophila involuta* (Hook) Jaeg.	23.7	1.5	4.5	23.4	7.7
8	*Brachymenium turgidum* Broth.	6.9	0.8	3.0	13.6	4.5
9	*Bryum coronatum* Schwaegr.	0.41	1.2	4.0	10.0	3.3
10	*Macromitrium sulcatum* Brid.	0.58	1.1	6.5	26.5	4.4

Values are mean of three replications expressed as mg/g dry wt.

Fig. 1: Contents of macronutrients (mg/g dry wt.) in mosses.



2. Micronutrient:

A. Iron(Fe):

The adequate level of iron for optimal growth of plants is 0.01% (Stout, 1961). The most well known function of Fe is as a cofactor in enzyme systems in which haem or haemin function as prosthetic groups. Another form i.e. structural form of Fe occurring in plants is ferredoxin, which is a non haem-iron protein which participates in oxido-reduction processes by transferring electrons. In green plants there is always an increase in the high levels of chlorophyll content when supplied with more Fe, suggesting its necessity for chlorophyll synthesis.

It is clear from the present results (Table 8) that the level of iron is the highest among all the microelements in mosses. Which is also similar in case of mosses studied (0.369-1.056 mg/g) from Artic regions (Rastorfer, 1974). *Steeriophyllum anceps* has the highest concentration of Fe (35.3 mg/g dry wt.) and with the lowest concentration in *Brachymenium turgidum* (10.5 mg/g dry wt.). It is also evident from the present study, shows that the level of Fe is higher than that reported by Rastorfer (1974). Level of Fe is higher than 1.55 mg/g, have been reported in bryophytes (Rao and LeBlance, 1967). Czarnowaska and Rejement-Grochowaska (1974) have reported that, the mosses are known to accumulate iron 5-10 times more readily than the vascular plant

B. Manganese (Mn):

The manganese concentration for optimal growth of a plant is 0.03 – 0.05 mg/g on the dry weight basis (Stout, 1961). Mumford *et al.* (1962) deficiency of manganese causes chlorosis associated with necrosis. Mn ions bridge ATP with the enzyme complex. It brings about the oxidation of IAA by activating IAA oxidase. Morgan *et al.* (1966) suggested that Mn toxicity leads to IAA deficiency due to high IAA oxidase activity.

Values of Table 8, gives clear idea about the levels of Mn in mosses. There is diverse range of manganese concentration from 0.310 to1.02 mg/g dry wt. *Funaria hygrometrica* and *Barbula unguiculata* accumulate more manganese (1.02 mg/g dry wt.) and *Bryum coronatum* accumulate minimum (0.310 µg/g dry wt.). According to Stout (1961) and Epstein (1972), critical level of manganese is 0.03-0.05 mg/g dry wt. for tracheophytes. Rastorfer (1974) studied the manganese levels in mosses which are between 0.053- 0.317 mg/g. Mayer and Gorham (1951) analyzed the manganese content of 19 moss species from England and found that these plants accumulated between 0.05-4 mg/g dry wt. manganese.

C. Zinc (Zn):

According to Epstein (1972) for optimal growth of plant zinc requirement is 0.02 mg/g dry wt. Zinc is required for the activity of many enzymes and may be required for chlorophyll biosynthesis. It participates in synthesis of indole acetic acid from its precursor, tryphtophan (Skoog, 1940). Zinc deficiency is characterized by a reduction in inter nodal growth resulting into rosette habit of growth. Zn toxicity results in reduction in root growth and leaf expansion which is followed by chlorosis. Rao and Leblanc (1967) have recorded higher levels of Zn at about 0.325 mg/g, while Gydesen *et al.* (1983) have reported its concentration as low as 0.083 mg/g which may even be increased.

The present results (Table 8), shows that the level of Zinc is the lowest among all the microelements in mosses. It ranges from 0.045-0.166 mg/g dry wt. The concentration of Zn for mosses from Artic region is in the range of 0.045-0.066 mg/g (Rastorfer, 1974). The highest concentration level of Zinc in *Hyophila involuta* (0.166 µg/g dry wt.) while, its concentration is lower in case *Bryum coronatum* (0.045 mg/g dry wt.). The result shows that, the level of Zn in all mosses is near to the values given by Gydesen *et al.* (1983).

D. Copper (Cu):

The Cu content of most in the plants is generally 0.002-0.02 mg/g in the dry plant material which is about one tenth of the Mn content. It is associated with enzymes

involved in redox reactions of photosynthesis (Haehnel, 1984). It participates in auxin synthesis (Skoog, 1940). Cu is a constituent of chloroplast protein, plastocyanin, which forms a part of the electron transport chain linking the two photochemical systems. It also plays a role in synthesis or the stability of chlorophyll and other plant pigments (Mengel and Krikby, 1982). Chlorosis as well as inhibition of root growth is some of the most rapid responses to toxic Cu levels.

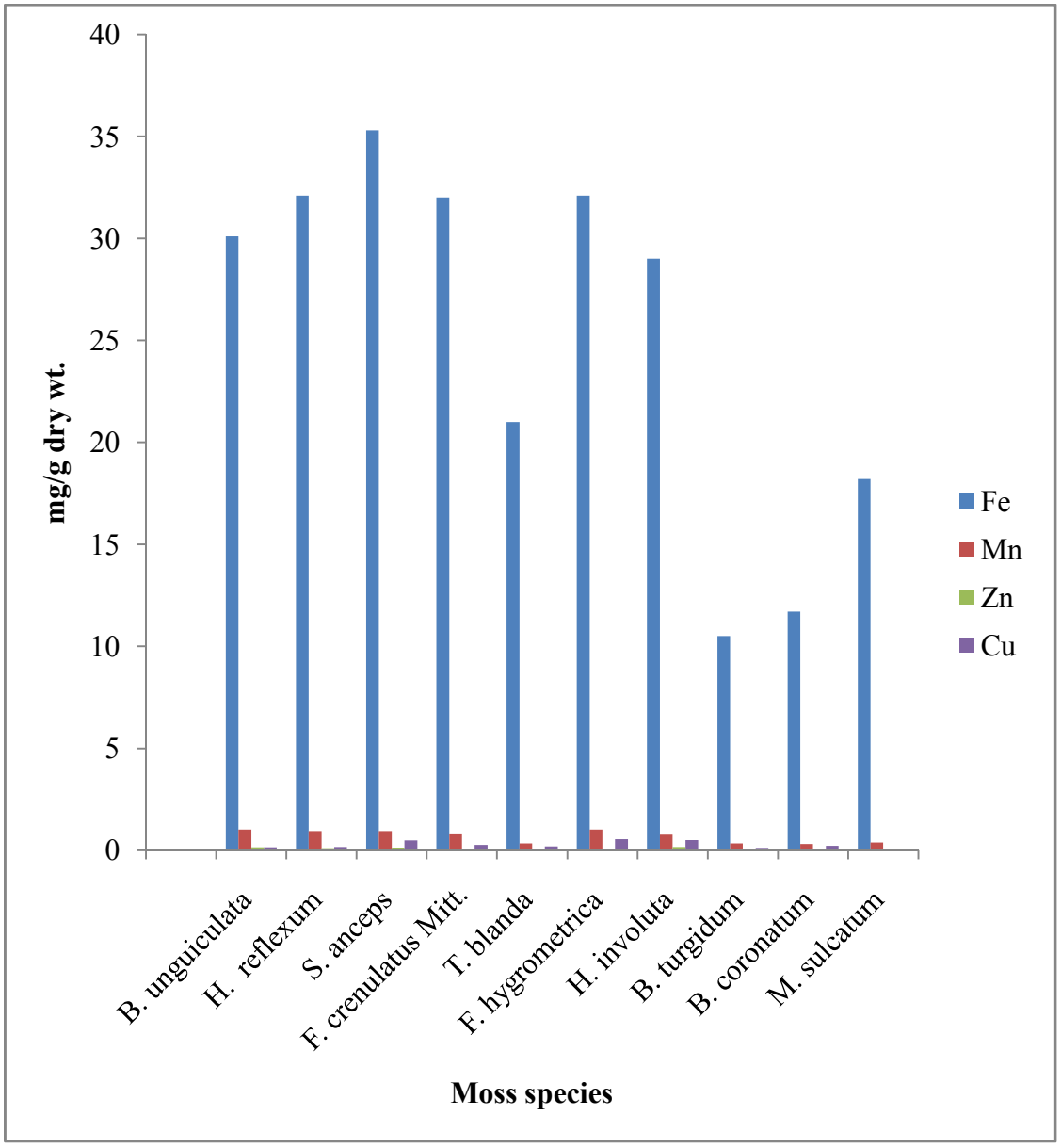
From the present study (Table 8), it is investigated that, the concentration of copper in the mosses was reported in the range of 0.071-0.541 mg/g dry wt. *Funaria hygrometrica* contains the highest amount of copper (0.541 mg/g dry wt.) from among the remaining mosses and then lower with 0.071 mg/g dry wt. of copper in *Macromitrium sulcatum.* It can be said that the level of copper in all mosses is higher than optimum level of dry plant material. Shacklette (1965) has determined the mineral content of 29 species of bryophytes and found that concentrations of Cu are higher in bryophytes than angiosperms. The concentration of copper in the mosses was reported to be in the range of 0.014-0.039 mg/g (Rastorfer, 1974).

Table 8 : Contents of micronutrients (mg/g dry wt.) in mosses.

Sr. No.	Name of species	Fe	Mn	Zn	Cu
1	*Barbula unguiculata* Hedw.	30.1	1.02	0.142	0.152
2	*Hypnum reflexum* F. E. Tripp.	32.1	0.945	0.102	0.158
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth	35.3	0.950	0.126	0.481
4	*Fissidens crenulatus* Mitt.	32.0	0.785	0.069	0.264
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	21.0	0.333	0.080	0.188
6	*Funaria hygrometrica* Hedw.	32.1	1.02	0.071	0.541
7	*Hyophila involuta* (Hook) Jaeg.	29.0	0.770	0.166	0.500
8	*Brachymenium turgidum* Broth.	10.5	0.340	0.049	0.120
9	*Bryum coronatum* Schwaegr.	11.7	0.310	0.045	0.216
10	*Macromitrium sulcatum* Brid.	18.2	0.390	0.075	0.071

Values are mean of three replications expressed as mg/g dry wt.

Fig. 2 : Contents of micronutrients (mg/g dry wt.) in mosses.



4.3.2 Photosynthetic pigments:

A. Chlorophylls :

The concentration of chlorophyll- a, b, and total chlorophyll was determined in the freshly collected moss by using Arnon (1949) method. The concentrations of chl-a is more than chl-b in all the given species. The highest chlorophyll- a was detected in *Barbula unguiculata* (1.04 mg/g FW) and lowest in *Steeriophyllum anceps* (0.61 mg/g FW). Maximum chlorophyll b was found in *Barbula unguiculata* (1.92 mg/g FW) and minimum in *Fissidens crenulatus* (1.01 mg/g FW). The concentration of total chlorophyll in the mosses collected from 14 localities of Maharashtra was in the order: *Barbula unguiculata* > *Hypnum reflexum* > *Steeriophyllum anceps* > *Fissidens crenulatus* > *Trachypodiopsis blanda* > *Brachymenium turgidum*> *Funaria hygrometrica* > *Hyophila involuta* > *Bryum coronatum* > *Macromitrium sulcatum* (Table 9). Chlorophyll a: b ratio recorded maximum in *Funaria hygrometrica* (6.8) and minimum (4.9) in *Bryum coronatum* (Fig. 3).

According to Deora and Chaudhary (1991) reported that chlorophyll content in 16 species of mosses and four species of liverworts and concluded that bryophytes exhibit low chlorophyll concentration and high a:b ratio in high solar irradiances. The chlorophyll content in number of Indian bryophytes that ranges from 0.402 ± 0.052 to 2.002 ± 0.700 mg g-1 dry mass for chlorophyll-a and 0. 265 ± 0.067 to 1.634 ± 0.070 mg g-1 dry mass for chlorophyll- b. Negi (2016) has argued that the highest chlorophyll- a was detected in *Brachythecium plumosum* (4.45 mg/g FW) and the lowest in *Entodon plicatus* (1.22 mg/g FW). Maximum chlorophyll b was found in *Ditrichum heteromallum* (1.85 mg/g FW) and minimum in *Pogonatum microstomum* (0.36 mg/g FW). The concentration of total chlorophyll in the mosses collected from Nainital was in the order: *Brachythecium plumosum* > *Ditrichum heteromallum* > *Anomodon minor* > *Trachypodopsis serrulata* > *Leptodontium viticulosoides* > *Thuidium tamariscinum* > *Leucodon secundus* > *Bryoerthythrophyllum gymnostomum* > *Pogonatum microstomum* > *Entodon plicatus* . The highest chlorophyll a/b ratio was present in *Pogonatum microstomum* (3.71) followed by *Thuidium tamariscinum* (3.23) and minimum (2.91) in *Brachythecium plumosum* Aroyehun *et al.* (2016) reported the accumulation of chlorophyll in *Thuidium gratum* and *Hyophila involuta* (forest moss species) was higher than that of the derived Savanna mosses,

Archidium ohioense. Higher chlorophyll accumulation in the forest moss species than that of the derived Savanna mosses also provide information about the changes that may be observed in mosses of the two vegetation zones with a possible strong influence from the water availability. Higher chlorophyll a/b ratio values were obtained in the current study under lower light intensities.

In the present study it is well evident that, a significant variation in individual chl. -a and chl.- b as well as total chlorophylls have been observed because of water unavailability and induced deficit affected this light harvesting machinery occurred in increasing order during rainy season.

B. Carotenoids:

The carotenoids of these mosses range in between 0.04 - 0.15 mg/g fresh tissue respectively. The maximum carotenoid content was observed in *Barbula unguiculata* and lowest in *Steeriophyllum anceps* and *Bryum coronatum* (Table 10 and Fig. 4).

Carotenoids are required in photosynthesis for the harvesting of light energy and as the primary electron donors and carotenoids as essential structural component of the photosynthetic apparatus, where they protect against photo-oxidation. However low carotenoids stability index follows the same pattern and it also indicates the protective role of photo-oxidation of chlorophyll in adverse conditions like drought in summer. This may be an adaptive feature of tolerance against drought. Carotenoid also plays a role in preparing the plant for stress management (Sharma *et al.*, 2012).

Negi (2016) reported that, the highest carotenoid content was found to occur in *Ditrichum heteromallum* (0.37 mg/g FW) and lowest in *Pogonatum microstomum* (0.05 mg/g FW). According to Devi *et al.* (2015) seasonal variation in the carotenoid content was found in the two species of *Marchantia* but *Dumortiera hirsuta* i.e. a very low (0.06 ± 0.005 mg/g fw) in winter and high (0.16 ± 0.005 mg/g fw) in rainy season while at end of the growing season (0.15 ± 0.005 mg/g fw). Low seasonal changes in the Carotenoids of both the species of *Marchantia* and apparent seasonal changes in the carotenoids of *Dumortiera hirsuta* are suggestive of the role of habitats in these variations.

The present investigation shows that the both photosynthetic pigments namely chlorophyll and carotenoid present in mosses. The role of carotenoids as photo-oxidative unit for chlorophylls is more marked in this mosses. A low concentration of carotenoids indicates that not much protection is provided to mosses by them.

Table 9: Chlorophyll content in different mosses.

Sr. No.	Name of species	Chl - a	Chl - b	Total Chlorophyll	Chlorophyll a:b ratio
1	*Barbula unguiculata* Hedw.	1.04 ± 0.00	1.92 ± 0.03	3.20 ± 0.04	5.2 ± 0.4
2	*Hypnum reflexum* F. E. Tripp.	0.74 ± 1.35	1.30 ± 0.02	2.59 ± 0.02	5.5 ± 0.3
3	*Steeriophyllum anceps* (Bosch et. Lac.) Broth.	0.61 ± 0.01	1.23 ± 0.04	2.44 ± 0.02	5.1 ± 0.1
4	*Fissidens crenulatus* Mitt.	0.63 ± 0.02	1.01 ± 0.05	2.34 ± 0.03	5.2 ± 0.1
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	0.86 ± 0.02	1.56 ± 0.06	2.22 ± 0.01	5.6 ± 0.4
6	*Funaria hygrometrica* Hedw.	0.70 ± 0.02	1.52 ± 0.01	2.09 ± 0.01	6.8 ± 0.1
7	*Hyophila involuta* (Hook) Jaeg.	0.76 ± 0.01	1.44 ± 0.03	1.99 ± 0.05	5.5 ± 0.3
8	*Brachymenium turgidum* Broth.	0.74 ± 0.02	1.08 ± 0.01	2.15 ± 0.04	5.3 ± 0.4
9	*Bryum coronatum* Schwaegr.	0.66 ± 0.01	1.20 ± 0.07	1.93 ± 0.02	4.9 ± 0.1
10	*Macromitrium sulcatum* Brid.	0.70 ± 0.01	1.31 ± 0.01	1.82 ± 0.01	6.2 ± 0.5

Values are mean of three replication expressed as mg $100g^{-1}$ fresh tissue.

Table 10 : Carotenoids content in mosses.

Sr. No.	Name of species	Carotenoid
1	*Barbula unguiculata* Hedw.	0.15 ± 0.05
2	*Hypnum reflexum* F. E. Tripp.	0.07 ± 0.02
3	*Steeriophyllum anceps* (Bosch et. Lac.) Broth.	0.04 ± 0.02
4	*Fissidens crenulatus* Mitt.	0.05 ± 0.03
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	0.09 ± 0.01
6	*Funaria hygrometrica* Hedw.	0.05 ± 0.04
7	*Hyophila involuta* (Hook) Jaeg.	0.05 ± 0.02
8	*Brachymenium turgidum* Broth.	0.05 ± 0.01
9	*Bryum coronatum* Schwaegr.	0.04 ± 0.01
10	*Macromitrium sulcatum* Brid.	0.05 ± 0.02

Values are mean of three replications expressed as mg $100g^{-1}$ fresh tissue.

Fig. 3: Chlorophyll contents in different mosses.

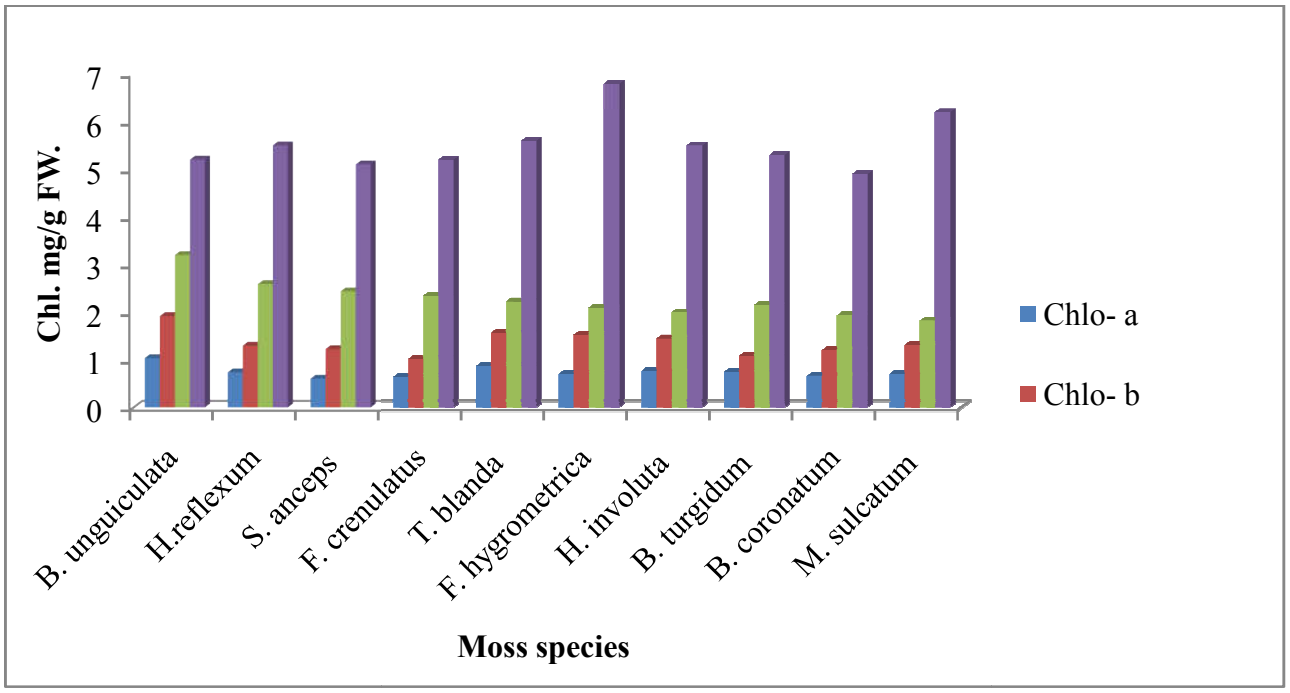
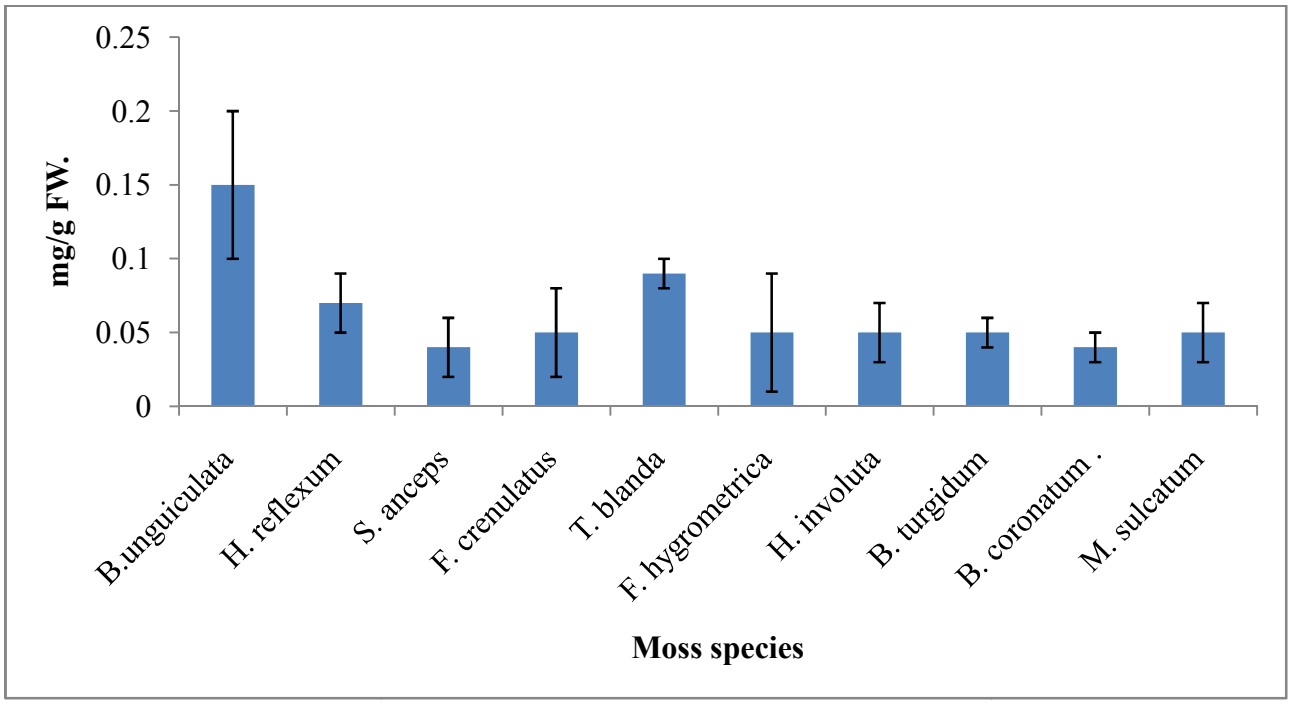


Fig. 4: Carotenoids contents in different mosses



4.3.3 Enzymes:

The existence of enzymes had been known for well over a century. Some of the earliest studies were performed in 1835 by the Swedish chemist Jon Jacob Berzelius who termed their chemical action catalytic. Enzymes occur in all living organisms and catalyze biochemical reactions necessary to support life (Olempska-Beer *et al*., 2006). Enzymes are the nature's sustainable catalysts. They are biocompatible, biodegradable and are derived from renewable resources (Sheldon and Pelt, 2013). Enzyme constitute a large biological globular protein molecule responsible for thousands of metabolic processes that sustain life and function as catalysts to facilitate specific chemical reactions within the cell. These reactions are essential for the life of the organism. The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical changes which go on continually in the living organism; enzyme facilitates life processes in essentially all life-forms from viruses to man (Smith, 1997).

A. Catalase:

Catalase was the first enzyme to be discovered amongst all the antioxidative enzymes and its action in plant and animal tissues was first observed in 1818 by Thenard, who noted that such tissues readily degraded hydrogen peroxide, a substance he had also discovered some years earlier (Aebi and Suter, 1971). The reaction of catalase occurs in two steps. A molecule of hydrogen peroxide oxidizes the heme to an oxyferryl species. A porphyrin cation radical is generated when one oxidation equivalent is removed from iron and one from the poryphyrin ring. A second hydrogen peroxide molecule acts as a reducing agent to regenerate the resting state enzyme, producing a molecule of oxygen and water (Switala and Loewen, 2002).

The present results (Table 11) indicate that its levels ranges from 13.6 – 51.51 H_2O_2 liberated min^{-1} g^{-1}. Highest level is in case of *Hypnum reflexum* and the lowest in case of *Barbula unguiculata.* Catalase enzymes activity is the highest among all the mosses than that of peroxides and polyphenol oxidase. Dhindsa and Matowe (1981) reported in *T. ruralis* the catalase activities increase during slow drying. Lu *et al*. (2008) studied the catalase eliminates H_2O_2 by breaking it down directly to form water and oxygen. In leaves of *E. adenophorum,* CAT activity was depressed by high temperature and increased to a high level in the low temperature treatment. Barón *et al.* (2009) reported that in *Racomitrium crispipilum* activities of catalase and peroxidase increase in this species in response to conditions of water deficit.

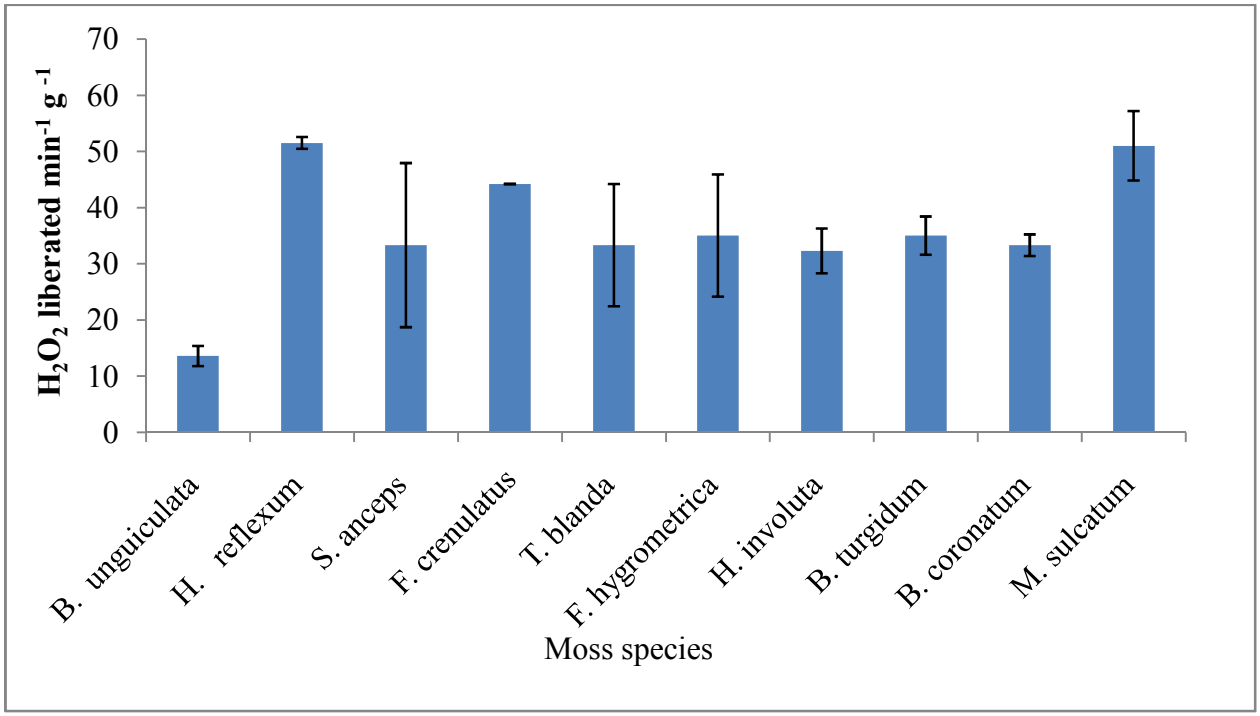
This is likely to be an important component in the mechanism by which this moss tolerates drought periods. Mafakheri *et al.* (2011) CAT activities play an essential protective role against drought stress in chickpea. Catalase activity determines the effect of pollution on the metabolism of species (Tandon and Misra, 2014). The enzyme catalase showed lower activity than the enzyme peroxidase but both the enzymes showed same seasonal pattern. *C. gollani* showed lowest activity of the enzyme catalase compared to *S. crenulata* and *F. himalayensis* (Thakur and Kapila, 2017).

Table 11: Catalase enzyme activity in mosses.

Sr. No	Name of species	Catalase H_2O_2 liberated min^{-1} g^{-1}
1	*Barbula unguiculata* Hedw.	13.6 ± 1.80
2	*Hypnum reflexum* F.E.Tripp.	51.51 ± 1.06
3	*Steeriophyllum anceps* (Bosch et lac.) Broth.	33.32 ± 14.6
4	*Fissidens crenulatus* Mitt.	44.2 ± 0.05
5	*Trachypodiopsis blanda (*Mitt) Fleish.	33.32 ± 10.87
6	*Funaria hygrometrica* Hedw.	35.02 ± 10.87
7	*Hyophila involuta* (Hook) Jaeg.	32.3 ± 3.98
8	*Brachymenium turgidum* Broth.	35.02 ± 3.4
9	*Bryum cornatum* Schwaegar	33.32 ± 1.92
10	*Macromitrium sulcatum* Brid.	51.0 ± 6.18

Values are mean of three replications.

Fig. 5 : Catalase enzyme activity in mosses .



B. Peroxidase :

Peroxidase is a hemoprotein catalyzing the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome C and the leuco form of many dyes.

The present results (Table 12), indicates that its levels ranges from 2.80 – 20.26 Δ OD. min $^{-1}$ g^{-1}. The highest level is in case of *Barbula unguiculata* and the lowest in case of *Trachypodiopsis blanda.* Pb and Ni had synergistic effect in moss *H. plumaeforme* to induce oxidative stress in high concentration. Single and combined metal stress was responsible for elevation of POD indicating its important role to resist ROS. (Sun *et al*., 2009 a).Increased concentration of Lead (Pb) and Nickel (Ni) was found to be responsible for increased POD and decreased SOD and catalase CAT activity in *Thuidium cymbifolium*. ROS and malondialdehyde (MDA) were accumulated in a dose dependent manner due to Pb and Ni stress. Thus POD plays an important role in elimination of ROS (Sun *et al*., 2009 b). Sawant (2010) find out POD activity in *Thuidium* was significantly higher than *Brachythecium* indicating that it has better capacity of protecting the cells from heavy metal stress. Vujicic *et al.* (2017) investigated that enzymes of oxidative stress POX in bryophytes possibly act as the first level of the anti-oxidative defence system.

Polyphenol oxidases :

Polyphenol oxidases (PPOs) are ubiquitous copper-containing enzymes which use molecular oxygen to oxidize common orthodiphenolic compounds such as caffeic acid and catechol to their respective quinones. PPO-generated quinones are highly reactive and may cross-link or alkylate proteins, leading to the commonly observed brown pigments in damaged plant tissues and plant extracts. The conspicuous pigments are generally undesirable in food products and the role of PPO in browning has prompted numerous studies on PPO in food and beverages. In parallel, the potential roles for PPO in plant defense against pests have motivated many studies on PPO in an ecological context; though few of these have used a transgenic approach. Functional and mechanistic studies on PPO in plant-insect interactions using PPO modified transgenic plants have recently been reported, providing new insight into the biology of this versatile enzyme (Constabel and Barbehenn, 2008). Enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD), may oxidize phenolics and thus take part in the regulation of the phenolic concentration in plants. Recent studies have also indicated that phenol oxidizing enzymes may participate in the response to various abiotic stresses including

drought (Sofo *et al.*, 2005; Veljovic - Jovanovic *et al.*, 2006, 2008). Polyphenol oxidase is an oxygen transferring enzyme. Besides using O_2 to catalyze the dehydrogenation of catechols to orthoquinones and the orthohydroxylation of phenols to catechols, a peroxidase activity has been reported on by Strothkamp and Mason (1974) Polyphenol oxidase (tyrosinase) is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity (Malmström and Rydén, 1968).

The results obtained in these study shows clearly that the rates of polyphenol oxidases of mosses differed from species to species. The polyphenol oxidases activity varied between 0.56 - 1.9 Δ O D. min $^{-1}$ g^{-1}. In *Barbula unguiculata* 1.9 Δ O D. min $^{-1}$ g^{-1} is synthesized it is more than other mosses and less for *Funaria hygrometrica* 0.56 Δ O D. min $^{-1}$ g^{-1} respectively.

Kaur *et al.* (2010) also observed that the specific activity of enzymes α-amylase, ß-amylase, proteases and polyphenol oxidases. The four enzymes tested with regard to their specific activity show the following sequential order in all the studied taxa. Protease > Polyphenol oxidase > α-amylase > β-amylase. Polyphenol oxidase (PPO) were analyzed in three leafy liverworts, namely *Solenostoma crenulata, Chiloscyphus gollani* and *Fossombronia himalayensis* showed highest activity in the rainy season and lowest during winter season and that of phenolic content showed inverse relationship (Thakur and Kapila, 2017). Wadavkar *et al.* (2017) studied that polyphenol oxidase activity is higher in *B. coronatum* while less in *F. hygrometrica* and the ten-moss species shows studied enzymes activity contains general trends of decrease as Catalase > Peroxidase > Polyphenol oxidase.

Table 12: Peroxidase enzyme activity in mosses.

Sr. No	Name of species	Peroxidase Δ OD. min $^{-1}$ g $^{-1}$
1	*Barbula unguiculata* Hedw.	20.26 ± 4.43
2	*Hypnum reflexum* F.E.Tripp.	14.3 ± 8.96
3	*Steeriophyllum anceps* (Bosch et lac.) Broth.	8.7 ± 6.05
4	*Fissidens crenulatus* Mitt.	4.0 ± 0.04
5	*Trachypodiopsis blanda (*Mitt) Fleish.	2.80 ± 0.81
6	*Funaria hygrometrica* Hedw.	3.02 ± 0.91
7	*Hyophila involuta* (Hook) Jaeg.	8.2 ± 0.05
8	*Brachymenium turgidum* Broth.	10.47 ± 5.14
9	*Bryum cornatum* Schwaegar	10.22 ± 5.07
10	*Macromitrium sulcatum* Brid.	9.4 ± 5.14

Values are mean of three replications.

Table 13: Polyphenol - oxidase enzyme activity in mosses.

Sr. No	Name of species	Polyphenol oxidase Δ OD. min $^{-1}$ g $^{-1}$
1	*Barbula unguiculata* Hedw.	1.9 ± 0.08
2	*Hypnum reflexum* F.E.Tripp.	1.21 ± 0.16
3	*Steeriophyllum anceps* (Bosch et lac.) Broth.	1.8 ± 0.15
4	*Fissidens crenulatus* Mitt.	1.49 ± 0.01
5	*Trachypodiopsis blanda (*Mitt) Fleish.	0.72 ± 0.24
6	*Funaria hygrometrica* Hedw.	0.56 ± 0.04
7	*Hyophila involuta* (Hook) Jaeg.	1.74 ± 0.26
8	*Brachymenium turgidum* Broth.	1.83 ± 0.01
9	*Bryum cornatum* Schwaegar	1.54 ± 0.11
10	*Macromitrium sulcatum* Brid.	0.58 ± 0.01

Values are mean of three replications.

Fig. 6: Peroxidase enzyme activity in mosses.

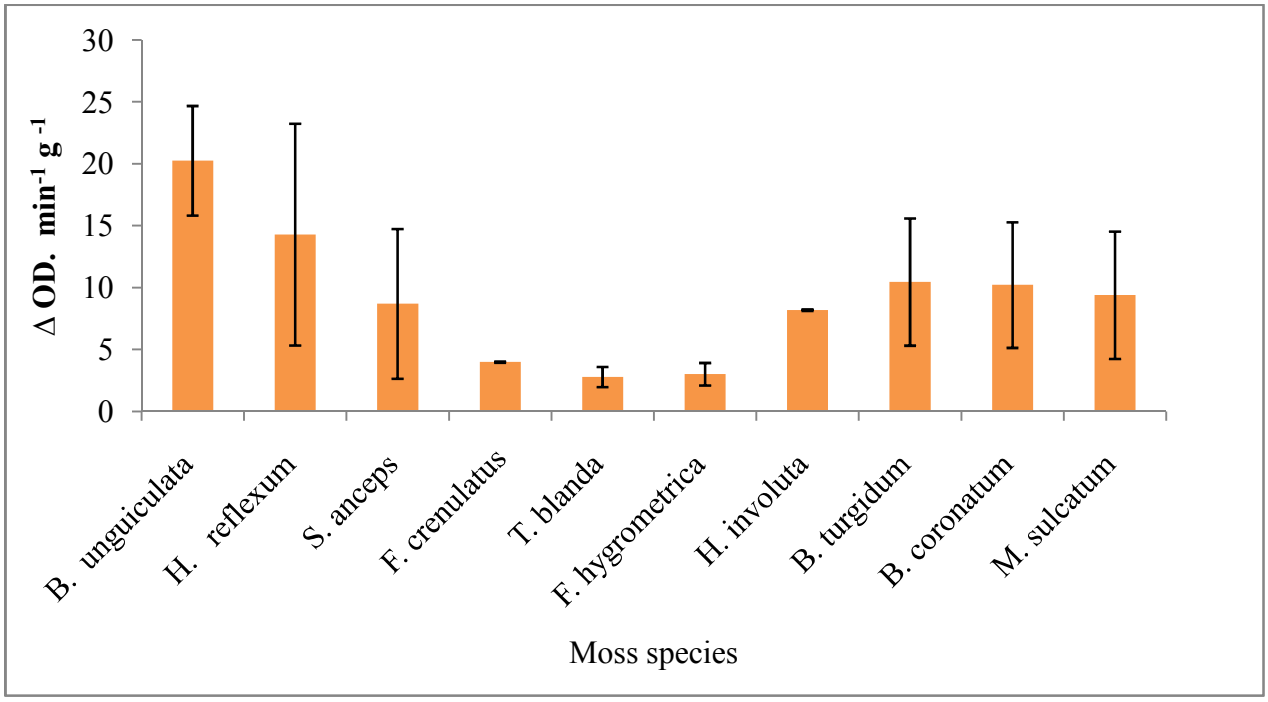
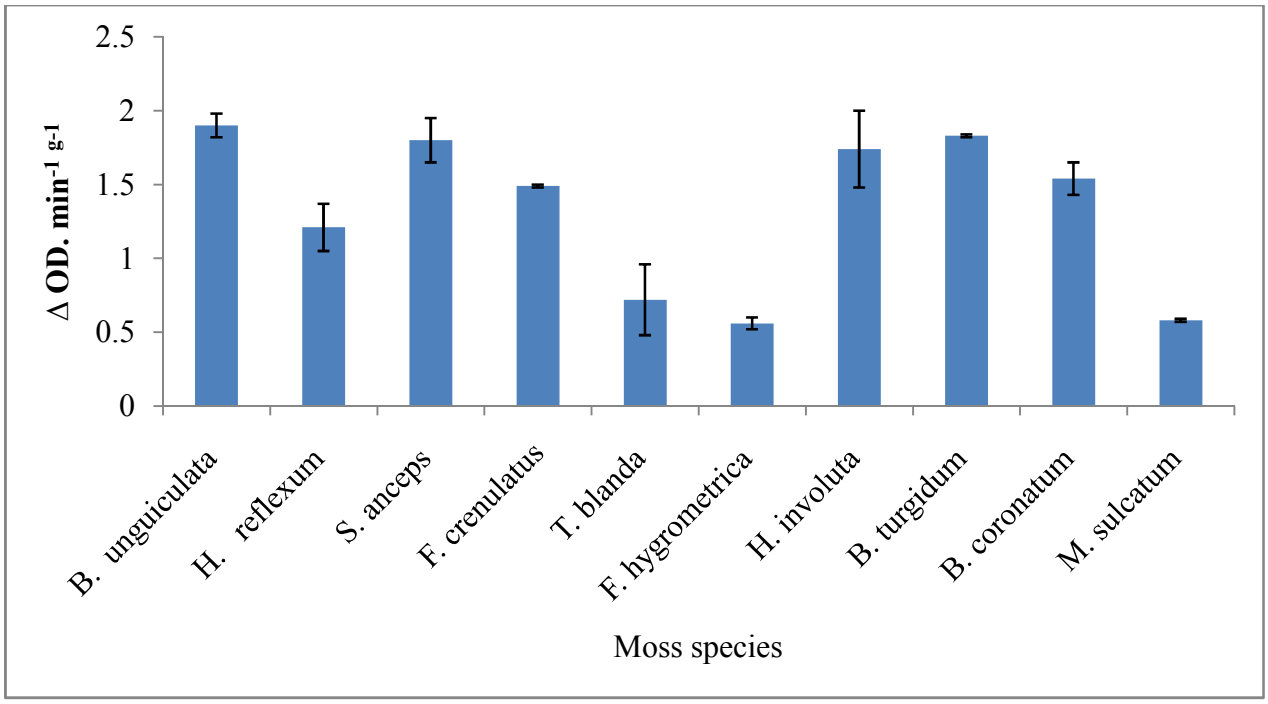


Fig. 7: Polyphenol - oxidase enzyme activity in mosses.



C. **Nitrate reductase (NR)** :

Nitrate reductase is one of the most important enzyme in the assimilation of exogenous nitrate. Activity of this enzyme in plants gives a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield (Srivastava , 1980).

The results obtained in these study shows clearly that, the all moss species demonstrated high activity of the enzyme nitrate reductase (Table 14) The nitrate reductase activity varied between 1.32 to 2.81 nmol per g dry mass per hour for *Hyophila involuta* 2.81 nmol per g dry mass per hour of nitrite synthesized maximum than other mosses but lowest in *Funaria hygrometrica.*

Krywult *et al*. (2013) investigated that nitrate reductase activity in green tissues of *Brachythecium rutabulum* and *Atrichum undulatum* for both species high activity of the enzyme was detected. The nitrate reductase activity varied between 99 to 9093 nmol per g dry mass per hour for *B. rutabulum* and 265 to 5135 nmol per g d.m. per hour of nitrite synthesized for *A. undulatum* respectively on Skalny waste tip. In the control area the results varied between 747 to 1077 for *B. rutabulum* and 171 to 518 nmol per g d.m. per hour of nitrite synthesized for *A. undulatum,* respectively. Panda and Choudhury (2005) investigate the effect of chromium (Cr), copper (Cu) and zinc (Zn) on nitrate reductase (NR) activity and oxidative stress responses in the moss *Polytrichum commune*. Cr, Cu and Zn resulted in the inhibition of NR activity. Mahan *et al.* (1998) observed effect of desiccation and hydration on nitrate reductase activity in the desiccation-tolerant moss *Tortula ruralis* has been investigated that the activity of nitrate reductase would decline during desiccation and recover rapidly following rehydration. Nitrate reducatase activity in *Racomitrium* from plots receiving the low N dose was 53% less than in the controls, but with no difference between ion types. At the high doses, nitrate reductase activity was even further reduced (Pearce and Van der Wal, 2002). NR activity in *Tortula ruralis* declined markedly in both dark and light (more steeply in the light) while in *Porella platyphylla* it remained at a relatively constant low level in the light and increased in the dark. After four days of dark starvation *Porella platyphylla* showed reduced but measurable NR activity. (Mariann.,1998)

D. Nitrite reductase (NiR):

Nitrite reductase refers to any of several classes of enzymes that catalyze the reduction of nitrite. There are two classes of NiR's. A multi haem enzyme reduces NO_2^- to a variety of products. Copper containing enzymes carry out a single electron transfer to produce nitric oxide (Atkins *et al*., 2006). Stohr *et al.* (2001) observed that plasma membranes of tobacco roots exhibited a nitrite-reducing enzyme activity that resulted in nitric oxide (NO) formation.

In the present study the Nitrite reductase (NiR) activity is recorded in different mosses as shown in the Table 15 and depicted in Fig. 9. Results clearly indicate that *Brachymenium turgidum* has shown the highest (NiR) activity than any other mosses. While *Funaria hygrometrica* has the lowest (NiR) activity and other mosses have shown slight lower values for (NiR) activity.

Nitrate uptake and reduction to nitrite and ammonium are driven in cyanobacteria by photosynthetically generated assimilatory power, i.e., ATP and reduced ferredoxin. High-affinity nitrate and nitrite uptake takes place in different cyanobacteria through either an ABC-type transporter or a permease from the major facilitator super family (MFS). Nitrate reductase and nitrite reductase are ferredoxin-dependent metalloenzymes that carry as prosthetic groups a [4Fe–4S] center and Mo-bis-molybdopterin guanine dinucleotide (nitrate reductase) and [4Fe–4S] and nitrite reductase (Flores *et al.,* 2005). Aslam and Huffaker (1989) observed that in barley leaves NiR is induced by NO_3^- directly i.e. without being reduced to NO_2^- and that absorbed NO_2^- induces the enzyme activity indirectly after being oxidized to NO_3^- within the leaf. Joy and Hageman (1966) find out nitrite reductase from higher plants, and its dependence on ferredoxin.

Table 14: Nitrate reductase enzyme activity in mosses.

Sr. No	Name of species	Nitrate Reductase nmole h^{-1} g^{-1}
1	*Barbula unguiculata* Hedw.	1.39 ± 0.34
2	*Hypnum reflexum* F.E.Tripp.	1.91 ± 0.22
3	*Steeriophyllum anceps* (Bosch et lac.) Broth.	1.72 ± 0.08
4	*Fissidens crenulatus* Mitt.	1.62 ± 0.05
5	*Trachypodiopsis blanda (*Mitt)Fleish.	1.43 ± 0.29
6	*Funaria hygrometrica* Hedw.	1.32 ± 0.36
7	*Hyophila involuta* (Hook) Jaeg.	2.81 ± 0.49
8	*Brachymenium turgidum* Broth.	1.75 ± 0.12
9	*Bryum cornatum* Schwaegar	1.55 ± 0.17
10	*Macromitrium sulcatum* Brid.	2.02 ± 0.11

Values are mean of three replications.

Table 15: Nitrite reductase enzyme activity in mosses.

Sr. No	Name of species	Nitrite Reductase nmole h^{-1} g^{-1}
1	*Barbula unguiculata* Hedw.	1.8 ± 0.01
2	*Hypnum reflexum* F.E.Tripp.	2.1 ± 0.04
3	*Steeriophyllum anceps* (Bosch et lac.) Broth.	1.91 ± 0.02
4	*Fissidens crenulatus* Mitt.	1.62 ± 0.01
5	*Trachypodiopsis blanda (*Mitt)Fleish.	1.81 ± 0.08
6	*Funaria hygrometrica* Hedw.	1.1 ± 0.04
7	*Hyophila involuta* (Hook) Jaeg.	1.32 ± 0.04
8	*Brachymenium turgidum* Broth.	2.19 ± 0.05
9	*Bryum cornatum* Schwaegar	1.76 ± 0.04
10	*Macromitrium sulcatum* Brid.	1.98 ± 0.16

Values are mean of three replications.

Fig. 8: Nitrate reductase enzyme activity in mosses.

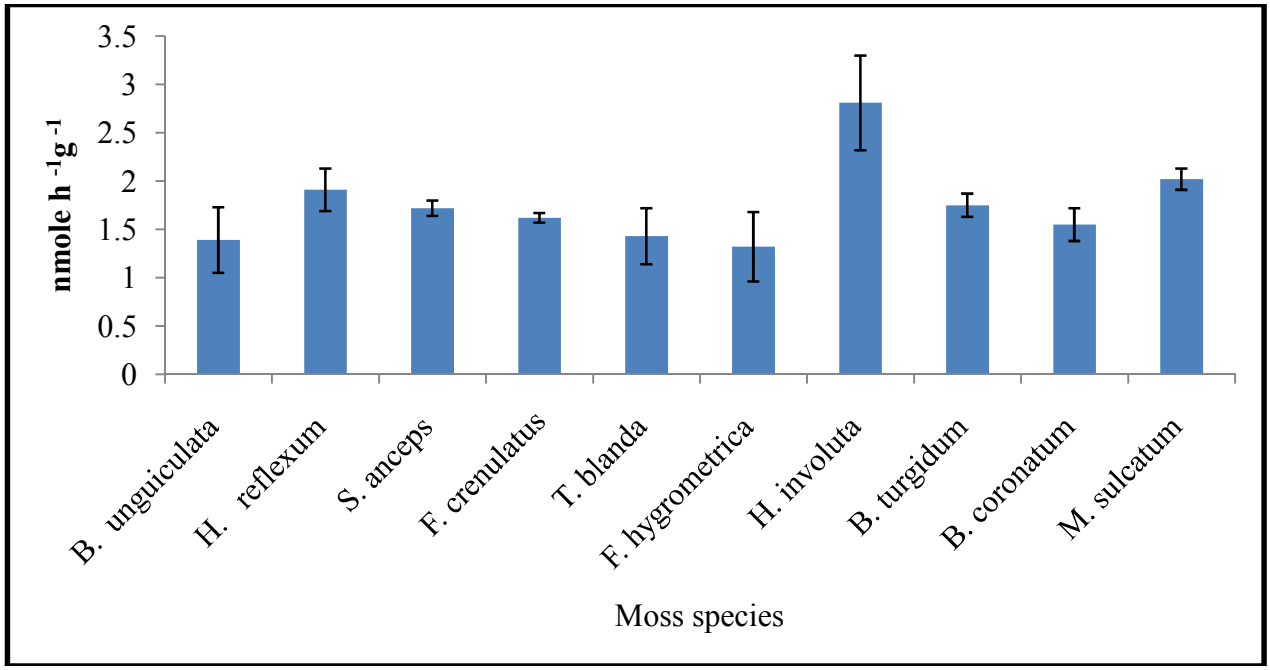
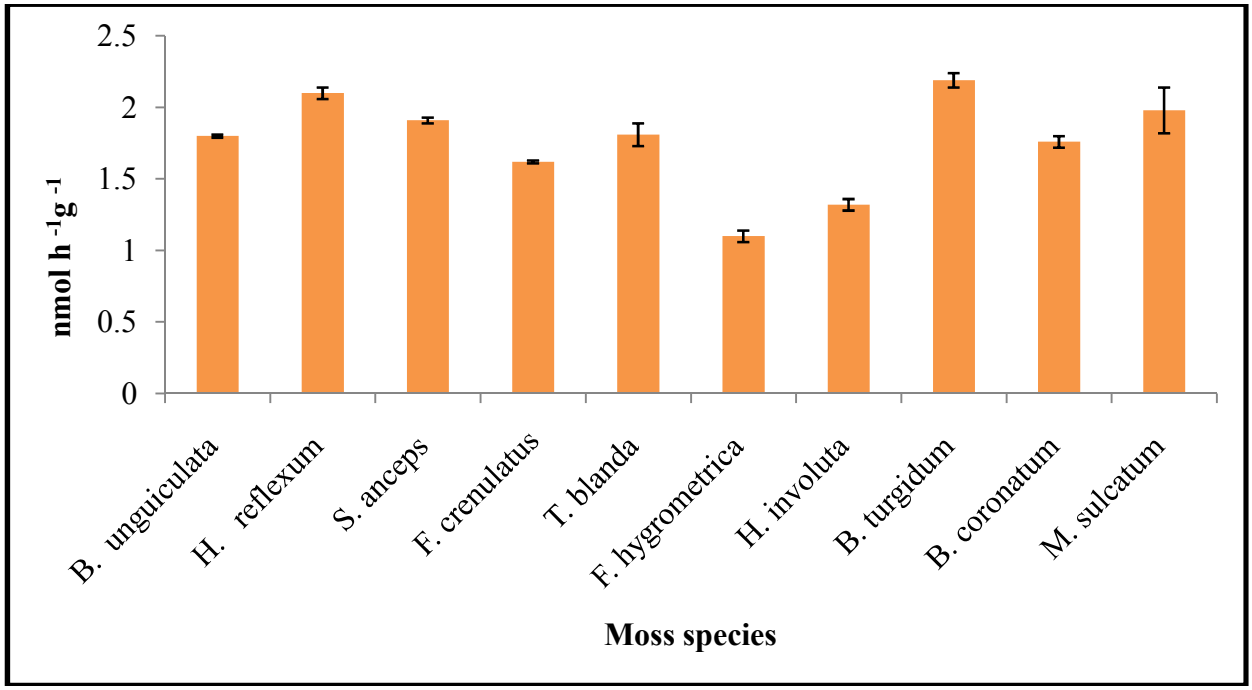


Fig. 9: Nitrite reductase enzyme activity in mosses.



4.4 Biochemical analysis:

4.4.1 Total Reducing sugars, Starch and Carbohydrates:

Carbohydrates, a final product of photosynthesis occupy a very important place in the primary metabolism of all green plants. These are the major products of photosynthetic carbon assimilation and the major substrates for respiration. Thus, the level of carbohydrates in the plant tissue gives an indirect idea of the metabolic status of the plant tissue as well as the energy content of the plant tissue. Carbohydrates provide carbon skeletons for wide range of carbon compounds present in the plant tissue. These compounds include various secondary metabolites, some of which have a definite medicinal value. Starch is the storage carbohydrate

The content of carbohydrates (sugars and starch) in mosses is mentioned in Table 16 and depicted in Fig. 10. It is clear from the results that the concentrations of reducing sugars and starch are lower as compared to those of higher plants. It is evident from the results that levels of starch are much higher than reducing sugars in all the mosses. *Bryum coronatum* exhibits the highest amount of starch and less in *Fissidens crenulatus.* While the highest values of reducing sugars are observed in *Funaria hygrometrica* and *Macromitrium sulcatum* contains very low amount of reducing sugars (0.64 mg / g) as compared to that in rest of the mosses.

A leafy liverwort was analyzed for carbohydrates by (Suleiman *et al.,* 1979). Roser *et al.* (1992) investigated sugar contents in some bryophyte members. Smirnoff (1992) studied the carbohydrates of bryophytes in response to drought tolerance suggesting that high sucrose content, allied with low levels of reducing sugars, contributes to the desiccation tolerance of mosses. Sawant (2010) investigated that the levels of starch are much higher in all the bryophytes. *A. wallichiana* exhibits the highest amount of starch while the highest values of reducing sugars are observed in *A. subtilis* and *A. wallichiana. C. cavernarum* contains very low amount of reducing sugars (0.026 %) as compared to that in rest of the bryophytes. The maximum carbohydrate content is found in *Brachythecium kamounense* and the minimum is observed in *Rhodobryum roseum*. This wide variation in Carbohydrate content may be due to the lesser photosynthetic area available in *R. roseum* because the stems are almost naked and the leaves are aggregated only at the top of the stem, while, in *Brachythecium kamounense*, *Funaria hygrometrica* and *Atrichum pallidum*, the

photosynthetic area is considerably enhanced by the increased number of leaves closely distributed on the stems. (Kaur *et al*., 2010). Kapila *et al.* (2014) recorded that carbohydrate content of the two species of *Marchantia* is almost the same in rainy season as well as in Winter *M. nepalensis* 25.14 ± 0.47 mg g –1 fresh weight in rainy season and 39.11 ± 2.36 mg g –1 fw in Winter season, *M. palmata* 24.32 ± 3.63 mg g–1 fw in rainy season and 21.91 ± 0.85 mg g–1 fw in winter season. At the end of the growing season i.e. Jan–March, carbohydrate content of both the species of *Marchantia* abruptly increased *M. nepalensis* 105.95 ± 2.81 mg g–1 fw and *M. palmata* 48.24 ± 2.89 mg g–1 fw .

4.4.2 Soluble Proteins:

The soluble proteins content in all mosses recorded in Table 17 it is observed that soluble protein content is more in mosses. It was considerably higher in *Fissidens crenulatus* and *Trachypodiopsis blanda* (7.6 mg/g) as compared to other moss species. Thus, *Barbula unguiculata* (6.2 mg/g) synthesis less amount of proteins.

Davey (1999) recorded similar observation in the protein content of hydric mosses as compared to that of drier habitat Antarctic mosses. The continuous flushing of nutrients in hydric habitats may be one of the reasons of higher protein content in these plans. Margaris and Kalaitzakis (1974) studied discrimination protein contents and increased activities of enzymes that created with result of low pollution levels which has not done much damage to plant Dhindsa and Bewely (1977) studied protein synthesis status in unstressed moss was considerably higher in *T. ruralis* (680 cpm/ug) than in *H. luridum* (255 cpm/ug). Thus, *H. luridum* has a less protein synthesis in the unstressed conditions. Thus it is very sensitive to water stress than *T. ruralis*. Kapila *et al.* (2014) recorded that the higher protein content observed that protein content of *D. hirsute* was found to be higher than for the two species of *Marchantia* which is suggesting that the liverworts growing along water streams and in more shaded areas contain higher protein content than the liverworts that growing on wet soil in mesic conditions.

Table 16 : Carbohydrate, reducing sugars and starch contents in mosses.

Sr. No.	Name of species	Carbohydrate	Reducing sugars	Starch
1	*Barbula unguiculata* Hedw.	13.44 ± 0.81	1.48 ± 0.01	7.8 ± 0.74
2	*Hypnum reflexum* F. E. Tripp.	11.06 ± 0.01	1.84 ± 0.01	10.4 ± 0.16
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth.	11.45 ± 1.63	1.34 ± 0.02	8.4 ± 0.24
4	*Fissidens crenulatus* Mitt.	6.84 ± 0.02	1.18 ± 0.03	5.6 ± 0.16
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	15.47 ± 0.01	1.18 ± 0.01	12.4 ± 0.32
6	*Funaria hygrometrica* Hedw.	14.01 ± 0.03	1.86 ± 0.03	12.2 ± 0.24
7	*Hyophila involuta* (Hook) Jaeg.	21.52 ± 0.83	1.34 ± 0.04	12.8 ± 1.77
8	*Brachymenium turgidum* Broth.	19.31 ± 1.64	1.46 ± 0.01	12.6 ± 0.04
9	*Bryum coronatum* Schwaegr.	18.62 ± 0.01	1.41 ± 0.05	13.0 ± 0.32
10	*Macromitrium sulcatum* Brid.	21.59 ±0 .83	0.64 ± 0.01	12.2 ± 0.73

Values are mean of three replications expressed as mg / g dry tissue.

Table 17: Soluble protein content in mosses.

Sr. No.	Name of species	Protein
1	*Barbula unguiculata Hedw.*	6.2 ± 0.08
2	*Hypnum reflexum F. E. Tripp.*	6.8 ± 0.16
3	*Steeriophyllum anceps (Bosch et. Lac.) Broth*	6.4 ± 0.08
4	*Fissidens crenulatus Mitt.*	7.6 ± 0.16
5	*Trachypodiopsis blanda (Mitt.) Fleisch.*	7.6 ± 0.32
6	*Funaria hygrometrica Hedw.*	7.2 ± 0.01
7	*Hyophila involuta (Hook) Jaeg.*	6.8 ± 0.04
8	*Brachymenium turgidum Broth.*	7.0 ± 0.09
9	*Bryum coronatum Schwaegr.*	7.0 ± 0.01
10	*Macromitrium sulcatum Brid.*	6.6 ± 0.09

Values are mean of three replications expressed as mg / g dry tissue.

Fig. 10 : Carbohydrate, reducing sugars and starch contents in mosses.

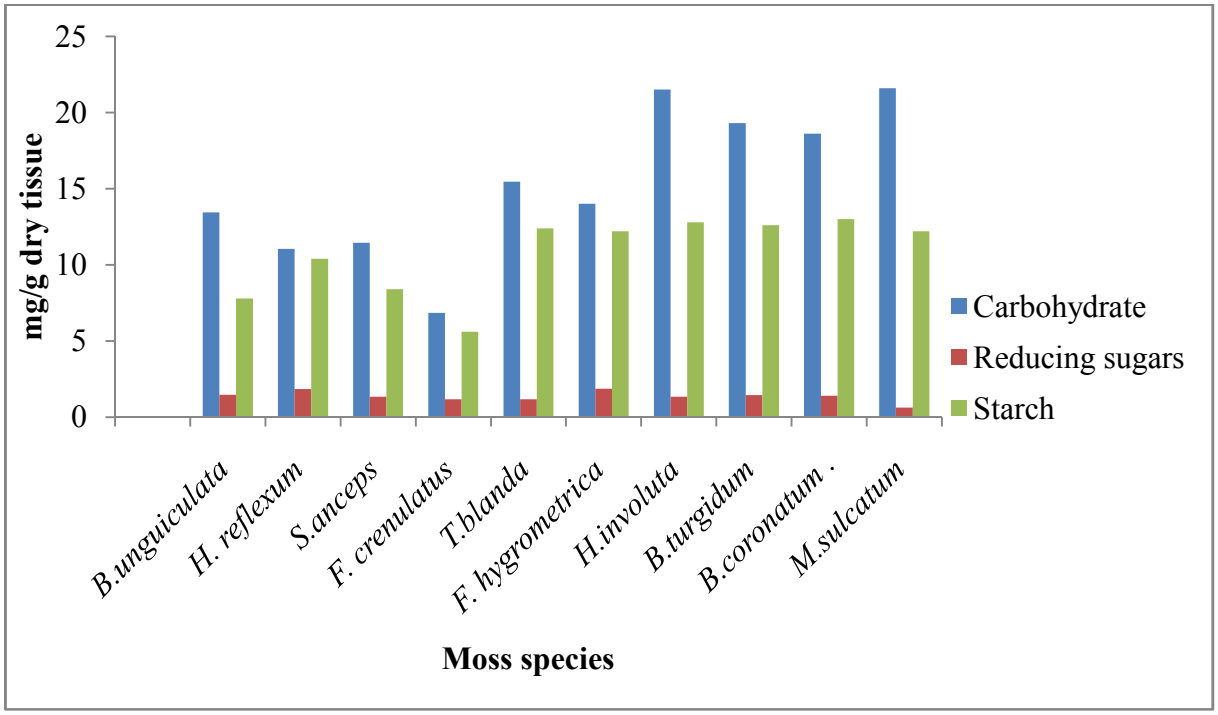
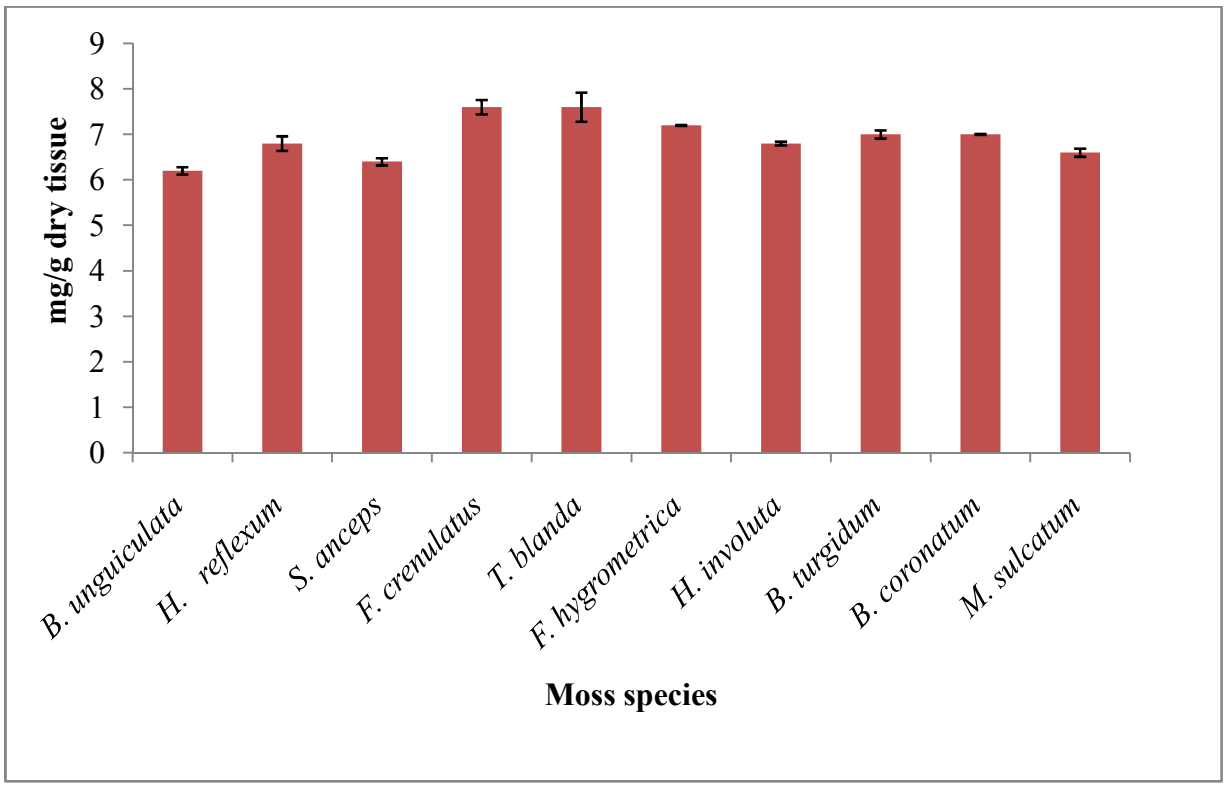


Fig11: Soluble protein content in mosses.



4.4.3 Free amino acids:

Amino acids known as building blocks of proteins are essential organic compounds. Organic nitrogen preferentially transported in the form of amino acids, which serves as core for many functions including nitrogen metabolism, protein synthesis and precursors for many important cellular constituents including nucleobases (Lalonde *et al.*, 2004).The twenty amino acids (actually, nineteen α-amino acids and one α-amino acid) that are utilized in living cells for protein synthesis under the control of genes are in a special category since they are fundamental to all life forms as building blocks for peptides and proteins. However, the reasons why all the other natural amino acids are located where they are rarely known, although this is an area of much speculation. For example, some unusual amino acids are present in many seeds and are not needed by the mature plant. They deter predators through their toxic or otherwise unpleasant characteristics and in this way are thought to provide a defence strategy to improve the chances of survival for the seed and therefore help to ensure the survival of the plant species (Barrett and Elmore, 1998).

The free amino acid content in mosses is depicted in Table 18 and Fig. 12. It is evident from the values that the amount of free amino acids is higher in case of *Funaria hygrometrica*, which is followed by *Steeriophyllum* anceps and rather similar amounts in case of *Trachypodiopsis blanda*. The lowest concentration of these constituents is observed in case of *Hypnum reflexum*. Almost no report has been found with respect to concentration of different amino acids except few reports regarding the qualitative estimation of amino acids in which mosses *Tortula princeps*, *Rhynchostegium* sp. *Platyhypendium ripariodoides*, *Homalothecium* sp. and *Camptothecium* sp. contain 0.51, 0.96, 1.10, 0.44 and 0.64 mg g^{-1} dry tissue respectively (Margaris, 1974). Sawant (2010) reported the amount of free amino acids is higher in case of *T. hypophylla,* which is followed by *A. subtilis* and rather similar amounts in case of *A. wallichiana*. In *C. cavernarum* amino acids content is 1.28 and 1.51 mg g^{-1} dry tissue respectively and finally the lowest concentration of these constituents is observed in case of *P. intermedium.* Kapila *et al.* (2014) observed the total free amino acids concentrations indicated significant seasonal changes during three bryological seasons ($p < 0.05$). The content of free amino acids was found to be lowest in the October–December (11.18 ± 1.85 mg g^{-1}fw in *M. palmate*, 7.7 ± 1.97 mg g^{-1} fw in *M. nepalensis* and 11.61 ± 1.38 mg g^{-1} fw in *D. hirsuta*) but low seasonal July–

September (14.87 ± 1.73 mg g^{-1} fw in *M. palmata*, 10.29 ± 5.28 mg g^{-1} fw in *M. nepalensis* and 14.02 ± 0.52 mg g^{-1} fw in *D. hirsuta*) and January–March (21.8 ± 2. mg g^{-1} fw in *M. palmata*, 16.39 ± 2.95 mg g^{-1} fw in *M. nepalensis* and12.22 ± 0.63 mg g^{-1} fw in *D. hirsuta*).

In mosses amino acids play an important role in protecting the photosynthetic apparatus against the destructive effects of light and ROS.

4.4.4 Free proline:

Proline is considered to play a major role in adjustment to osmotic stresses (Voetberg and Sharp, 1991). Proline is one of the important amino acids in plants. This cyclic amino acid is synthesized from glutamate generated from ornithine in biochemical pathways. It is observed that free proline accumulates in plants in response to various types of environmental stresses, such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals and high acidity (Oncel *et al*., 2000 and Ruiz *et al*., 2002). In view of Matysik *et al.* (2002) proline is a proteinogenic amino acid which functions as an osmolyte, free radical scavenger, electron sink and stabilizer of macromolecules and component of cell wall.

The content of free proline in different mosses is mentioned in Table 19 and depicted in Figure 13. In *Brachymenium turgidum* contains the more concentration of proline (9.4 mg/ g dry tissue) in comparison with *Hypnum reflexum* (3.5 mg/ g dry tissue). Irigoyen *et al.* (1992) have shown that, under stress conditions, proline helps to stabilize proteins and regulate cytosolic pH and the NAD/ NADH ratio. Liu *et al.* (2001) reported the accumulation of free proline in *Plagiomnium acutum* and *Thuidium cymbifolium* under high temperature stress showed that the high temperature as well as water stress could cause the proline accumulation in mosses under high temperature stress. Panda (2003) reported that as heavy metals induce water-deficit conditions, an increase in proline accumulation may help in osmoprotection. The osmoprotectant was accumulated uniformly in moss *Taxithellium* sp. under heavy metal treatment, with a maximum in chromium treatment (0.001nM). Sawant (2010) observed that content of proline in *T. hypophylla* contains the highest amount of proline (11.29 mg g^{-1} dry tissue) while about half and fairly similar amount is present in case of *A. subtilis* and *A. wallichiana*. This is followed by *C. cavernarum* with 4.33 mg g^{-1} dry tissue and the lowest amount in *P. intermedium* (3.93 mg g^{-1} dry tissue). In the present findings free proline is chief organic solute that seems to

report that in mosses. It acts as osmolyte in response to the environmental stress i.e. drought / high temperature as well as to heavy metal concentration.

Table 18: Free amino acids content in mosses.

Sr. No.	Name of species	Free amino acids
1	*Barbula unguiculata* Hedw.	16.5 ± 1.79
2	*Hypnum reflexum* F. E. Tripp.	10.6 ± 0.24
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth.	19.4 ± 0.32
4	*Fissidens crenulatus* Mitt.	16.7 ± 1.46
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	19.0 ± 0.40
6	*Funaria hygrometrica* Hedw.	28.5 ± 0.16
7	*Hyophila involuta* (Hook) Jaeg.	16.1 ± 0.77
8	*Brachymenium turgidum* Broth.	13.5 ± 0.16
9	*Bryum coronatum* Schwaegr.	17.9 ± 0.24
10	*Macromitrium sulcatum* Brid.	15.3 ± 0.32

Values are mean of three replications expressed as mg / g dry tissue.

Table 19: Free proline content in mosses.

Sr. No.	Name of species	Free proline
1	*Barbula unguiculata*	8.4 ± 1.79
2	*Hypnum reflexum* F. E. Tripp.	3.5 ± 0.32
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth	9.1 ± 0.24
4	*Fissidens crenulatus* Mitt.	5.5 ± 1.55
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	8.6 ± 0.20
6	*Funaria hygrometrica* Hedw.	8.3 ± 0.44
7	*Hyophila involuta* (Hook) Jaeg.	5.8 ± 0.73
8	*Brachymenium turgidum* Broth.	9.4 ± 0.04
9	*Bryum coronatum* Schwaegr.	7.5 ± 0.32
10	*Macromitrium sulcatum* Brid.	8.5 ± 0.48

Values are mean of three replications expressed as mg / g dry tissue.

Fig. 12 : Free amino acids content in mosses.

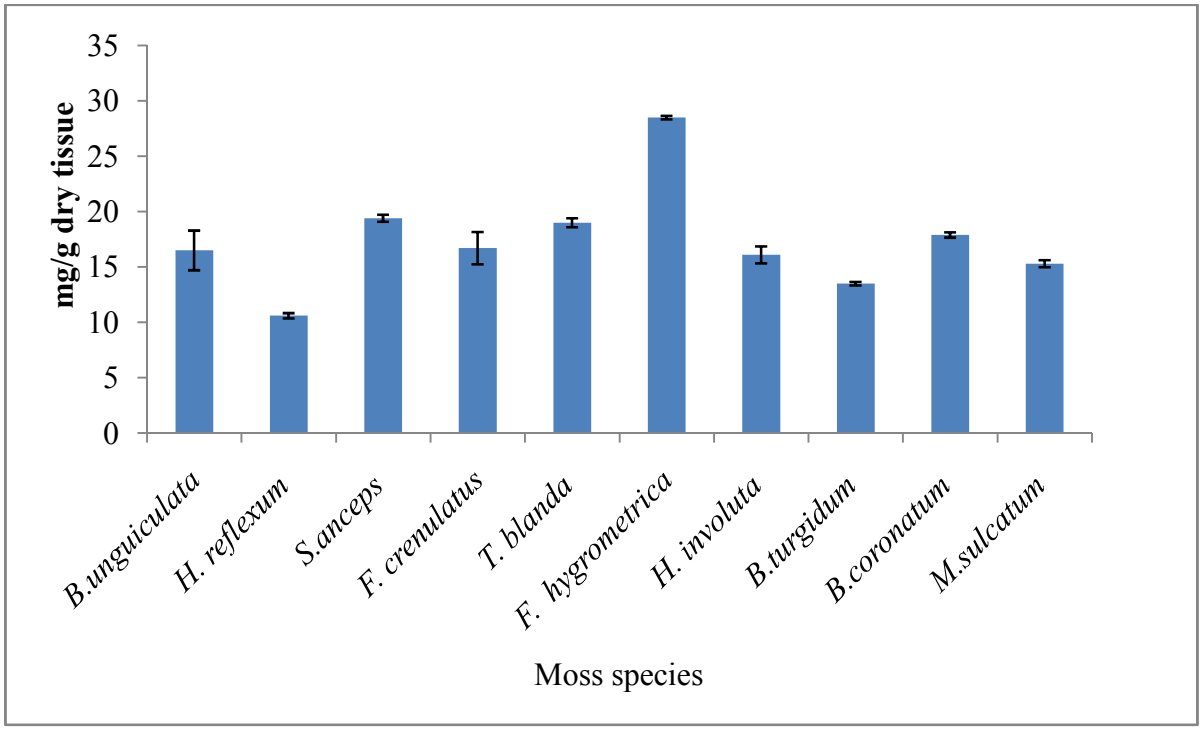
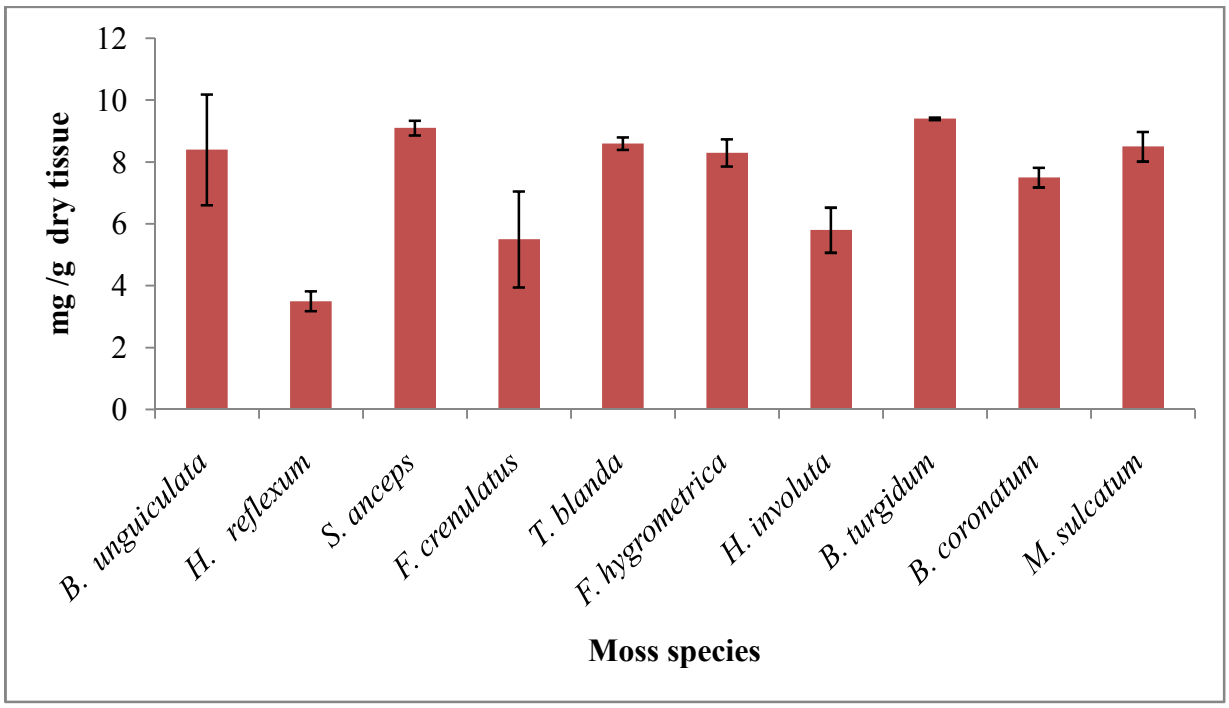


Fig. 13: Free proline content in mosses.



4.5 Secondary metabolites:

Secondary metabolites refer to compounds present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism but are thought to be required in defence mechanism. They not only defend against the plant competition, microbial attack, and insect or animal predation, but also function in UV protection, drought tolerance, and freezing survival (Xie and Lou, 2009). Secondary metabolites in defense may involve deterrence/anti-feedant activity, toxicity or acting as precursors to physical defences. Many specialist herbivores and pathogens do not merely circumvent the deterrent or toxic effects of secondary metabolites but actually utilize these compounds as either host recognition clues or nutrients or both. This is true of both cyanogenic glucosides and glucosinolates, which are discussed in detail as examples of defense compounds. Their biochemistry is compared and contrasted. An enormous variety of secondary metabolites are derived from shikimic acid or aromatic amino acids, many of which have important roles in defence mechanisms (Bennett and Wallsgrove, 1994).

4.5.1 Total Polyphenols :

Polyphenols are a group of chemical substances which are widespread found in plants characterized by the presence of more than one phenol units or building blocks per molecule and are of great significance. They are generally divided into hydrolysable tannis and phenyl propanoids such as lignin flavonoids which induce several thousand compounds. The most abundant polyphenols are the condensed tannins found in all families of plants and comprise in upto 50 % of the dry weight of the leaves. Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. As stated by Harborne (1989), the term "phenolic" or "polyphenol" can be precisely defined chemically as a substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl ethers, glycosides, etc.): as a general rule, the terms phenolics and polyphenols refer to all secondary natural metabolites arising biogenetically from the shikimate-phenylpropanoids-flavonoids pathways, producing monomeric and polymeric phenols and polyphenols.

The total polyphenols content of mosses is recorded in Table 20 depicted in Fig.14. It is clear from the results that, the highest concentration of polyphenols is 9.0 mg/g dry

tissue in *Brachymenium turgidum* while the lowest observed in *Fissidens crenulatus, Trachypodiopsis blanda* and *Hypnum reflexum* which are 2.6. mg/g dry tissue. Sawant (2010) investigated that the highest concentration of polyphenols is 1g 100 g^{-1} fresh tissue in *P. intermedium* while the lowest is observed in *C. cavernarum* which is 0.17 g 100 g^{-1} fresh tissue. *A. wallichiana* and *T. hypophylla* have moderately similar concentrations while the hornwort, *A. subtilis* contains 0.39 g 100 g^{-1} of polyphenols. Kadam (2016) investigated the polyphenol content of *Anthocerous, Astrella, Cyathodium, Plagiocasma and Targinia* has shown a decrease in the month of September but slightly increases in October. *Cythodium* shows maximum content of polyphenols followed by *Plagiochasma and Targionia*

4.5.2 Tannins:

The tannins content of mosses is recorded in Table 20 and depicted in Fig. 15. The tannins of these mosses range in between 6.0 – 11.5 mg/g dry tissue respectively. The highest concentration of tannins in *Fissidens crenulatus* while lowest is observed in *Barbula unguiculata.*

Tannin is the major phenolic polymer, for defensive properties in plants. The term tannin was first used to describe compounds that could convert raw animal hides into leather in the process known as tanning. Tannin has molecular masses between 600-3000 KD. Tannins are general toxin that significantly reduces the growth and survivorship of many herbivores when added to their diets. Unripe fruits frequently have very high tannin levels, which may be concentrated in skin or epicarp. Interestingly, it is often desired a certain level of astringency in tannin containing foods such as apples, blackberries, tea and red wine, which are consumed as stimulatory agents in human diet (Taiz and Zeiger, 2006).

Tannins are the most abundant secondary metabolite synthesized by plants, commonly ranging from 5% to 10% dry weight of tree leaves. Tannin can defend leaves against insect herbivores by deterrence and/or toxicity. Contrary to early theories, tannins have no effect on protein digestion in insect herbivores. By contrast, in vertebrate herbivores tannins can decrease protein digestion. Tannins are especially prone to oxidize in insects with high pH guts, forming semiquinone radicals and quinones, as well as other reactive oxygen species. Tannin toxicity in insects is thought to result from the production of high levels of reactive oxygen species (Barbehenn and Constabel , 2011).

Table 20 : Secondary metabolites content of total polyphenols and tannins in mosses.

Sr. No.	Name of species	Total polyphenols	Tannins
1	*Barbula unguiculata*	3.5 ± 0.04	6.0 ± 0.73
2	*Hypnum reflexum* F. E. Tripp.	2.6 ± 0.73	6.3 ± 0.04
3	*Steeriophyllum* anceps (Bosch et. Lac.) Broth	4.5 ± 0.20	9.0 ± 0.77
4	*Fissidens crenulatus* Mitt.	2.6 ± 0.04	11.5 ± 0.16
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	2.6 ± 0.57	9.0 ± 0.08
6	*Funaria hygrometrica* Hedw.	8.1 ± 0.32	8.3 ± 0.89
7	*Hyophila involuta* (Hook) Jaeg.	3.0 ± 0.16	9.6 ± 0.01
8	*Brachymenium turgidum* Broth.	9.0 ± 0.04	9.0 ± 0.04
9	*Bryum coronatum* Schwaegr.	7.5 ± 0.65	9.3 ± 0.16
10	*Macromitrium sulcatum* Brid.	6.1 ± 0.69	9.5 ± 0.48

Values are mean of three replications expressed as mg / g dry tissue.

Fig. 14 : Total polyphenols content in mosses.

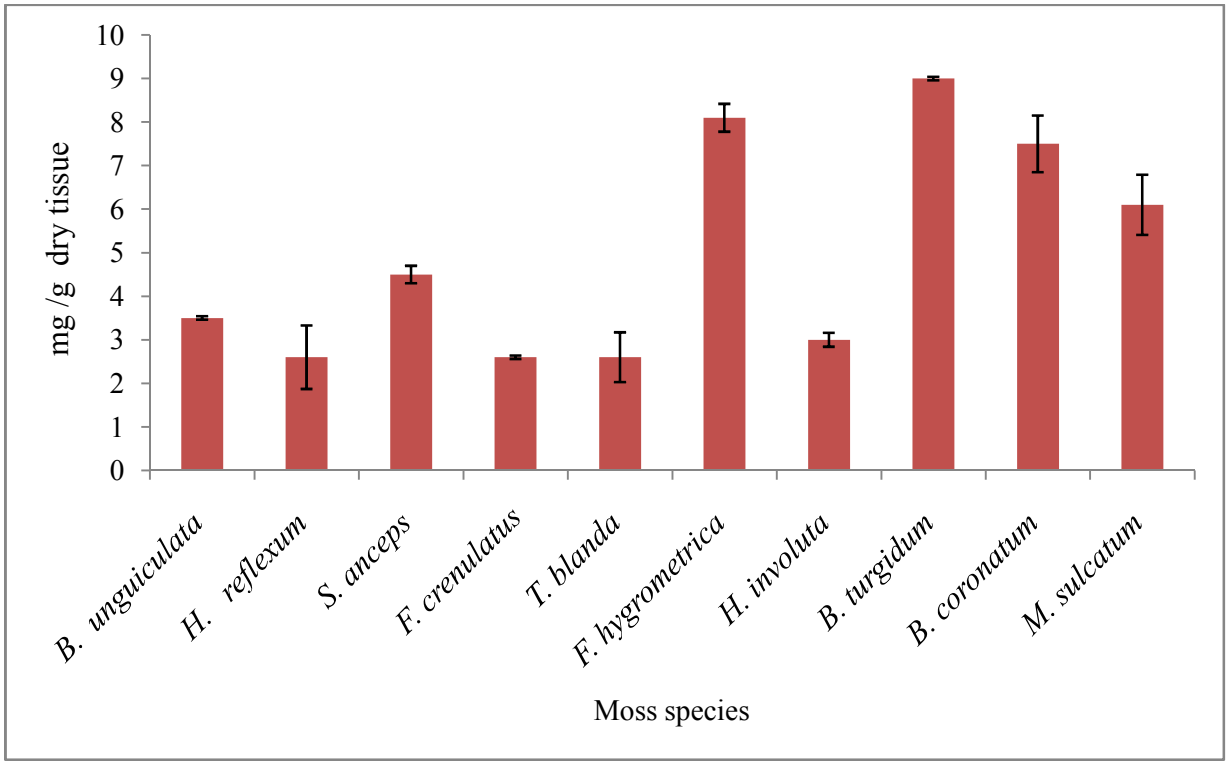
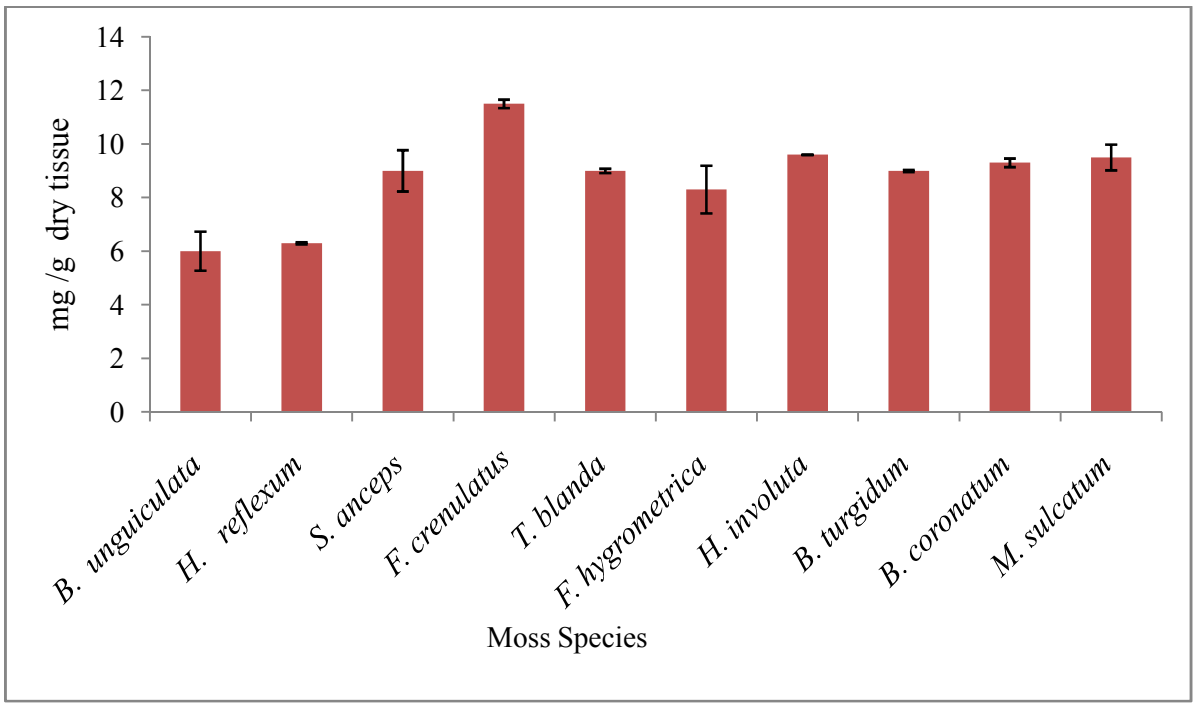


Fig. 15 : Tannin content in mosses.



4.6 Effect of aqueous moss extracts on seed germination and seedling growth of semi-arid region crops:

Germination and growth are inhibited by plant metabolites is frequently associated to allelopathy. This process plays an important role in both natural and agro-ecosystems and shows either stimulatory or inhibitory effect by one plant on another through production of chemical compounds released into the habitats (Rice, 1984, Oliveira *et al*., 2004). Suitable manipulation of the allelopathy towards improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases and synthesis of novel agrochemicals based on natural products they have played major role because of this researchers gained prominent attention.

The phenomenon of allelopathy cover all types of chemical interactions among plants and could account for the observation that certain plants sometimes do not grow in habitats of others. Moreover, it was shown that extracts of some plants inhibit the growth of others (Dias *et. al.,* 2005). Now it is generally recognized that some terpenoids, mainly monoterpenes and sesquiterpenes present in the volatile fractions and phenolic compounds are mainly responsible for growth inhibition of competing plants (Fischer, 1991; Von Poser *et al.,* 1996). Bryophytes, which are oldest lineages among existing land plants, are the second largest phylum of land plants after angiosperms among, and inhabit all continents. Bryophytes are invading variety of habitats and are exposed to different abiotic and biotic factors with the advantage of miniature nature. In order to defend these factors many of the secondary metabolites especially terpenoids and phenolic compounds are synthesized (Herout, 1990).

4.6.1 Effect of various aqueous moss extracts on germination and seedling growth of cereals:

The seeds germinated in control and various concentrations of aq. extracts of mosses for five days in petriplates and found the effect of aq. extracts on germination are mentioned in Tables 21 and depicted in Fig. 16. The semi-arid region cereals crop commonly cultivated in the Maharashtra viz. *Sorghum vulgare* L., *Pennisetum glaucum* L. and *Triticum aestivum* L. for germination bioassay in the present piece of work. Hundred seeds of each crop were

treated with aqueous moss extracts in sterilized petriplates lined with germination paper where as water serving as control.

Observations made for five days after sowing the seeds in petriplates of respected extract indicated that all extracts show influence effect on seed germination as compared to control.

A. Germination percentage:

The result showed that (Table 21) maximum germination was *S. vulgare* L., *P. glaucum* L *T. aestivum* L. observed in aq. moss extracts as compared to control. The germination growth of cereals seeds was highly influence under aqueous moss extracts treatment. According to Chavan *et.al* (2014) aq. extracts of some mosses highly influence the growth and seed germination of different plants.

It is evident that, extracts caused minimum growth effect on seeds germination of cereals crop plants during the initial stages (1st day). After 1st day a maximum seed germination of *S. vulgare* L. and *T. aestivum* L was recorded in aq. extracts treatment petriplates but it is also showed that maximum number of seed germinated in last 4 days recorded with few exceptions over the control. According to Sharma *et al*., (2009) studied growth regulatory activities of three liverworts (*T. hypophylla, M. polymorpha* and *P. appendiculatum*) and five mosses (*L. secundus, T. anomala, R. roseum* and *P integrumB. buchananii*) on germination behavior of *B. biternata* seeds. Irrespective of the concentration, in aqueous extracts of all bryophyte species germination was approximately 100 % was recorded.

B. Root length:

There is gradual increase the length of root in all tested aqueous extract that depicted in Table 26. The aqueous extract has been remarkably increased length of root than control. The aqueous extracts of *Barbula unguiculata* (8.2 cm), *Hypnum reflexum* (8.99 cm), *Steeriophyllum anceps* (8.51cm), *Trachypodiopsis blanda* (7.57 cm), *Funaria hygrometrica* (7.12 cm), *Hyophila involuta* (7.1 cm), *Brachymenium turgidum* (7.15 cm), *Bryum coronatum* (8.25 cm) and *Macromitrium sulcatum* (7.9 cm) showed insignificant result in root length of *Sorghum vulgare* and the aqueous extract of *Fissidens crenulatus* (9.95) showed remarkably highly significant results at $P < 0.001$.

The root lengths of *Pennisetum glaucum* seedlings were significant results by all treatments. The positive effect was much more pronounced in the treatment of *Barbula unguiculata* (13.14 cm), *Steeriophyllum anceps* (11.5 cm), *Fissidens crenulatus* (9.9 cm), *Trachypodiopsis blanda* (14.27cm), *Funaria hygrometrica* (11.34 cm), *Hyophila involuta* (12.09 cm), *Brachymenium turgidum* (10.04 cm), *Bryum coronatum* (12.76 cm) and *Macromitrium sulcatum* (10.79 cm) as compared to control. *Hypnum reflexum* (9.61 cm) were not much influenced the root length of *Pennisetum glaucum* seedlings than control.

Triticum aestivum showed highly significant results at $P < 0.001$ in aqueous extracts of *Steeriophyllum anceps* (14.57 cm), *Fissidens crenulatus* (13.27 cm), *Brachymenium turgidum* (13.95 cm) and *Bryum coronatum* (13.65 cm) however other aqueous extracts of mosses showed insignificant result in root length of wheat.

C. Shoot length:

There is gradual increase the length of shoot in all tested aqueous extracts that is depicted in Table 27. The shoot length of *Sorghum vulgare* seedling was significantly influenced by some treatments. The positive effect was much more pronounced in the treatment of *Barbula unguiculata* (8.7 cm), Hypnum *reflexum* (8.7 cm), *Steeriophyllum anceps* (8.3 cm), Fissidens *crenulatus* (11.35 cm) and *Trachypodiopsis blanda* (8.3 cm) as compared to control. Some treatment showed insignificantly effect against the shoot length i.e. *Funaria hygrometrica* (6.4 cm) *Hyophila involuta* (6.1 cm) *Brachymenium turgidum* (6.65) *Bryum coronatum* (6.4 cm) *Macromitrium sulcatum* (6.4 cm)

The aqueous extracts of *Barbula unguiculata* (8.26 cm), *Hypnum reflexum* (8.5 cm) *Fissidens crenulatus* (8.54 cm) *Trachypodiopsis blanda* (9.8 cm) *Hyophila involuta* (8.43 cm) *Brachymenium turgidum* (7.88 cm) *Bryum coronatum* (7.96 cm) showed remarkably highly significant results at $P < 0.001$ in the *Pennisetum glaucum* shoot length except in the aqueous extract of *Steeriophyllum anceps* (7. 71 cm) *Funaria hygrometrica* (7.43 cm) and *Macromitrium sulcatum* (6.78 cm).

Triticum aestivum seedling was high significantly at $p < 0.001$ shoots length than other seedling. The positive effect was much more pronounced in the treatment of *Hypnum reflexum* (9.54 cm) *Steeriophyllum anceps* (9.35 cm) *Funaria hygrometrica* (12.17 cm) *Hyophila involuta* (12.12 cm) *Brachymenium turgidum* (12.45cm) *Bryum coronatum* (12.5 cm) *Macromitrium sulcatum* (12.38 cm) as compared to control.

4.6.2 Effect of various aqueous moss extracts on germination and seedling growth of pulses:

The seeds germinated in control and various aq. extracts of mosses for five days in petriplates and findings effect of aq. extracts on germinations of Pulses seeds mentioned in Tables 22 and depicted in Fig. 17.

A. Germination percentage:

The result showed that (Table 23) maximum germination like *Cajanus cajan* L. and *Cicer arientinum* L. was observed in aq. moss extracts as compared to control. The germination growth of pulses seeds was promoted under aqueous moss extracts treatment.

It is evident that, extracts caused minimum growth effect on seeds germination of Pulses crop plants during the initial stages (1st day). After 1st day a maximum seed germinated was recorded in all aq. moss extracts on seeds of *Cicer arientinum* L. but minimum seed germinated was observed in *Cajanus cajan* L. There is 100 % seeds are germinated in all aq. moss extracts for 5 days with few exceptions as compared to control.

According to Bhadauriya *et al.* (2016) studied the inhibition in germination rate was observed at high concentration of methanol and aqueous extract in the both seeds of Mung bean and Bengal gram; however, highly diluted aqueous extracts showed an increase in the germination and promoted the growth in both crop species

B. Root length:

There is gradual increase the length of pulses root in all tested aqueous extract that depicted in Table 26. The aqueous extract has been remarkably highly significant results at $P < 0.001$ length of root than control. The positive effect was much more pronounced in the treatment of *Hypnum reflexum* (4.51 cm), *Steeriophyllum anceps* (4.45 cm), *Fissidens crenulatus* (5.09 cm), *Trachypodiopsis blanda* (5.41 cm), *Funaria hygrometrica* (4.68 cm), *Hyophila involuta* (4.87 cm), *Brachymenium turgidum* (5.24 cm), *Bryum coronatum* (5.16 cm), *Macromitrium sulcatum* (5.17 cm) showed result in the *Cajanus cajan* root length except in the aqueous extract of *Barbula unguiculata* (4.87 cm) it is moderately significant.

The root lengths of *Cicer arientinum* seedlings were incredibly inhibited by some treatment. The insignificant effect was much more pronounced in the aqueous extract treatment of *Barbula unguiculata* (5.11 cm), *Steeriophyllum anceps* (5.11 cm). Among these

treatments, some extracts were showed highly significant results in root lengths growth when compared to control. i. e *Hypnum reflexum* (11.8 cm), *Fissidens crenulatus* (9.53 cm), *Trachypodiopsis blanda* (8.63 cm), *Funaria hygrometrica* (8.85cm), *Hyophila involuta* (9.7 cm), *Brachymenium turgidum* (8.98 cm), *Bryum coronatum* (10.7 cm) and *Macromitrium sulcatum* (9.78 cm)

C. Shoot length:

The effect of aqueous moss species extracts on shoots length is shown in Table 27. It is observed that the highly significant results at $P < 0.001$ in aqueous extracts of *Barbula unguiculata* (4.57 cm), *Hypnum reflexum* (4.54 cm), *Steeriophyllum anceps* (4.77 cm), *Fissidens crenulatus* (4.72 cm), and *Trachypodiopsis blanda* (4.49 cm) but other aqueous extracts of mosses showed insignificant result in shoot length of *Cajanus cajanas* compared to control.

The aqueous extracts of *Barbula unguiculata* (3.36 cm), *Hypnum reflexum* (5.5 cm), *Steeriophyllum anceps* (3.4 cm), *Funaria hygrometrica* (4.0 cm), *Hyophila involuta* (3.44 cm), *Brachymenium turgidum* (3.08 cm), *Bryum coronatum* (3.38 cm), and *Macromitrium sulcatum* (4.57 cm) showed remarkably highly significant results at $P < 0.001$ in the *Cicer arientinum* shoot length except in the aqueous extract of *Fissidens crenulatus* (2.33 cm), *Trachypodiopsis blanda* (2.72 cm).

4.6.3 Effect of various aqueous moss extracts on germination and seedling growth of legumes:

The seeds germinated in control and various aq. extracts of mosses for five days in petriplates and findings effect of aq. extracts on germinations of legumes seeds mentioned in Tables 23 and depicted in Fig. 18.

A. Germination percentage:

The result showed that (Table 23) maximum germination in the *Arachis hypogaea* L. and *Phaseolus aureus* Roxb. was observed in aq. moss extracts as compared to control. The germination growth of legumes seeds was promoted under aqueous moss extracts treatment.

It is evident that, extracts caused minimum growth effect on seeds germination of legumes crop plants during the initial stages (1^{st} day). After 1^{st} day a maximum seed germinated was recorded in all aq. moss extracts on seeds of *Phaseolus aureus* Roxb. but

minimum seed germinated was observed in *Arachis hypogaea* L. There is 100 % seeds are germinated in *Phaseolus aureus* Roxb. after 5 days with few exceptions as compared to control.

According to Huneck and Meinunger (1990) tested 52 species of mosses and 29 species of liverworts on growth regulation activity. Study done by them illustrated that, different concentrations of the bryophyte extracts vary the amount of promotion viz. inhibition and hence, it is difficult to explain. It was concluded from the study that, the growth regulation activity depends on the concentrations, i.e. inhibiting growth at higher and promoting growth at lower concentrations of bryophyte extract done in aqueous and alcoholic solvent. Same results were also suggested by Matsuo *et al.* (1981) and Asakawa (1982). Bryophytes have unexpected high potential for applied research with implications for the improvement of crop plants (Frahm, 2004).

B. Root length:

There is gradual increase the length of legumes root in all tested aqueous extract that depicted in Table 26. The *Arachis hypogaea* showed highly significant results at $P < 0.001$. In aqueous extracts of *Barbula unguiculata* (3.19 cm), *Steeriophyllum anceps* (3.79 cm), *Fissidens crenulatus* (3.92 cm), *Trachypodiopsis blanda* (3.46 cm), *Funaria hygrometrica* (5.61 cm), *Hyophila involuta* (5.48 cm), *Brachymenium turgidum* (5.72 cm), *Bryum coronatum* (5.65 cm), *Macromitrium sulcatum* (4.83 cm) but *Hypnum reflexum* (2.31 cm) showed insignificant result in root length as compared to control.

The root lengths of *Phaseolus aureus* seedlings were greatly significant results by most of treatments. The positive effect was much more pronounced in the treatment of *Steeriophyllum anceps* (6.46 cm), *Fissidens crenulatus* (6.04 cm), *Trachypodiopsis blanda* (5.68 cm), *Funaria hygrometrica* (5.98 cm), *Hyophila involuta* (7.25 cm), Brachymenium *turgidum* (7.7 cm), *Bryum cornatum* (7.47 cm), and *Macromitrium sulcatum* (7.51 cm), *as* compared to control. But in *Barbula unguiculata* (4.69 cm) and *Hypnum reflexum* (5.11 cm) were insignificant root length of *Phaseolus aureus* seedlings than control.

C. Shoot length:

The effect of aqueous moss species extracts on shoots length is shown in Table 27 and depicted in Figure 30. The shoot length of *Arachis hypogaea* seedling was significantly

influenced by all aqueous mosses extract treatments. The positive effect was much more pronounced in the extract of *Barbula unguiculata* (2.17 cm), *Hypnum reflexum* (1.92 cm), *Steeriophyllum anceps* (2.1 cm), *Fissidens crenulatus* (2.19 cm), *Trachypodiopsis blanda* (2.28 cm), *Funaria hygrometrica* (2.27 cm), *Hyophila involuta* (2.28 cm), *Brachymenium turgidum* (2.45 cm), *Bryum cornatum* (2.5 cm), and *Macromitrium sulcatum* (2.4 cm).

Phaseolus aureus seedling was high significantly at $p < 0.001$ shoots length than control seedling. The positive effect was much more pronounced in the treatment of *Barbula unguiculata* (9.15 cm), *Steeriophyllum anceps* (6.77 cm), *Fissidens crenulatus* (8.64 cm), *Funaria hygrometrica* (7.51 cm), *Hyophila involuta* (8.05 cm), *Brachymenium turgidum* (8.14 cm), *Bryum coronatum* (7.22 cm), and *Macromitrium sulcatum* (6.35 cm). but other aqueous extracts of *Hypnum reflexum* (5.93 cm) *Trachypodiopsis blanda* (5.06 cm) showed insignificant result in shoot length of *Phaseolus aureus* .

4.6.4 Effect of various aqueous moss extracts on germination and seedling growth of spices:

The seeds germinated in control and various concentrations of aq. extracts of mosses for five days in petriplates and findings effect of aq. extracts on germinations are of mosses mentioned in Tables 24 and depicted in Fig.19. The semi-arid region Spices crop commonly cultivated in the Maharashtra viz. *Allium cepa* and *Brassica juncea* L. for germination bioassay in the present piece of work.

A. Germination percentage:

The result showed that (Table 24) maximum germination was *Brassica juncea* L. observed in aq. moss extracts as compared to control. The germination growth of spices seeds was highly influence under aqueous moss extracts treatment.

It is evident that, extracts caused minimum growth effect on seeds germination of spices crop plants during the initial stages (1st day). After 1st day maximum seed germination in *Brassica juncea* L. was recorded in aq. extracts treatment petriplates but minimum seed germination was observed in *A. cepa* L. It is also showed that maximum number of seed germinated in last 4 days. According to Gavrillova (1970) reported that aqueous extracts of *P. commune* and *Sphagnum* sp. inhibited the growth of *Pinus* and *Picea* seedlings, but stimulated the growth of *Larix* seedlings and *P. integram* were found most effective where the seed germination was completely checked.

B. Root length:

There is gradual increase the length of root in all tested aqueous extract that depicted in Table 26. The aqueous extract has been remarkably increased length of root than control.

The root lengths of *Allium cepa* seedlings were incredibly inhibited by some treatment. The insignificant effect was much more pronounced in the aqueous extract treatment of *Barbula unguiculata* (1.68 cm), *Hypnum reflexum* (1.65 cm), *Steeriophyllum anceps* (1.91 cm) *and Trachypodiopsis blanda* (2.23 cm). Among these treatments, some extracts were showed highly significant results in root lengths growth when compared to control. i. e. *Fissidens crenulatus* (2.45 cm), *Funaria hygrometrica* (4.37 cm), *Hyophila involuta* (4.52 cm), *Brachymenium turgidum* (3.85 cm), *Bryum coronatum* (4.21 cm) and *Macromitrium sulcatum* (3.8 cm) .

The aqueous extracts of *Barbula unguiculata* (3.91 cm), *Hypnum reflexum* (6.68 cm), *Fissidens crenulatus* (5.67 cm), *Funaria hygrometrica* (7.38 cm), *Hyophila involuta* (7.66 cm), *Brachymenium turgidum* (7.4 cm), *Bryum coronatum* (7.37 cm) and *Macromitrium sulcatum* (8.5 cm) showed remarkably highly significant results at $P < 0.001$ in the *Brassica juncea* root length except in the aqueous extract of *Steeriophyllum anceps* (3.73 cm) is moderately significant and *Trachypodiopsis blanda* (2.78 cm) were insignificant root length as compared to control.

C. Shoot length:

There is gradual increase the length of shoot in all tested aqueous extract that depicted in Table 27. The shoot lengths of *Allium cepa* seedlings were insignificant effect was much more pronounced in the aqueous extract treatment of *Barbula unguiculata* (5.28 cm), *Hypnum reflexum* (5.57 cm), *Steeriophyllum anceps* (4.68 cm) and *Trachypodiopsis blanda* (5.7 cm). Among these treatments, some extracts were showed highly significant results in shoot lengths growth when compared to control. i. e. *Fissidens crenulatus* (6.27 cm), *Funaria hygrometrica* (7.93 cm) ,*Hyophila involuta* (8.6 cm), *Brachymenium turgidum* (8.31 cm) , *Bryum coronatum* (8.04 cm) and *Macromitrium sulcatum* (7.7 cm) .

The shoot length of *Brassica juncea* seedling was significantly influenced by all aqueous mosses extract treatments. The positive effect was much more pronounced in the extract of *Barbula unguiculata* (7.68 cm), *Hypnum reflexum* (6.29 cm), *Steeriophyllum anceps* (6.62 cm), *Fissidens crenulatus* (5.02 cm), *Trachypodiopsis blanda* (5.36 cm), *Funaria*

hygrometrica (8.85 cm), *Hyophila involuta* (7.57 cm), *Brachymenium turgidum* (9.24 cm), *Bryum cornatum* (8.23 cm), and *Macromitrium sulcatum* (8.45 cm).

4.6.5 Effect of various aqueous moss extracts on germination and seedling growth of vegetable *Solanum*:

The seeds germinated in control and various concentrations of aq. extracts of mosses for five days in petriplates and findings effect of aq. extracts on germinations are of mosses mentioned in Tables 25 and depicted in Fig. 20. The semi-arid region vegetables crop commonly cultivated in the Maharashtra viz. *Solanum lycopersicum L.* for germination bioassay in the present piece of work.

A. Germination percentage:

The result showed that (Table 25) maximum germination was *Solanum lycopersicum* L. observed in aq. moss extracts as compared to control.

It is evident that, the extracts caused minimum growth effect on seeds germination of vegetables crop plants during the initial stages (1st day). After 1st day there is a no seeds germinated in *Solanum lycopersicum* L. but it is also showed that, maximum number of seed germinated in last 4 days. According to (Frahm *et al.,* 2012) reported that, the bryophyte extracts exhibit dual effects on seedling growth and germination depending on the species. It was shown that extract of the liverwort *P. platyphylla* inhibits the growth of radice seedlings, whereas the extract of *B. rutabulum* promotes the growth of radice seedlings

B. Root length:

There is gradual increase the length of root in all tested aqueous extract that depicted in Table 26. The aqueous extract has been remarkably increased length of root than control.

The root lengths of *Solanum lycopersicum* seedlings were incredibly inhibited by some treatment. The insignificant effect was much more pronounced in the aqueous extract treatment of *Barbula unguiculata* (5.04 cm), *Steeriophyllum anceps* (4.68 cm). Among these treatments, some extracts were showed highly significant results in *Funaria hygrometrica* (7.88cm), *Hyophila involuta* (8.41 cm), *Brachymenium turgidum* (8.19 cm), *Bryum coronatum* (7.02 cm) and *Macromitrium sulcatum* (7.3 cm). It is moderately significant in the aqueous

extract of *Hypnum reflexum* (5.04 cm), *Fissidens crenulatus* (5.09 cm) and significant in *Trachypodiopsis blanda* (4.91 cm) as compared to control.

C. Shoot length :

There is gradual increase the length of shoot in all tested aqueous extract that depicted in Table 27. The shoot lengths of *Solanum lycopersicum* seedlings were insignificant effect was much more pronounced in the aqueous extract treatment of *Barbula unguiculata* (4.98 cm), *Steeriophyllum anceps* (4.36 cm) and *Fissidens crenulatus* (4.95 cm). Among these treatments, some extracts were showed highly significant results in shoot lengths growth when compared to control. i. e. *Hypnum reflexum* (5.27 cm), *Trachypodiopsis blanda* (5.24 cm), *Funaria hygrometrica* (7.8 cm), *Hyophila involuta* (8.04 cm), *Brachymenium turgidum* (7.07 cm), *Bryum coronatum* (7.15 cm) and *Macromitrium sulcatum* (7.16 cm).

In our experiment, when studying the effect of aqueous extract of mosses on germination and seedling growth of some cereals, pulses, legumes**,** spices and vegetable for five days clearly indicated that all extracts had promoting effect on germination and seedling growth.

In the present findings effect of aqueous moss extracts have showed that the values are remarkably significant at $P < 0.001$ in the shoot and root length of seedlings of cereals (*Sorghum vulgare* L., *Pennisetum glaucum* L., *Triticum aestivum* L.*),* pulses *(Cajanus cajan* L., *Cicer arientinum* L.), legumes (*Arachis hypogaea* L., *Phaseolus aureus* Roxb.), spices (*Allium cepa, Brassica juncea* L.) and vegetables (*Solanum lycopersicum* L.).

Table 21 : Effect of various aqueous moss extracts on germination of cereals .

Cereals	Germination percentage										
	Control (D.W.)	*B. unguiculata*	*H. reflexum*	*S. anceps*	*F. crenulatus*	*T. blanda*	*F.hygrometrica*	*H. involuta*	*B. turgidum*	*B. coronatum*	*M. sulcatum*
	After 1st day										
Sorghum vulgare	74	78	77	64	73	71	71	76	74	62	74
Pennisetum glaucum	15	20	15	21	15	19	23	15	17	19	17
Triticum aestivum	58	53	90	67	59	83	90	93	86	88	94
	After 2nd day										
Sorghum vulgare	90	82	85	81	84	83	90	80	88	93	88
Pennisetum glaucum	90	92	90	90	92	91	94	94	94	92	93
Triticum aestivum	90	89	94	100	94	95	94	96	91	98	96
	After 3nd day										
Sorghum vulgare	91	97	93	88	93	90	90	88	88	93	92
Pennisetum glaucum	95	98	96	97	97	96	98	95	95	97	95
Triticum aestivum	91	95	97	100	96	97	95	96	94	99	97
	After 4th day										
Sorghum vulgare	95	98	100	89	95	95	91	88	91	96	93
Pennisetum glaucum	95	98	96	97	97	96	98	97	95	99	98
Triticum aestivum	91	95	98	100	96	97	96	97	94	99	97
	After 5th day										
Sorghum vulgare	95	98	100	93	95	95	91	88	91	92	93
Pennisetum glaucum	96	98	100	98	97	99	100	100	96	100	100
Triticum aestivum	91	95	98	100	97	98	96	97	94	99	97

Fig. 16: Effect of various aqueous moss extracts on seeds germination of cereals after 5th day.

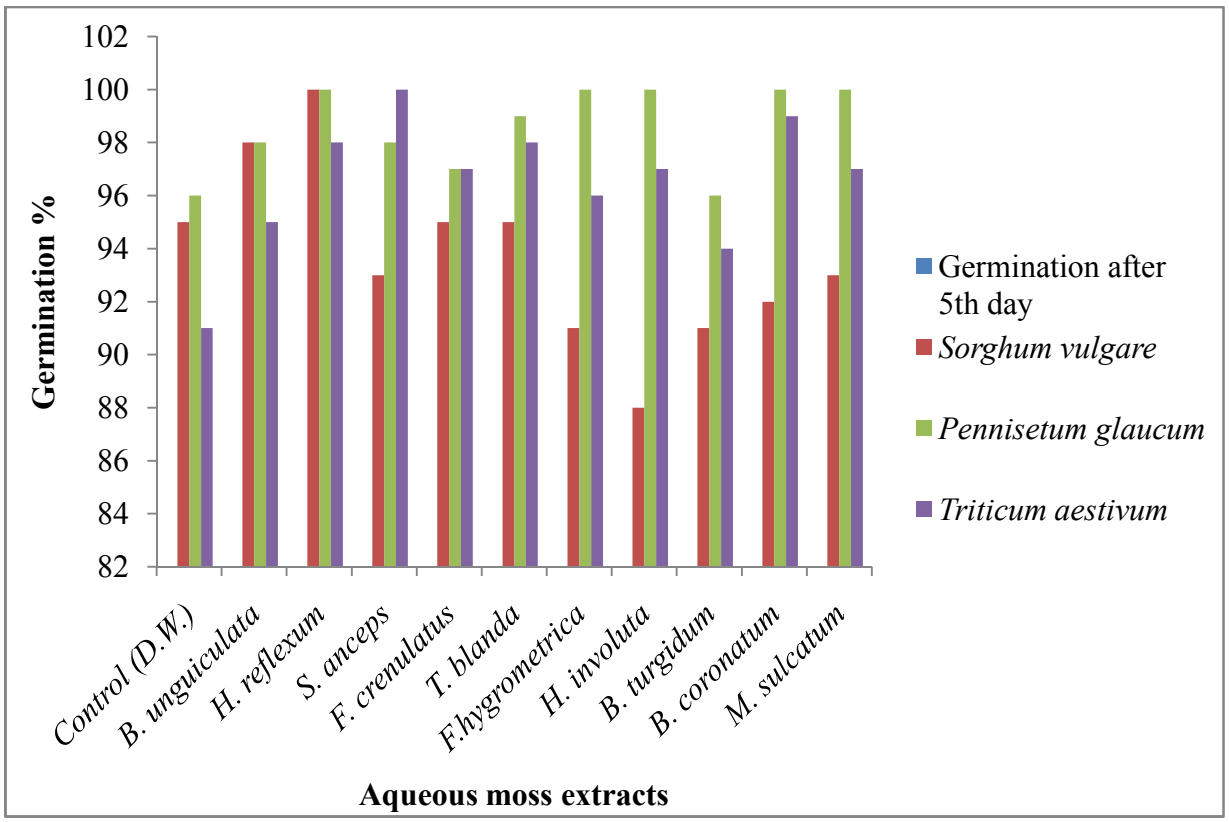


Table 22: Effect of various aqueous moss extracts on germination of pulses:

Pulses	Germination percentage (%)										
	Control (D.W.)	***B. unguiculata***	***H. reflexum***	***S. anceps***	***F. crenulatus***	***T. blanda***	***F.hygrometrica***	***H. involuta***	***B. turgidum***	***B. coronatum***	***M. sulcatum***
	After 1st day										
Cajanus cajan	73	92	83	85	92	81	77	65	70	70	70
Cicer arientinum	75	20	99	64	80	98	76	86	70	100	85
	After 2nd day										
Cajanus cajan	81	93	90	95	93	83	92	85	86	70	70
Cicer arientinum	97	53	100	96	97	99	93	87	86	100	97
	After 3rd day										
Cajanus cajan	88	93	90	95	93	83	97	97	97	98	99
Cicer arientinum	97	64	100	98	98	100	100	100	99	100	97
	After 4th day										
Cajanus cajan	90	100	98	98	100	97	97	97	97	98	99
Cicer arientinum	99	70	100	98	98	100	100	100	99	100	97
	After 5th day										
Cajanus cajan	90	100	98	100	100	99	97	97	97	98	99
Cicer arientinum	99	96	100	98	98	100	100	100	99	100	97

Fig. 17: Effect of various aqueous moss extracts on seeds germination of Pulses after 5th day.

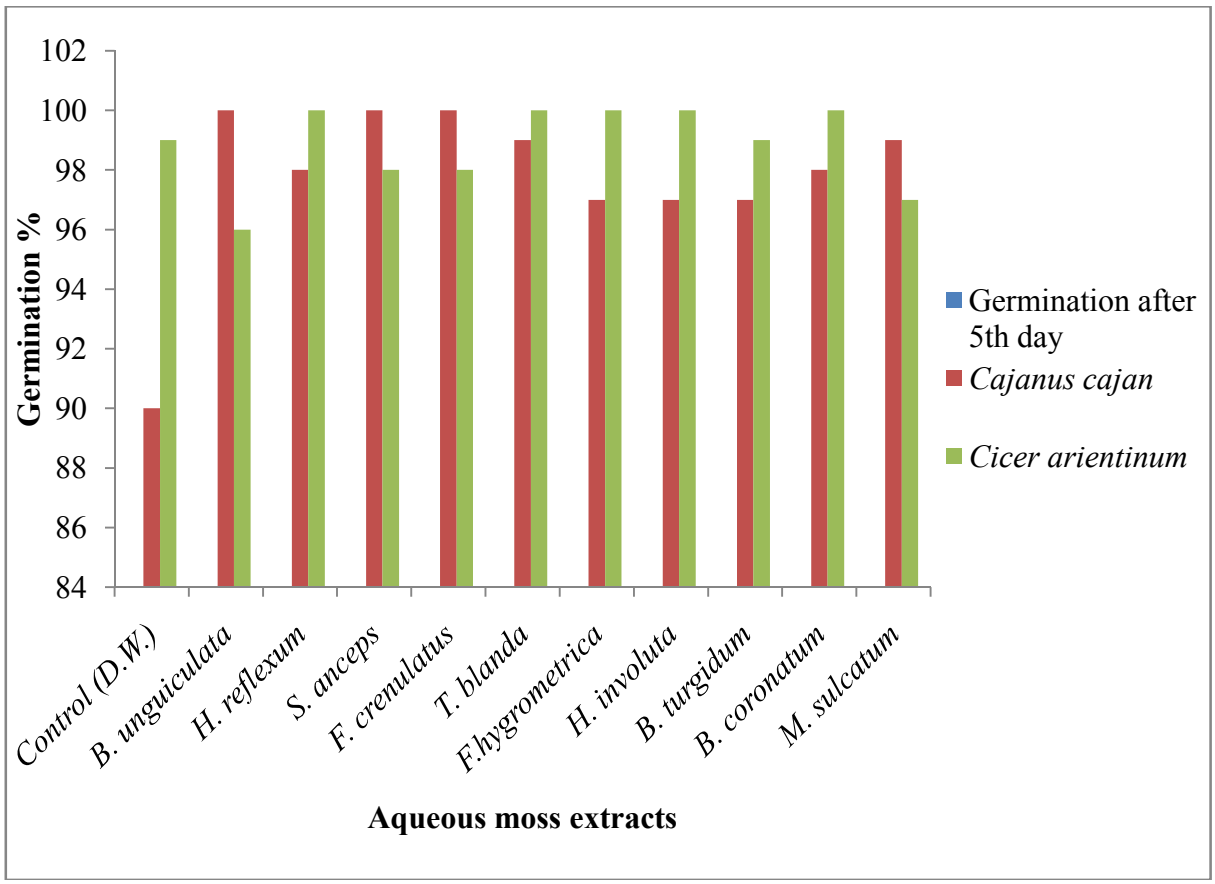


Table 23 : Effect of various aqueous moss extracts on germination of Legumes .

Legumes	Germination percentage (%)										
	Control (D.W.)	***B. unguiculata***	***H. reflexum***	***S. anceps***	***F. crenulatus***	***T. blanda***	***F.hygrometrica***	***H. involuta***	***B. turgidum***	***B. coronatum***	***M. sulcatum***
	After 1st day										
Arachis hypogaea	56	50	60	62	70	57	76	80	80	88	88
Phaseolus aureus	98	98	99	97	100	100	92	90	95	85	90
	After 2nd day										
Arachis hypogaea	71	73	76	74	79	72	94	88	82	88	92
Phaseolus aureus	99	98	99	98	100	100	97	96	99	93	95
	After 3rd day										
Arachis hypogaea	77	82	79	74	89	74	94	96	84	92	94
Phaseolus aureus	99	98	99	98	100	100	98	99	99	97	98
	After 4th day										
Arachis hypogaea	83	86	80	82	89	78	95	96	85	92	94
Phaseolus aureus	99	98	99	99	100	100	99	100	100	99	98
	After 5th day										
Arachis hypogaea	83	98	91	85	89	87	95	96	89	95	95
Phaseolus aureus	99	98	99	100	100	100	99	100	100	99	98

Fig. 18: Effect of various aqueous moss extracts on seeds germination of Legumes after 5th day.

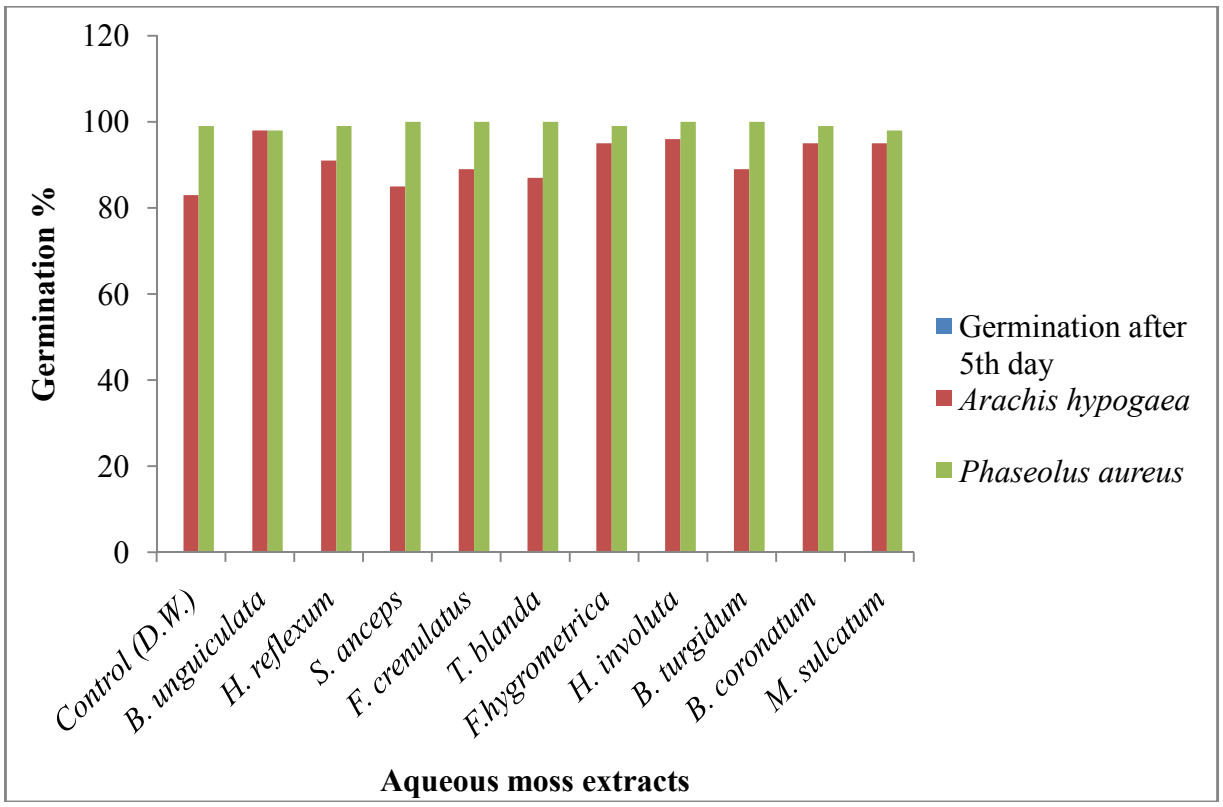


Table 24 : Effect of various aqueous moss extracts on germination of spices .

Spices	Germination percentage										
	Control (D.W.)	***B. unguiculata***	***H. reflexum***	***S. anceps***	***F. crenulatus***	***T. blanda***	***F. hygrometrica***	***H. involuta***	***B. turgidum***	***B. coronatum***	***M. sulcatum***
	After 1st day										
Allium cepa	3	6	10	10	3	9	11	29	18	17	34
Brassica juncea	48	50	38	50	40	42	10	51	28	33	10
	After 2nd day										
Allium cepa	22	15	22	18	39	24	55	67	66	62	60
Brassica juncea	71	78	67	78	72	63	91	90	99	97	99
	After 3rd day										
Allium cepa	34	32	28	29	44	39	75	82	81	76	78
Brassica juncea	80	78	80	80	80	71	99	95	99	98	99
	After 4th day										
Allium cepa	39	48	38	38	52	43	82	92	89	79	90
Brassica juncea	83	81	80	80	84	79	100	100	100	100	100
	After 5th day										
Allium cepa	55	90	57	70	60	80	82	92	89	79	90
Brassica juncea	86	87	87	86	87	83	100	100	100	100	100

Fig. 19: Effect of various aqueous moss extracts on seeds germination of spices after 5th day.

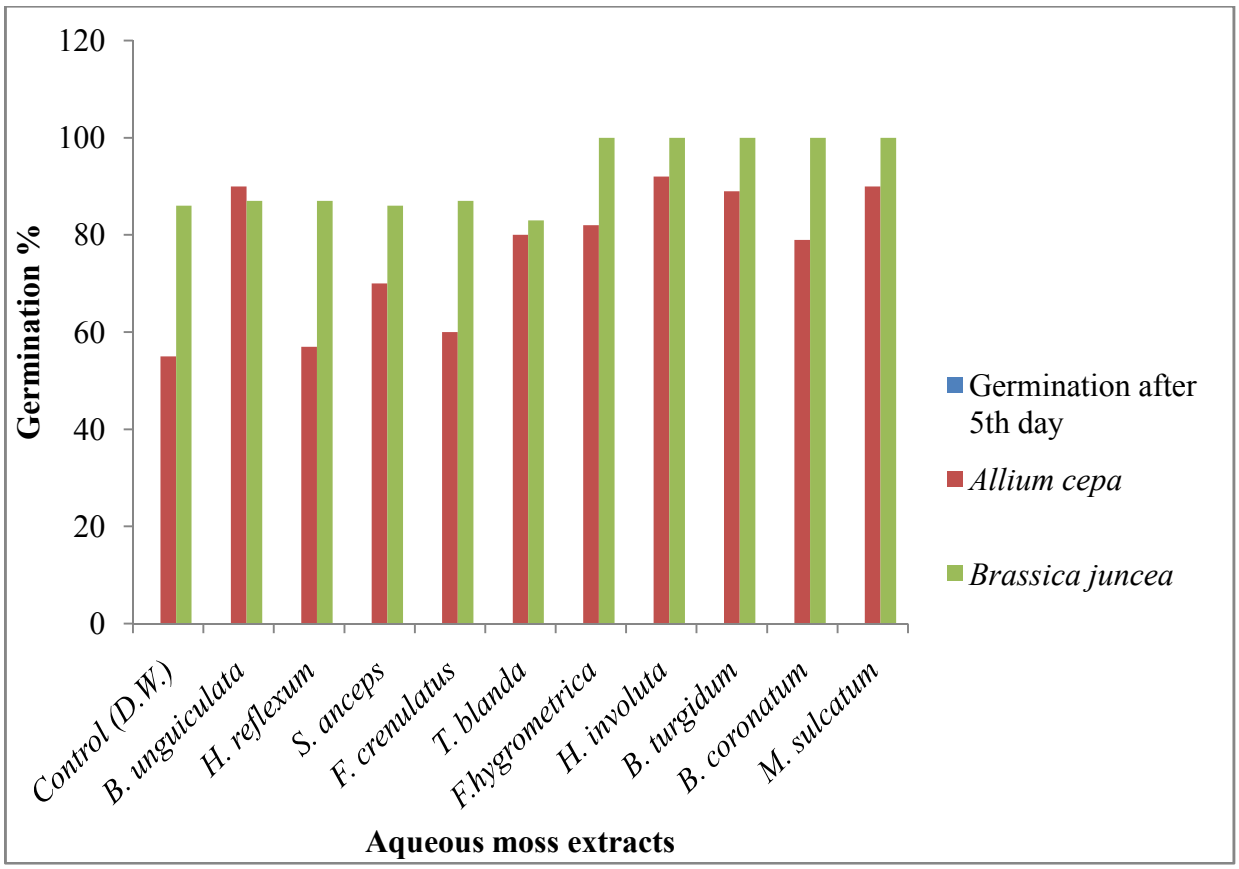


Table 25 : Effect of various aqueous moss extracts on germination of vegetable.

Vegetable	Germination percentage (%)										
	Control (D.W.)	***B. unguiculata***	***H. reflexum***	***S. anceps***	***F. crenulatus***	***T. blanda***	***F. hygrometrica***	***H. involuta***	***B. turgidum***	***B. coronatum***	***M. sulcatum***
	After 1st day										
Solanum lycopersicum	-	-	-	-	-	-	-	-	-	-	-
	After 2nd day										
Solanum lycopersicum	10	08	07	13	09	06	10	09	07	09	24
	After 3nd day										
Solanum lycopersicum	33	30	35	40	39	36	36	27	53	22	40
	After 4th day										
Solanum lycopersicum	83	84	88	90	95	70	41	79	82	88	79
	After 5th day										
Solanum lycopersicum	91	90	90	95	97	88	94	90	95	95	97

Fig. 20: Effect of various aqueous moss extracts on seeds germination of vegetable after 5th day.

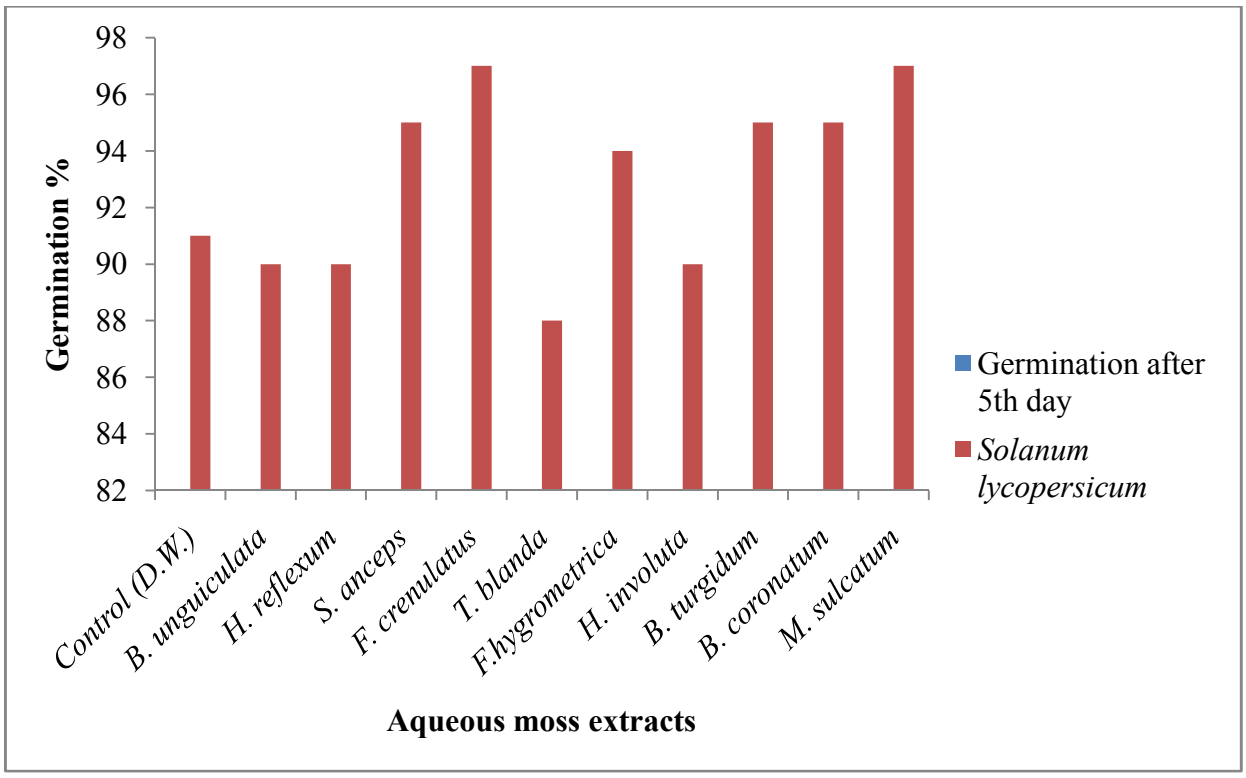


Table No. 26: Effect of various aqueous moss extracts on root length of 5 days old seedlings (Mean):

Time after seedling growth	Crop plant		root length (cm)										
			Control (D.W.)	*B. unguiculata*	*H. reflexum*	*S. anceps*	*F. crenulatus*	*T. blanda*	*F.hygrometrica*	*H. involuta*	*B. turgidum*	*B. cornatum*	*M. sulcatum*
5 days	Cereals	*Sorghum vulgare*	6.93 ± 1.92	8.2 ± 1.94 Ns	8.99 ± 2.64 Ns	8.51 ± 3.56 Ns	9.95 ± 1.87 ***	7.57 ± 3.70 Ns	7.12 ± 1.36 Ns	7.1 ± 1.67 Ns	7.15 ± 1.37 Ns	8.25 ± 0.95 Ns	7.9 ± 1.31 Ns
		Pennisetum glaucum	8.37 ± 0.85	13.14 ± 0.86 ***	9.61 ± 2.22 Ns	11.5 ± 1.34 ***	9.9 ± 1.11 ***	14.27 ± 0.64 ***	11.34 ± 1.35 ***	12.09 ± 1.94 ***	10.04 ± 2.30 ***	12.76 ± 1.75 ***	10.79 ± 2.51 ***
		Triticum aestivum	11.28 ± 1.37	12.15 ± 2.0 Ns	12.1 ± 1.76 Ns	14.57 ± 3.2 ***	13.27 ± 2.7 ***	11.69 ± 2.9 Ns	12.0 ± 0.42 Ns	12.01 ± 2.0 Ns	13.95 ± 1.8 ***	13.65 ± 1.78 ***	12.53 ± 1.61 Ns
	Pulses	*Cajanus cajan*	3.45 ± 0.42	4.87 ± 2.06 **	4.51 ± 0.86 ***	4.45 ± 0.91 ***	5.09 ± 1.09 ***	5.41 ± 1.82 ***	4.68 ± 0.74 ***	4.87 ± 0.61 ***	5.24 ± 0.22 ***	5.16 ± 0.62 ***	5.17 ± 0.44 ***
		Cicer arientinum	5.99 ± 1.84	7.17 ± 1.15 Ns	11.8 ± 4.81 ***	7.3 ± 1.60 Ns	9.53 ± 2.87 ***	8.63 ± 2.70 **	8.85 ± 1.24 ***	9.7 ± 1.32 ***	8.98 ± 0.87 ***	10.7 ± 1.70 ***	9.78 ± 1.30 ***
	Legumes	*Arachis hypogaea*	2.12 ± 0.24	3.19 ± 0.28 ***	2.31 ± 0.4 2 Ns	3.79 ± 0.84 ***	3.92 ± 0.67 ***	3.46 ± 0.72 ***	5.61 ± 0.63 ***	5.48 ± 0.79 ***	5.72 ± 0.86 ***	5.65 ± 1.15 ***	4.83 ± 0.84 ***
		Phaseolus aureus	3.88 ± 1.19	4.69 ± 1.96 Ns	5.11 ± 2.31 Ns	6.86 ± 1.84 ***	6.04 ± 1.72 ***	5.68 ± 2.41 *	5.98 ± 0.87 ***	7.25 ± 1.27 ***	7.7 ± 2.18 ***	7.47 ± 0.84 ***	7.51 ± 1.53 ***
	Spices	*Allium cepa*	1.51 ± 0.58	1.68 ± 0.41 Ns	1.65 ± 0.67 Ns	1.91 ± 0.59 Ns	2.45 ± 0.66 ***	2.23 ± 1.01 Ns	4.37 ± 0.59 ***	4.52 ± 0.12 ***	3.85 ± 0.41 ***	4.21 ± 0.42 ***	3.8 ± 0.35 ***
		Brassica juncea	1.87 ± 0.95	3.91 ± 2.36 ***	6.68 ± 2.77 ***	3.73 ± 2.02 **	5.67 ± 2.77 ***	2.78 ± 1.56 Ns	7.38 ± 1.06 ***	7.66 ± 1.48 ***	7.4 ± 1.35 ***	7.37 ± 1.60 ***	8.5 ± 1.87 ***
	Vegetables	*Solanum lycopersicum*	3.58 ± 1.62	5.04 ± 1.82 Ns	5.42 ± 1.33 **	4.68 ± 0.66 Ns	5.09 ± 1.32 **	4.91 ± 1.12 *	7.88 ± 0.89 ***	8.41 ± 0.85 ***	8.19 ± 0.73 ***	7.02 ± 1.24 ***	7.3 ± 0.78 ***

Values are mean of ten replications

Two sample test (t- test) : Ns - Non significant at $p > 0.05$ * Significant at $p < 0.05$, ** Moderately significant at $p < 0.05 > 0.01$, *** Highly significant at $p < 0.001$ when compared to control.

Table 27: Effect of various aqueous moss extracts on shoot length of 5 days old seedlings (Mean).

Time after seedling growth	Crop plant		Shoot length (cm)										
			Control (D.W.)	*B. unguiculata*	*H. reflexum*	*S. anceps*	*F. crenulatus*	*T. blanda*	*F.hygrometrica*	*H. involuta*	*B. turgidum*	*B. cornatum*	*M. sulcatum*
5 days	Cereals	*Sorghum vulgare*	5.85 ± 1.00	8.7 ± 1.74 ***	8.7 ± 2.94 ***	8.3 ± 2.57 ***	11.35 ± 2.49 ***	8.3 ± 1.30 ***	6.4 ± 1.56 Ns	6.1 ± 0.8 Ns	6.65 ± 0.92 Ns	6.4 ± 0.8 Ns	6.4 ± 1.15 Ns
		Pennisetum glaucum	6.5 ± 1.02	8.26 ± 1.20 ***	8.5 ± 1.37 ***	7.71 ± 1.58 Ns	8.54 ± 0.90 ***	9.8 ± 1.50 ***	7.43 ± 1.11 Ns	8.43 ± 1.33 ***	7.88 ± 1.59 **	7.96 ± 1.79 **	6.78 ± 1.14 Ns
		Triticum aestivum	7. 58 ±1.02	8.46 ± 1.42 Ns	9.54 ± 1.57 ***	9.35 ± 1.75 ***	8.11 ± 0.86 Ns	7.5 ± 1. 30 Ns	12.17 ± 0.56 ***	12.12 ± 1.10 ***	12.45 ± 0.75 ***	12.5 ± 0.92 ***	12.38 ± 0.91 ***
	Pulses	*Cajanus cajan*	2.59 ± 0.52	4.57 ± 0.80 ***	4.54 ± 1.02 ***	4.77 ± 0.79 ***	4.72 ± 1.24 ***	3.49 ± 0.61 ***	2.80 ± 0.40 Ns	2.41 ± 0.29 Ns	2.81 ± 0.30 Ns	2.73 ± 0.41 Ns	2.71 ± 0.4 Ns
		Cicer arientinum	2.28 ± 0.20	3.36 ± 0.99 ***	5.5 ± 3.15 ***	3.4 ± 0.86 ***	2.33 ± 1.57 Ns	2.72 ± 1.12 Ns	4.0 ± 0.59 ***	3.44 ± 0.71 ***	3.08 ± 0.66 ***	3.38 ± 0.65 ***	4.57 ± 0.84 ***
	Legumes	*Arachis hypogaea*	1.62 ± 0.19	2.17 ± 0.14 ***	1.92 ± 0.35 **	2.1 ± 0.20 ***	2.19 ± 0.14 ***	2.28 ± 0.27 ***	2.27 ± 0.21 ***	2.28 ± 0.23 ***	2.45 ± 0.10 ***	2.5 ± 0.17 ***	2.4 ± 0.07 ***
		Phaseolus aureus	5.29 ± 0.95	9.15 ± 1.59 ***	5.93 ± 1.87 Ns	6.77 ± 1.09 ***	8.64 ± 3.38 ***	5.06 ± 1.17 Ns	7.51 ± 1.31 ***	8.05 ± 1.19 ***	8.14 ± 1.51 ***	7.22 ± 0.97 ***	6.35 ± 0.86 ***
	Spices	*Allium cepa*	4.72 ± 0.48	5.28 ± 1.03 Ns	5.57 ± 1.71 Ns	4.68 ± 0.85 Ns	6.27 ± 1.39 ***	5.7 ± 1.84 Ns	7.93 ± 0.40 ***	8.6 ± 0.14 ***	8.31 ± 0.42 ***	8.04 ± 0.77 ***	7.7 ± 0.26 ***
		Brassica juncea	4.33 ± 0.47	7.68 ± 1.52 ***	6.29 ± 1.70 ***	6.62 ± 0.50 ***	5.02 ± 1.21 ***	5.36 ± 0.88 ***	8.85 ± 1.23 ***	7.57 ± 1.02 ***	9.24 ± 1.08 ***	8.23 ± 1.12 ***	8.45 ± 1.38 ***
	Vegetables	*Solanum lycopersicum*	4.56 ± 2.17	4.98 ± 1.05 Ns	5.27 ± 0.75 **	4.36 ± 0.73 Ns	4.95 ± 1.23 Ns	5.24 ± 1.29 **	7.8 ± 0.86 ***	8.04 ± 1.06 ***	7.07 ± 0.51 ***	7.15 ± 1.29 ***	7.16 ± 1.01 ***

Values are mean of ten replications

Two sample test (t- test): Ns - Non significant at $p > 0.05$ * Significant at $p < 0.05$, ** Moderately significant at $p < 0.05 > 0.01$, *** Highly significant at $p < 0.001$ when compared to control.

CHAPTER V

SUMMARY AND CONCLUSIONS

5. Summary and conclusions:

The material and method adapted for present work includes survey of moss species at different altitudinal areas of Maharashtra. The collection, storage and identification of moss species was done during the period from July 2015 to October 2018.

The collection of mosses was done from different 14 localities of Western Ghats namely Khandala, Lonawala, Lohagad, Tamhini ghat, Lavasa, Sinhagad, Purandar, Raireshwar, Dhom Dam (Wai), Pratapgad, Mahabaleshwar, Kaas Plateau, Aundh (Satara), and Koynanagar from Maharashtra states. After collection specimens were rinsed, cleaned and studied taxonomical characters for its identification. Some healthy, fully growth samples dried thoroughly and then stored in paper bags at room temperature. Identification of the specimens was done by referring authentic literatures and also by consultation with experts. The specimens were further confirmed by Western Circle, Botanical Survey of India, Pune. About 19 species of mosses collected from the different localities and among these 10 dominant species are selected for study of further analysis. The soil adjacent to moss species was also collected, cleaned and maintained separately in sterile environment.

The dominant moss species selected for physiological study are:

1. *Fissidens crenulatus* Mitt.
2. *Brachymenium turgidum* Broth.
3. *Bryum coronatum* Schwaegr
4. *Funaria hygrometrica* Hedw.
5. *Hypnum reflexum* F. E. Tripp.
6. *Macromitrium sulcatum* Brid.
7. *Hyophila involuta* (Hook.) Jaeg.
8. *Barbula unguiculata* Hedw.
9. *Trachypodiopsis blanda* (Mitt.) Fleisch.
10. *Steeriophyllum anceps* (Bosch et. Lac.) Broth.

After identification and confirmation the soil samples were studied for their physical properties viz. pH, electrical conductivity and mineral content analysis of rhizoplane soil.

Further, these selected mosses were subjected for study of physiological and biochemical parameters like total chlorophylls, carotenoids, total soluble proteins, free amino acids, free proline contents. total reducing sugars, starch and total carbohydrates, inorganic nutrients i.e. micro and micronutrients have been studied. In order to know the status of secondary metabolites i.e. total polyphenols and tannins have been investigated our work extended further for study of the antioxidant properties in the species through study of enzymes like catalase, peroxidase and polyphenol-oxidase. The enzymes of nitrogen metabolism namely nitrate and nitrite reductase has also been investigated for understanding their status in moss species.

Germination studies of semi-arid crops under influence of moss extract has also been carried out. The effect of aqueous extracts from these mosses on germination and seedling growth has been studied up to 5 days of some cereals (*Sorghum vulgare* L.*, Pennisetum glaucum* L.*, Triticum aestivum* L.*),* pulses *(Cajanus cajan* L., *Cicer arientinum* L.), legumes (*Arachis hypogaea* L., *Phaseolus aureus* Roxb.), spices (*Allium cepa, Brassica juncea* L.) and vegetables (*Solanum lycopersicum* L.) .

The significant findings of the present work are summarized as follows.

1. The mosses grow on different habitats such as bark of trees, plastered and un-plastered house walls, blocks, rock surfaces and sand soil. There are two terrestrial species *Hyophila involuta* (Hook) Jaeg. and *Bryum coronatum* Schwaegr. which grow on solid substratum and holsters of soil. Six epiphytic species such as *Hypnum reflexum* F. E. Tripp., *Steeriophyllum anceps* (Bosch et. Lac.), *Fissidens crenulatus* Mitt. Broth, *Trachypodiopsis blanda* (Mitt.) Fleisch., *Brachymenium turgidum* Broth. and *Macromitrium sulcatum* Brid. occur on portion of bark or trees. Two lithophytic species *Barbula unguiculata* Hedw. and *Funaria hygrometrica* Hedw. grow on basalt and latirus rocks.
2. The geological parameters were observed in 14 localities of Maharashtra from where these mosses were collected showed that these mosses grow at higher altitude, rainfall and humidity conditions .The altitude ranged from 550 m to 1,438 m. The average annual rainfall varied from 534 mm to 6498 mm. Averages humidity varied from 30 to 78.5 %. The autecological analysis clearly visualizes that distribution of mosses at different altitude is governed by rainfall and humidity.

3. Rhizosphere soil analysis showed that three mosses namely *Steeriophyllum anceps*, *Macromitrium sulcatum* and *Barbula unguiculata* grow in saline condition. Also, low EC values are indicating of less free ion availability and poor soil condition.
4. In present study, it was found that the rhizosphere soil of these mosses was rich in inorganic matter with high amount of available total Nitrogen, Phosphorus, Potassium and Organic carbon for this may be helpful for favourable growth of mosses.
5. The inorganic nutrients were investigated in these mosses .overall major and minor nutrients like Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, and Iron, Manganese Copper, Zinc content showed high values in mosses. The values of nutrient elements studied in moss species show a common trend indicating no specialized metabolic structure in these primary land plants.
6. The accumulation of macronutrient nutrients varies from species to species. Ca content was comparatively higher in all studied moss species. Accumulation of macronutrients in moss species showed a general trend of decrease Ca > Mg > N > K > P.
7. High value of Nitrogen was recorded in *Hyophila involuta* indicating high metabolic activity needed for the enzymatic reactions and in protein metabolism.
8. Highest Phosphorus content is recorded in *Trachypodiopsis blanda* this gives clear idea of receiving adequate quantity of P from soil and thereby maintaining its normal metabolic activity.
9. Accumulation of more Potassium in *Macromitrium sulcatum* may help in transport of solutes maintaining osmotic potential.
10. Record of higher Calcium content in all mosses indicates regulation of water flow across cell membranes thereby also helping in stomatal movement.
11. Magnesium content is high in *Funaria hygrometrica* Hedw. The Mg content in shoot helps in K translocation.
12. The accumulation of micronutrient nutrients varies from species to species. Fe content was comparatively higher in all moss species studied. Accumulation of micronutrients accumulation general trend was Fe > Mn > Cu > Zn.
13. High Iron content is recorded in *Hyophila involuta*. It is an important element in oxido-reduction reactions in plants.

14. The maximum amount of Chl- b than the Chl- a was reported in studied mosses. The contrast result were reported in higher plants in case synthesis of Chl- a and Chl- b pigments.
15. The carotenoids content of these mosses was in between 0.04 - 0.15 mg/g fresh wt. Carotenoids are associated with chlorophylls and are required for photosynthesis. Chlorophylls capture the light energy and are the primary electron donors. Carotenoids are essential components of the photosynthetic apparatus, where they also protect chlorophyll molecules against photo-oxidation.
16. In present study, enzymatic performance of oxidases like Catalase, Peroxidase and Polyphenol oxidases showed highest activity. It seems in water scarcity of moss species show increase in activity of oxidase enzymes like Catalase, Peroxidase, and Polyphenol oxidase. This indicates the accumulation of reactive oxygen species and protection against drought indicating high drought tolerance mechanism in this moss species
17. The six enzymes tested with regards to their specific activity showed a response of decrease sequential order in all the studied mosses. The studied enzymes activity contains general trends of decrease as catalase > peroxidase > polyphenol oxidase > nitrate reductase > nitrite reductase
18. High levels of peroxidase enzyme in *Barbula unguiculata* where it is 20.26 Δ OD. min $^{-1}$ g^{-1} indicate its active role in this moss and this can be attributed to effective defence mechanism in drought conditions.
19. Higher Catalase activity has been reported in *Hypnum reflexum* in present findings. As catalase is a predominant enzyme is scavenging reactive oxygen species (ROS) and acting as strong antioxidant, its high activity in hot arid xeric condition is quite justifying. Also, its occurrence in mosses indicates the general oxidative role as oxidation of H_2O_2 generated during catalytic activity of other metabolites.
20. In present investigation, high activity of polyphenol oxidase was observed in *Barbula unguiculata*. It may be towards plant defense mechanism against pests and diseases, regulating phenolic compounds and alkaloids.
21. The nitrate reductase (NR) activity is considerably high in mosses. This active co-ordination the reductive assimilation of inorganic carbon and nitrogen.

22. Also Nitrite reductase (NIR) activity is higher in mosses. As NIR activity is higher than NR, it reflects the efficiency of reduction of NO_2 to NH_4 thereby regulating nitrogen metabolism and also responsible for synthesis of secondary metabolites.
23. The reducing sugars and starch content showed lower values as compared to those of higher plants. The total carbohydrates were much higher than those of reducing sugars and starch in all the mosses. The carbohydrates produced in photosynthesis are well known for their essential role as vital sources of energy and to provide carbon skeleton for synthesis of other organic compounds including storage components.
24. The maximum amount of proteins in two species namely *Fissidens crenulatus* and *Trachypodiopsis blanda* (7.6 mg/g) observed as compared to remaining mosses. It provides important role in structural developments and enzymatic activity of mosses.
25. The amount of free amino acids was higher in *Funaria hygrometrica*, followed by *Steeriophyllum anceps* and *Trachypodiopsis blanda*. The free amino acid play an important role in leveraging secondary metabolites protecting the photosynthetic apparatus against the destructive effects of light and ROS.
26. *Brachymenium turgidum* showed highest amount of fee proline. The range value of proline is from (3.5 -9.4 mg/ g dry tissue) which suggesting more drought tolerant capability than other mosses studied.
27. The secondary metabolites, including total polyphenol and tannin content high in mosses. Which is provides defense of moss species against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses.
28. In our experiment, when studying the effect of aqueous extract of mosses on germination and seedling growth of some cereals, pulses, legumes, spices and vegetables for five days clearly indicated that all extracts had promoting effect on germination and seedling growth.
29. In the present findings effect of aqueous moss extracts have showed that the values are remarkably significant at $P < 0.001$ in the shoot and root length of seedlings of cereals (*Sorghum vulgare* L., *Pennisetum glaucum* L., *Triticum aestivum* L.*),* pulses *(Cajanus cajan* L., *Cicer arientinum* L.), legumes (*Arachis hypogaea* L., *Phaseolus aureus* Roxb.), spices (*Allium cepa, Brassica juncea* L.) and vegetables (*Solanum lycopersicum* L.).

The present research is based on occurrence and taxonomy of mosses at different localities differing in altitude, rainfall, humidity and substratum. The occurrence and

dominance of the species is associated with its autecological parameters as showed in findings of rhizosphere analysis. Each species under study has also shown a remarkable composition of inorganic nutrients, organic compounds and also activities related to photosynthesis, nitrogen metabolism, carbohydrates metabolism, secondary metabolites and oxidative enzyme profile. Even though these species are restricted to specific altitude, they express a unique profile of organic compounds to survive and establish in given environment.

As these species are specialized in their organic and inorganic profile and also expressing medical properties, it was worth to record the effect of these mosses in the form of aqueous extracts on a important activity towards growth and development of higher plants that is germination of seedling growth of some important crop plants of semi-arid region.

Our findings of effect on germination and seedling growth are quite promising and show positive promontory effects. In today's world of polluted environment, these mosses can be a promising organic growth promoter for various crops at early stage the bioremediation of these mosses can be an important aspect of further study.

Thus, the present work is important from various prospectives and throws light on several aspects still unknown to society. So we feel that this present work based on original research will add new information towards identification and taxonomy of these mosses and also their influence on sustainability of crop plants under stress conditions.

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