

6. Format for submission of final report of completed project

(Report must be for complete duration of the project)

- Title: ***Establishment of Advance Institutional Level Biotech Hub (Phase-II) at Dhing College, Nagaon (Assam)***
- 1. Project Investigators and address: **Dr. Manoj Kumar Saikia PI (Retd) , Dr. Sanjeeb Kumar Nath CO-PI (PI- In charge), Dr Ujjayanta Das CO-PI , Dhing College, Nagaon (Assam)**
- 2. Date of sanction : **01/02/2023**
- 3. Date of completion: **30/04/2026**
- 4. Approved cost: **5784720.00**
- 5. Budget released: **21,10,960.00 [First Year Grant]**
- 6. Objectives (including targets set at the starting of the projects):
 - ❑ **PRIMARY OBJECTIVES [Capacity building programme]**
 - To strengthen the laboratory infrastructure of the hub for achieving excellence in teaching and training.
 - To enhance the quality of the learning through ‘Hands On” exposure to experimental work and participation in Summer Schools/ Projects.
 - To promote networking and strengthen ties with neighbouring institutions and other Biotech hub laboratories.
 - To conduct specialized training programmes/Lecture programme /Outreach/awareness/STC for the Students, faculty & teachers to improve their skills.
 - To provide access and exposure to students to R& D activities of the hub/ laboratories and industries in the NER region of India.
 - To help in devising standard Operating Procedures (SOP’s)/kit’s for practicals.
 - To conduct community programme their by linking with societal people/NGOetc.
 - ❑ **SPECIFIC OBJECTIVES [R&D Component]:**
 - **Title: In vitro antimicrobial & antagonistic activity of Endolichenic Fungus (ELF) against pathogenic bacteria & fungus and evaluation of their bioactive compound using Thin Layer Chromatography (TLC) & HPLC”**
 - i. *“Isolation, Identification & In-vitro evaluation of antimicrobial& antagonistic potentiality of Endolichenic fungus and their efficiency against some clinically significant human and plant pathogen.*
 - ii. Isolation of bioactive compounds from Lichen and their identification.

7. Detailed methodologies (full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs):

[Work plan (methodology/experimental design to accomplish the stated aim)]

- **Field Survey & Preparation of maps for Sample sites and Collection of Lichens:** The lichen specimens of the present study were collected from twigs of bark and stem from 5 (five) different sample station/location of Nagaon district of Assam. The healthy looking thalli were cut out from the tree bark with a sterile knife and placed into sterile plastic collection bags. The collected samples were brought to the laboratory in sterile polythene bags and processed within 24 hours of collection. The identification was done using standard key (*Awasthi, 1988, 1991, 2000a*). The species preserved in college laboratory for further studies.
- **Isolation and identification of endolichenic fungi:** The isolation procedure of endolichenic fungi was carried out following the method prescribed by *Liet al.(2007)*. Lichen thalli was washed thoroughly with Milli-Q water to remove dirt or debris from surfaces and then surface sterilized by immersing sequentially in 10% of 30% hydrogen peroxide (H_2O_2) solution for 4–5 minutes followed by rinsing with 70% alcohol for 5 seconds and 1% of 4% sodium hypochlorite ($NaOCl$) for 6 minutes and washed with sterile distilled water. The lichen thalli are then dried by placing over sterile filter papers and was dissected aseptically to a fragment of $1mm^2$ size. Each fragment was then inoculated onto Potato Dextrose Agar (PDA) medium. The plates were sealed with parafilm and incubated in BOD incubator at $30^\circ C$ until growth of endolichenic fungi. The plates were observed once a day for fungal growth. After several days of incubation the colonies growing out of surface sterilized fragments were isolated, sub-cultured and maintained at $4^\circ C$ in PDA slants. The isolates were then identified based on their morphological and reproductive characters using the standard identification manuals given by *Barnett Hunter (1998)&Gilman (1971)*. The cultures that failed to sporulate were categorized as sterile mycelia.

- **Screening of for antimicrobial /MIC activity:** Pure cultures of endolichenic fungi were cultivated on Potato Dextrose Broth (PDB) by placing agar blocks of actively growing culture (3mm in diameter) in 250ml Erlenmeyer flasks containing 100 ml of the medium. The flasks will be incubated in BOD shaking incubator for 14 days at $29 \pm 1^\circ\text{C}$ with periodic shaking at 150rpm. The cultures were then filtered through sterile cheese cloth to remove the mycelia mats. The liquid broth was collected and extracted with equal volume of acetate in a separating funnel by vigorous shaking for 15min. The cell masses were separated, and the ethyl acetate extracts were collected for each isolate. Ethyl acetate was evaporated, and the resultant compounds were allowed to be dried individually with magnesium sulfate (MgSO_4) and concentrated to yield the crude extracts. The crude extracts were then dissolved in 15% Dimethyl sulfoxide (DMSO) for anti microbial bioassay. Antimicrobial activities of the crude metabolites isolated from the endolichenic fungi were determined by agar cup diffusion method against two bacterial pathogens and two fungal pathogens obtained from KVV Kendra, AAU, Nagaon (Assam). Muller Hinton Agar (MHA) plates were inoculated with overnight culture of each bacterial suspension. Similarly for the fungal pathogens, Sabourad Dextrose Agar (SDA) plates were used for each fungal suspension. The plates with the inoculated organisms were evenly spread out with sterile cotton swabs. Agar cups were prepared by scooping out the media with a sterile cork borer (7mm diameter). The cups were then filled with 100 μl of the crude metabolites that were dissolved in DMSO to get a concentration of 1mg/ml. The plates were then incubated at $36 \pm 1^\circ\text{C}$ for 24h and 48h for bacterial and fungal pathogens respectively. Antimicrobial activity was determined as growth inhibition of the target organism around agar cup as appearance of clear zones. The Optical Density (OD_{600}) of crude lichenoid extract upon test pathogen was also conducted using UV-VIS spectrophotometer to determine the % inhibition on two bacterial and two fungal pathogens. The results were expressed in terms of growth of organisms (+) or inhibition of growth (-). The lowest concentration inhibiting fungal growth was noted as MIC (Rath et al., 1999).

8. Detail analysis of the Results with good quality images:

The findings of this study offer valuable insights into the lichen diversity and endolichenic fungal community in the Nagaon district of Assam. The collection of 29 lichen species across five distinct areas demonstrates the region's rich lichen flora and the associated endolichenic fungal community. Through meticulous collection efforts across five distinct areas (**Map 1&2**) namely *Dhing*, *Kothiatoli*, *Nonoi*, *Podumoni*, and *Chapanala* – a total of 29 lichen species were identified, with *crustose* species comprising 60% and *foliose* species 40% of the total (**Table-2, Photo plate 2-7**). Notably, a prevalence of *corticolous* lichen was observed within the sampled regions. Expert identification conducted at lichenology laboratories at Nowgong University, Nagaon, and the ICFRE-Rain Forest Research Institute, Jorhat, Assam, facilitated accurate categorization of specimens, with *Parmotrema* being the predominant genus among foliose lichens.

Following collection, surface sterilization techniques (**Photo plate-8**) were employed to isolate sporulated endolichens, leading to the successful isolation and storage of pure colonies for further analysis. Biochemical and growth pattern evaluations of the endolichens revealed consistent positive delayed *catalase* and *oxidase* activity across all endolichenic fungi (ELFs). To enhance understanding and distinction among various lichen associated ELFs, ongoing efforts include the implementation of additional biochemical and MIC test. These endeavors aim to deepen our comprehension of the diverse endolichenic fungal community within the region, paving the way for potential applications in ecological research and biotechnology. The findings of this study offer also provide us an valuable insights into the lichen diversity and endolichenic fungal community in the Nagaon district of Assam. The collection of 29 lichen species across five distinct areas demonstrates the region's rich lichen flora. The predominance of crustose species, comprising 60% of the total, suggests a preference for substrates conducive to their growth, while the presence of foliose species, particularly those belonging to the *Parmotrema* genus, highlights

the diversity within this group. The identification of lichen specimens was facilitated by collaboration with expert lichenology laboratories at Nowgong University, Nagaon , and the ICFRE- Rain Forest Research Institute, Jorhat, Assam. This ensured accurate taxonomic classification and laid the foundation for further investigations into the ecological roles and potential applications of these lichen. The successful isolation and storage of pure colonies of sporulated endolichens signify a crucial step towards understanding the endolichenic fungal community associated with these lichens. The consistent positive delayed catalase and oxidase activity exhibited by all endolichenic fungi (ELFs) suggest metabolic activity indicative of potential symbiotic or parasitic relationships with their lichen hosts. These findings corroborate previous studies highlighting the metabolic diversity of endolichenic fungi and their potential ecological significance. The planned implementation of additional biochemical and molecular tests aims to elucidate the diversity and genetic makeup of the endolichenic fungal community further. These tests will provide valuable insights into the functional roles of ELFs within lichen symbiotic associations and their potential biotechnological applications. Overall, this study contributes to our understanding of lichen diversity and endolichenic fungal communities in the Nagaon district of Assam. The data generated pave the way for future research endeavors aimed at exploring the ecological, evolutionary, and biotechnological significance of these fascinating organisms in natural ecosystems. The study reveals rich lichen diversity with *Parmotrema* and *Pyxine* being dominant genera, thriving due to favourable climatic conditions. Most samples were collected from rural areas having favourable annual rainfall, temperature and humidity which favours the growth of others lichen species namely *Corticolous*, *Saxicolous* and *Lignicolous* lichens in this locality. In the present study the Simson's Diversity Index (**Table- 4**) of Lichens species from 5(five)sample sites of Nagaon District was calculated as- $D=0.87547$ which signify the high diversity of lichen species . The study reflects both richness and evenness, a few dominant species lead to low diversity, while many species with similar number lead to high diversity.

The interpretation of the value of Simpson's indices was calculated. The value 0.87547 is likely from the most commonly used diversity index (1-D), as this range makes intuitive sense. If the value is for Simpson's Diversity Index (Gini-Simpson Index, or 1-D): The index ranges from 0 to 1. A value of 0.87547 in the present study is close to 1, which signifies a high degree of diversity (many different species with a relatively even distribution of individuals among those species).

A value between approximately 0.7 and 1.0 is often considered high diversity. If the value is for Simpson's Index (D, or Dominance Index): The index also ranges from 0 to 1, but the interpretation is counterintuitive: higher values indicate *lower* diversity (or higher dominance by one or two species). In this case, 0.87547 would suggest very low diversity/high dominance (a value > 0.7 is typically very low diversity). The high diversity index indicates a robust ecosystem, suggesting potential for discovering n Further the antimicrobial activity of the lichen extracts was evaluated against two bacterial pathogens, *Ralstonia solanacearum* (MTCC 13168) and *Pseudomonas syringae* (MTCC 673), and two fungal pathogens, *Fusarium oxysporum* (MTCC 12994) and *Alternaria alternata* (MTCC 13407), using agar well diffusion assay. The extracts prepared using *acetone*, *methanol*, *petroleum ether* and *diethyl ether* exhibited varying degrees of antimicrobial activity against the tested pathogens (**Table-5, Photoplate-1& Figure-1**). Among the tested microorganisms, the bacterial pathogens showed greater susceptibility to the lichen extracts than the fungal pathogens. The highest antibacterial activity was observed against *R. solanacearum* and *P. syringae*, with petroleum ether and acetone extracts producing comparatively larger zones of inhibition. Methanol and diethyl ether extracts also exhibited inhibitory activity, although the zones were relatively smaller. Streptomycin, used as the positive control, produced the largest inhibition zones against both bacterial pathogens. In contrast, the fungal pathogens exhibited lower sensitivity to the lichen extracts.

Only weak to moderate inhibition was observed against *F. oxysporum* and *A. alternata*, irrespective of the solvent used for extraction. Carbendazim, used as the positive control, showed superior antifungal activity compared to the lichen extracts. Few species in future studies to come which may contribute ICBN. The comparatively lower inhibition observed against the fungal pathogens may be attributed to differences in cell wall composition and inherent resistance mechanisms. The results indicate that the lichen extracts possess promising antibacterial activity, particularly against phytopathogenic bacteria.

The variation in antimicrobial activity among the solvent extracts may suggest that the extraction efficiency of bioactive secondary metabolites depends on the polarity of the solvent employed. The MIC test of lichenoid extract upon selected bacterial pathogen *Ralstonia solanacearum* & *Pseudomonas syringae* and two fungal pathogen *F. oxysporum* and *A. alternata* as identified in the ELF list (**Table-3**) from the study sites. the above said pathogens (bacteria and ELF) was also conducted in laboratory using VIS-UV spectrophotometer (**Table-7**) which also confirm that lichen extracts has greater inhibition effect on bacterial pathogen than fungal pathogen which need further investigation for biotechnological application. These tests will provide valuable insights into the functional roles of ELF's within lichen symbiotic associations and their potential biotechnological applications. Overall, this study contributes to our understanding of lichen diversity and endolichenic fungal communities in the Nagaon district of Assam. The data generated pave the way for future research endeavors aimed at exploring the ecological, evolutionary, and biotechnological significance of these fascinating organisms in natural ecosystems.

Results and Tables with good quality images

- **Table: 1. No. of workshop/symposia/brainstorming meeting/ trainings organized in financial year 2023-2026.**

[Outcome Indicators of CAPACITY DEVELOPMENT PROGRAMME]

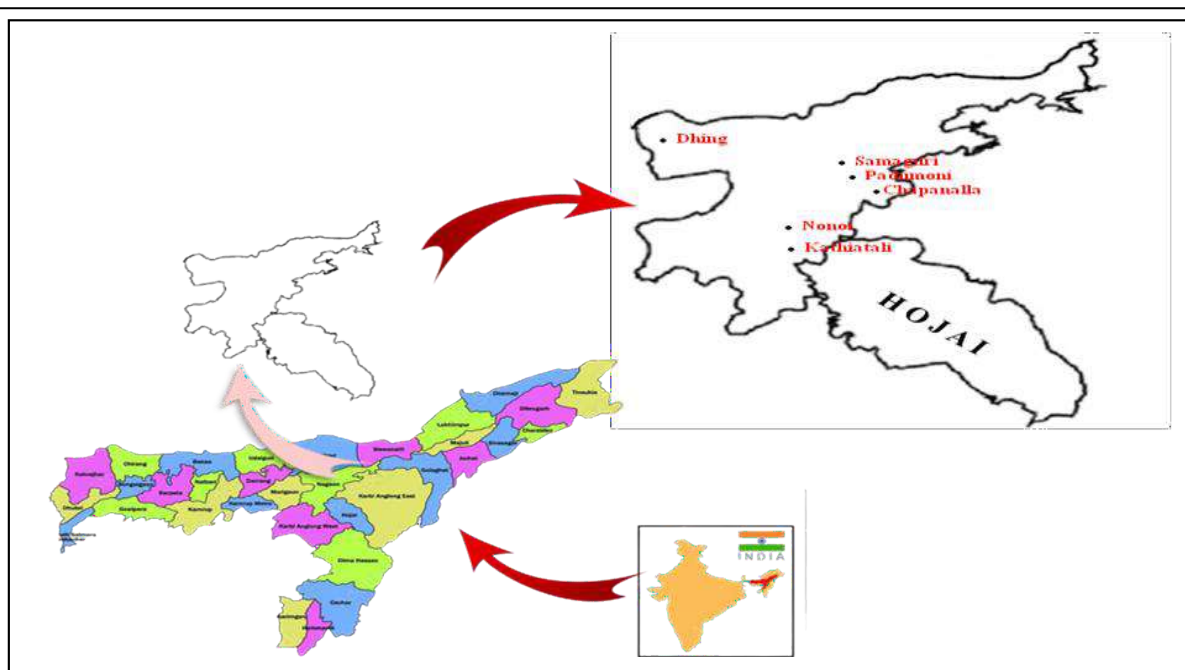
Year	1 st Year		2 nd Year		3 rd Year		Total	
Target (T)/ Achievements (A)	A	T	T	A	T	A	T	A
No. of manpower supported/ trained				287		141	428	428
No. of female beneficiaries in the DBT funded project						20	20	20
No. of publications					01	01	02	02
No. of Patents filed/ granted/commercialized						01	Gran ted 01 Filed 01	Granted 01 Filed 01
No. of products or technologies developed/ transferred/commercialized								
Others (Training/STC/ wprkshop/ OP)	3			4		3	10	10
Patents				01		01(A/F)	02	02

Table 2: List of Identified Lichens from various sample sites [R&D PROGRAMME]

Lichen Samples	Site	Scientific Name	Figures
Sample 1	Kathiatoli (Nagaon)	<i>Parmotrema tinctorum</i> (Nyl.)Hale	Figure1-3
Sample 2	Dhing (Nagaon)	<i>Oxneriopsisbassiae</i> (Ach.)S.Y. Kondr.Upreti &Hur	Figure4
Sample 3	Padumoni (Nagaon)	<i>Dirinariaaegiliata</i> (Afzel.ExAch.) Moore	Figure5-6
Sample 4	Dhing (Nagaon)	<i>Cryptotheciastrata</i> Thor.	Figure7
Sample 5	Padumoni (Nagaon)	<i>Parmotrematinctorum</i> (Nyl.)Hale	Figure1-3
Sample 6	Chapanala (Nagaon)	<i>Dirinariapapillulifera</i> (Nyl.)D.D. Awasthi	Figure8-9
Sample 7	Chapanala (Nagaon)	<i>Pyxinesubcinerea</i> Stirton	Figure10-11
Sample 8	Kathiatoli (Nagaon)	<i>Lecanoraachroa</i> Nyl.	Figure12
Sample 9	Chapanala (Nagaon)	<i>Graphisscripta</i> (L.) Ach.	Figure13-14
Sample 10	Chapanala (Nagaon)	<i>Phaeographiscaesioradians</i> (Leight.) Archer	Figure15-16
Sample 11	Dhing	<i>Parmotrematinctorum</i> (Nyl.)Hale	Figure1-3
Sample 12	Bordua	<i>Pyxinesorediata</i> (Ach.)Mont.	Figure17-18
Sample 13	Chapnala	<i>Bacidiacconnexula</i> (Nyl.) Zahlbr.	Figure19-20
Sample 14	Chapnala	<i>Lecanoraachroa</i> Nyl.	Figure12
Sample 15	Padumoni	<i>Thelotremasp.</i>	Figure21-22
Sample 16	Dhing	<i>Pyxinecoco</i> es(Sw.)Nyl.	Figure23
Sample 17	Dhing	<i>Pyxinereticulate</i> (Vain.)Vain.	Figure24
Sample 18	Dhing	<i>Parmotremapraesorediosum</i> (Nyl.) Hale	Figure25
Sample 19	Chapnala	<i>Parmotremapraesorediosum</i> (Nyl.) Hale	Figure25
Sample 20	Chapnala	<i>Pyxinecoco</i> es(Sw.)Nyl.	Figure23
Sample 21	Chapnala	<i>Pyxinefarinose</i> Kashiw.	Figure26
Sample 22	Bordua	<i>Dirinariapapillulifera</i> (Nyl.)D.D.aWAwasthi	Figure8-9
Sample 23	Bordua	<i>Graphisnigroglauca</i> Leighton	Figure27-28

Sample 24	Bordua	<i>Bacidia incongruens</i> (Stirton) Zahlbr.	Figure29
Sample 25	Chapnala	<i>Parmotrema crinitoides</i> J.C. Wei	Figure30
Sample 26	Chapnala	<i>Graphis</i> sp.	Figure31-32
Sample 27	Dhing	<i>Graphinarufopallida</i> (Vainio) Zahlbr.	Figure33-34
Sample 28	Dhing	<i>Chrysothrix candelaris</i> (L.) Laundon	Figure35
Sample 29	Chapanala (Nagaon)	Sample is not a lichen but probably [A wood decaying fungus]	Figure36
Sample 30	Dhing	(Fruticose Lichen)[unidentified]	Figure37

Map1: Geographical Map of Lichen Collection Sites:



Map2: Location, GPS Details of Lichen Collection Sites:-

Sample site	Name	Nature	Latitude & Longitude
1	Dhing	Plain area	Lat:26.457140, Long: 92.487541
2	Chapanala	Foot hills	Lat:26.339437, Long: 92.894914
3	Padumoni	Foot hills	Lat:26.369124, Long: 92.844875
4	Kathiatoli	Foot hills	Lat:26.225751, Long: 92.808177
5	Bordua	Plain area	Lat:26.402127, Long: 92.536625

➤ **Table 3: Isolation of ELF corresponding to Lichen Species**

Genus	Identified Lichen Species	ELF identified
Bacidia	Bacidia connexula (Nyl.) Zahlbr.	Colletotrichum
	Bacidia incongruens (Stirton) Zahlbr.	Trichoderma Sp
Chrysothrix	Chrysothrix candelaris (L.) Laundon	Phomopsis
Cryptothecia	Cryptothecia striata Thor.	Trichoderma Sp
Dirinaria	Dirinaria aegiliata (Afzel. Ex Ach.) Moore	Alternaria spp
	Dirinaria papillulifera (Nyl.) D.D. Awasthi	Cladosporium sp.,
Graphina–	Graphina rufopallida (Vainio) Zahlbr.	Fusarium Sp
Graphis	Graphis scripta (L.) Ach.	Aspergillus
	Graphis scripta (L.) Ach.	Alternaria spp
	Graphis spp.	Fusarium Sp
Lecanora	Lecanora achroa Nyl.	Cladosporium sp.,
Oxneriopsis	Oxneriopsis bassiae (Ach.) S.Y. Kondr. Upreti & Hur	Trichoderma Sp
Parmotrema	Parmotrema tinctorum (Nyl.) Hale	Alternaria spp
	Parmotrema praesorediosum (Nyl.) Hale	Alternaria spp
	Parmotrema crinitoides J.C. Wei	Fusarium Sp
Phaeographis	Phaeographis caesioradians (Leight.) Archer	Alternaria spp
Pyxin	Pyxine cocoes (Sw.) Nyl.	Xylaria sp
	Pyxine farinosa Kashiw.	Fusarium Sp
	Pyxine subcinerea Stirton	Daldinia
	Pyxine reticulata (Vain.) Vain.	Fusarium
	Pyxine soorediata (Ach.) Mont.	Aspergillus
Thelotrema	Thelotrema sp.	Trichoderma Sp

Table 4: Simpsons Diversity Index of Lichen Species of Nagaon District:

$$\text{Diversity Index} = 1 - \sum (n/N)^2$$

n=Total Number of organisms of a particular species

N= Total number of organisms of all species

D=Diversity Index

➤ **Data Collection:**

Lichen samples	Total species identified
<i>Parmotrema</i>	6
<i>Oxneriopsis</i>	1
<i>Dirinaria</i>	3
<i>Cryptothecia</i>	1
<i>Pyxine</i>	6
<i>Lecanora</i>	2
<i>Graphis</i>	3
<i>Phaeographis</i>	1
<i>Bacidia</i>	2
<i>Thelotrema</i>	1
<i>Graphina</i>	1
<i>Chrysothrix</i>	1
FruticoseLichenspp	1
Total=29	

➤ **Calculation of $(n/N)^2$ for each species:**

Lichen samples	$(n/N)^2$	
<i>Parmotrema</i>	$(6/29)^2$	0.042849
<i>Oxneriopsis</i>	$(1/29)^2$	0.001156
<i>Dirinaria</i>	$(3/29)^2$	0.010609
<i>Cryptothecia</i>	$(1/29)^2$	0.001156
<i>Pyxine</i>	$(6/29)^2$	0.042849
<i>Lecanora</i>	$(2/29)^2$	0.004761
<i>Graphis</i>	$(3/29)^2$	0.010609
<i>Phaeographis</i>	$(1/29)^2$	0.001156
<i>Bacidia</i>	$(2/29)^2$	0.004761
<i>Thelotrema</i>	$(1/29)^2$	0.001156
<i>Graphina</i>	$(1/29)^2$	0.001156
<i>Chrysothrix</i>	$(1/29)^2$	0.001156
FruticoseLichenspp	$(1/29)^2$	0.001156
Sum the squared proportions $(\sum(n/N)^2)$:		0.12453
Calculation of $D(1-\text{Sum})1-0.12453$		0.87547

Table 5 : Qualitative antimicrobial activity of lichen extracts against selected plant pathogens

Pathogen	Acetone	Methanol	Petroleum Ether	Diethyl Ether	Positive Control
<i>Pseudomonas syringae</i> (MTCC 673)	+++	++	+++	++	++++ (Streptomycin)
<i>Ralstonia solanacearum</i> (MTCC 13168)	+++	++	+++	++	++++ (Streptomycin)
<i>Alternaria alternata</i> (MTCC 13407)	-	-	±	-	++ (Carbendazim)
<i>Fusarium oxysporum</i> (MTCC 12994)	±	-	±	-	++ (Carbendazim)

Scale: ++++very strong inhibition,+++Strong,++Moderate, + Weak, -No inhibition.

TABLE 6: STATION WISE COLLECTION OF DATA:

☐ Sample Station (L-01): Dhing Area (Campus and nearby forest):

Code	Habitat	GPS	Type	Location
D-01	Corticolous	Lat. 26.457140, Long. 92.487541	Crustose	Dhing College Campus
D-02	Corticolous	Lat. 26.457747, Long. 92.485705	Crustose	Dhing College Campus
D-03	Corticolous	Lat. 26.457747, Long. 92.485705	Foliose	Dhing College Campus

☐ Sample Station (L-02): Chapanala and Samaguri Area (Nearby forest):

Code	Habitat	GPS	Type	Location
C-01	Saxicolous	Lat. 26.339437, Long. 92.894914	Crustose (White and orange)	Chapanala
C-02	Corticolous	Lat. 26.339251, Long. 92.894884	Crustose (Whitish green)	Chapanala
C-03	Ramicolous	Lat. 26.369144, Long. 92.844882	Foliose	Chapanala
C-04	Corticolous	Lat. 26.339251, Long. 92.894884	Foliose	Champawati falls area
C-05	Corticolous	Lat. 26.321012, Long. 92.904060	Crustose (Whitish green)	Champawati falls area
C-06	Corticolous	Lat. 26.368863, Long. 92.845258	Foliose	Champawati falls area
C-07	Corticolous	Lat. 26.320604, Long. 92.904717	Crustose (Green)	Champawati falls area
C-08	Corticolous	Lat. 26.320604, Long. 92.904717	Crustose (Whitish)	Champawati falls area
C-09	Ramicolous	Lat. 26.340091, Long. 92.894852	Foliose (Green)	Champawati falls area
C-10	Corticolous	Lat. 26.340091, Long. 92.894852	Crustose (Whitish green)	Champawati falls area

❑ **Padumoni area (L-03) nearby forest**

Code	Habitat	GPS	Type	Location
P-01	Corticolous	Lat. 26.369128, Long.92.844901	Crustose (Whitish green)	Podumoni Village
P-02	Corticolous	Lat. 26.369058, Long. 92.844810	Foliose (Green)	Podumoni Village

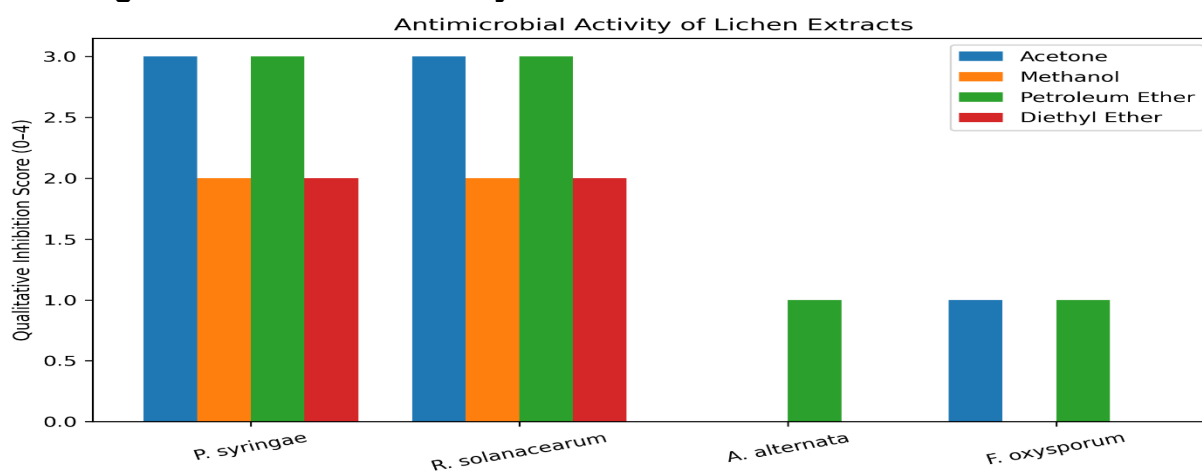
❑ **Kothiatoli area (L-04) nearby forest**

Code	Habitat	GPS	Type	Location
K-01	Corticolous	Lat. 26.225755, Long.92.808166	Crustose (white)	Kothiatoli Foothills
K-05	Ramicolous	Lat. 26. 225746, Long.92.808177	Foliose	Kothiatoli Foothills

❑ **Nonoi area (L-05) nearby forest**

N-01	Corticolous	Lat. 26.225750, Long.92.808167	Crustose	Nonoi Area
N-02	Corticolous	Lat. 26. 225760, Long.92.808182	Crustose	Nonoi Area

➤ **Fig 1 : Antimicrobial activity of Lichen Extract.**



➤ **Photo Plate 1: : Antimicrobial activity of Lichen Extract.**



Table 7: Percent Inhibition (%) of bacterial and fungal pathogen treated with lichen extract at OD_{600nm} using UV-VIS spectrophotometer [mean value of 3 replicas in petroleum and ethanol extract]

Pathogen/Test organism	ppm	OD _{Control}	OD _{Sample}	% Inhibition (mean of 3 replica)
<i>Pseudomonas syringae</i>	25	0.625	0.489	21.76
	75	0.634	0.464	26.81
	50	0.649	0.455	29.89
	100	0.697	0.408	<u>41.46</u>
<i>Ralstonia solanacearum</i>	25	0.615	0.478	22.27
	75	0.629	0.455	27.66
	50	0.640	0.452	29.37
	100	0.678	0.425	<u>37.31</u>
<i>Alternaria alternata</i>	25	0.630	0.621	0.90
	50	0.645	0.640	0.77
	75	0.650	0.649	0.15
	100	0.670	0.666	<u>0.59</u>
<i>Fusarium oxysporum</i>	25	0.623	0.620	0.48
	50	0.639	0.635	0.62
	75	0.657	0.651	0.91
	100	0.662	0.659	<u>0.45</u>

➤ **Perform calculation:**

➤ A control= Brooth+ Test organism [Absorbance before adding extract]

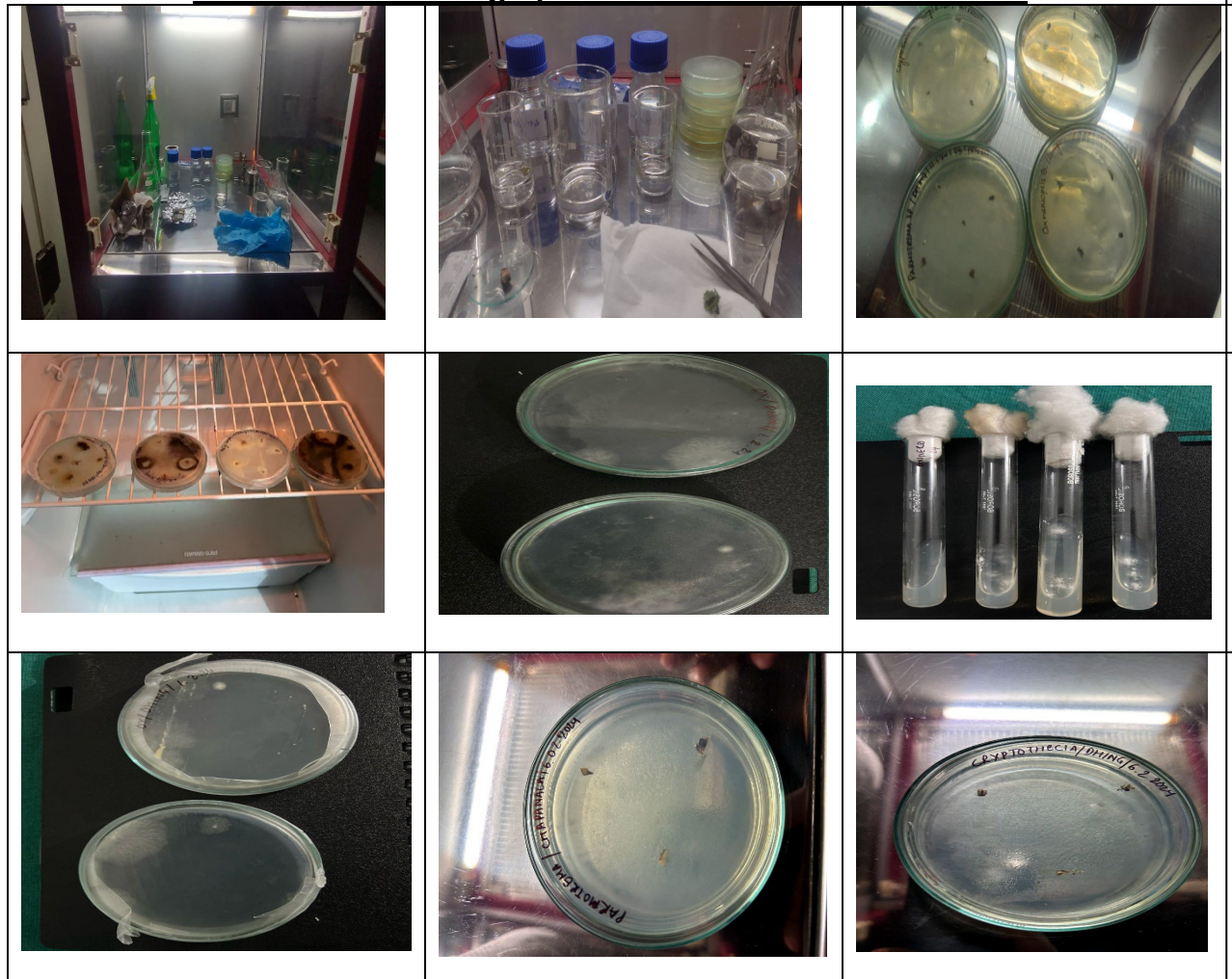
➤ A sample= Brooth+ Test organism+ extracts [Absorbance after adding extract]

❖ Apply Formula:

$$\text{➤ \% Inhibition} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

A control

➤ **PhotoPlate-2: Photographs of Aidentified Lichen Thallus**



➤ **PhotoPlate-2: Photographs of identified Lichen Thallus**

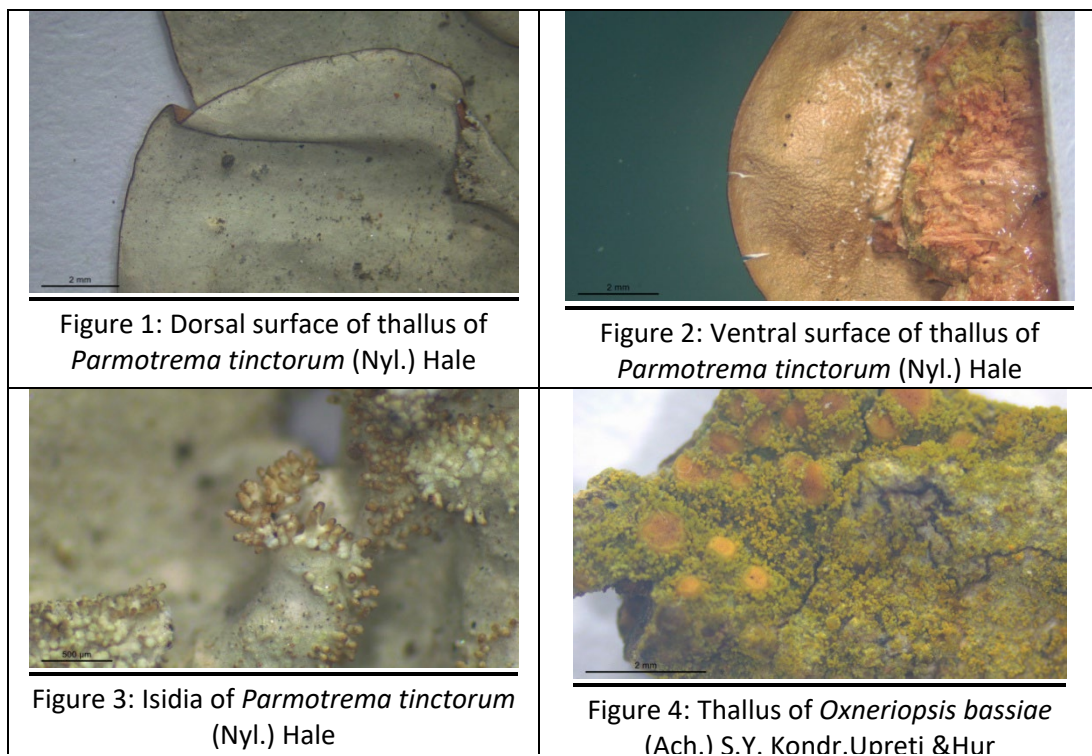




Figure 5: Thallus of *Dirinaria aegiliata* (Afzel. Ex Ach.) Moore



Figure 6: Soralia of *Dirinaria aegiliata* (Afzel. Ex Ach.) Moore

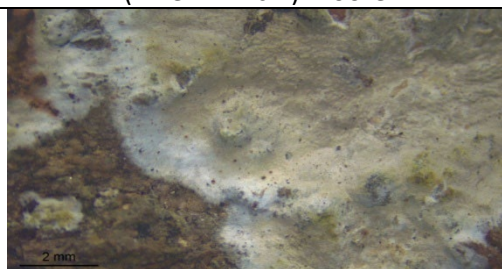


Figure 7: Thallus of *Cryptothecia striata* Thor.



Figure 8: Thallus of *Dirinaria papillulifera* (Nyl.) D.D. Awathi

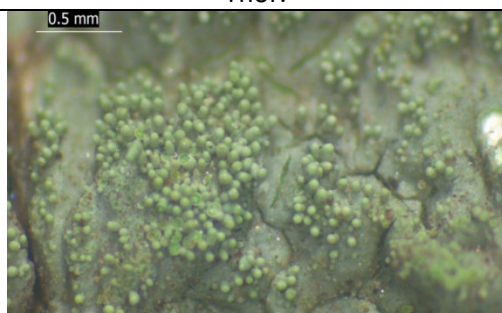


Figure 9: Isidia of *Dirinaria papillulifera* (Nyl.) D.D. Awathi

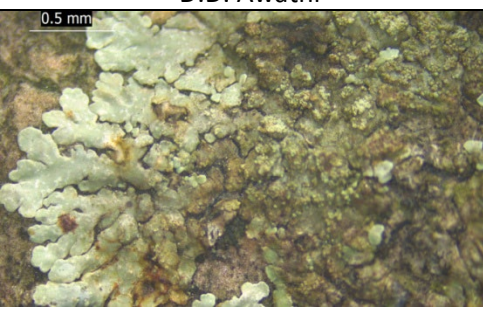


Figure 10: Thallus of *Pyxine subcinerea* Stirton

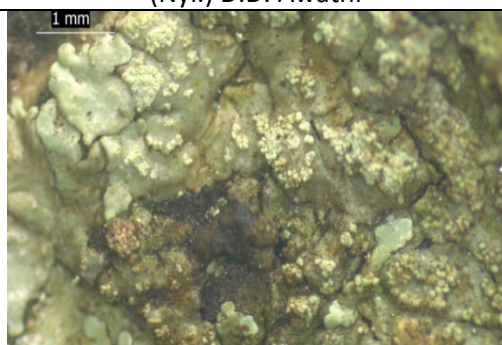


Figure 11: Soralia of *Pyxine subcinerea* Stirton

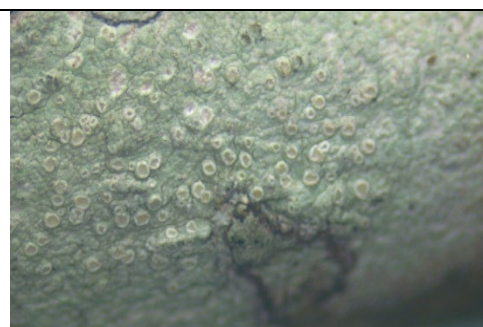


Figure 12: Thallus of *Lecanora achroa* Nyl.

➤ **PhotoPlate-3: Photographs of identified Lichen Thallus**

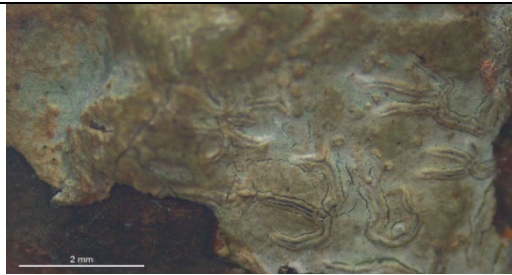


Figure 13: Thallus of *Graphis scripta* (L.) Ach.



Figure 14: Spore of *Graphis scripta* (L.) Ach.



Figure 15: Thallus of *Phaeographis caesioradians* (Leight.) Archer



Figure 16: Spore of *Phaeographis caesioradians* (Leight.) Archer

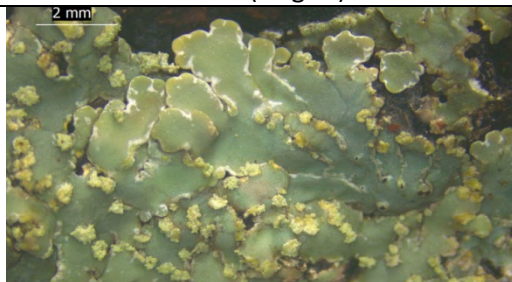


Figure 17: Thallus of *Pyxine soorediata*



Figure 18: Spores of *Pyxine soorediata*



Figure 19: Thallus of *Bacidia connexula* (Nyl.) Zahlbr.

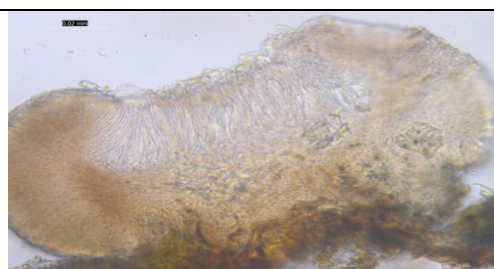


Figure 20: Apothecia of *Bacidia connexula* (Nyl.) Zahlbr

➤ **PhotoPlate-4: Photographs of identified Lichen Thallus**



Figure 21: Thallus of *Thelotrema* sp.



Figure 22: Spores of *Thelotrema* sp.



Figure 23: Thallus of *Pyxine cocoes* (Sw.) Nyl.



Figure 24: Thallus of *Pyxine reticulata* (Vain.) Vain.



Figure 25: Thallus of *Parmotrema praesorediosum* (Nyl.) Hale



Figure 26: Thallus of *Pyxine farinose* Kashiw.

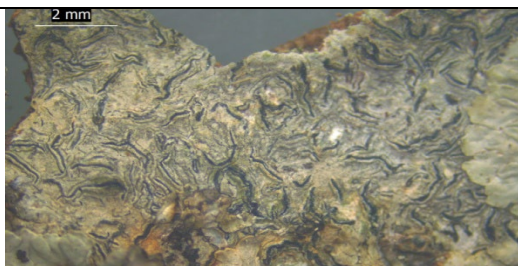


Figure 27: Thallus of *Graphis nigroglauca* Leighton



Figure 28: Spore of *Graphis nigroglauca* Leighton.



Figure 29: Thallus of *Bacidia incongruens* (Stirton) Zahlbr.



Figure 30: Thallus of *Parmotrema crinitoides* J.C.

➤ **PhotoPlate-5: Photographs of identified Lichen Thallus**

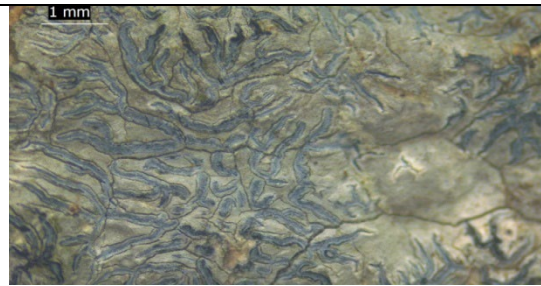


Figure 31: Thallus of *Graphis* sp.



Figure 32: Spore of *Graphis* sp.



Figure 33: Thallus of *Graphina rufopallida* (Vainio) Zahlbr



Figure 34: Spore of *Graphina rufopallida* (Vainio) Zahlbr.



Figure 35: Thallus of *Chrysothrix candelaris* (L.) Laundon

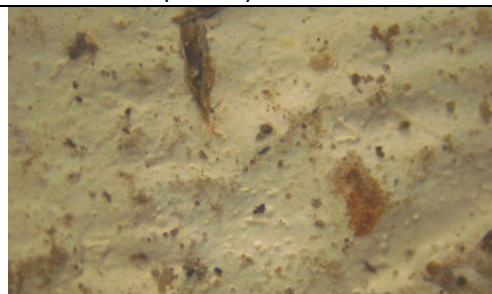


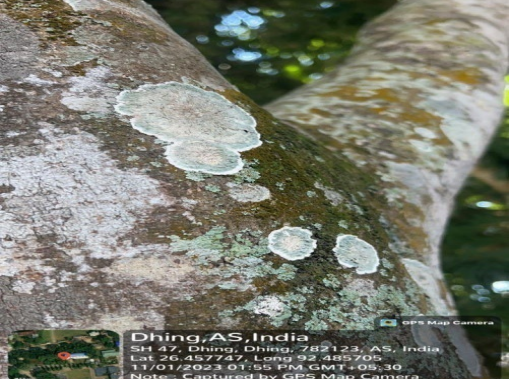
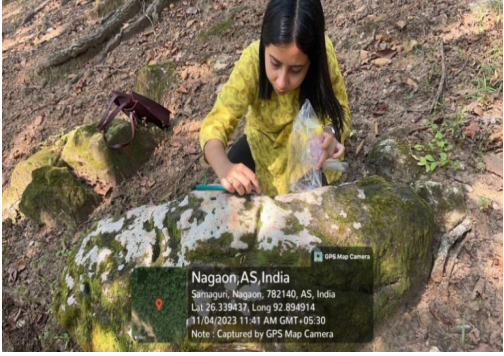
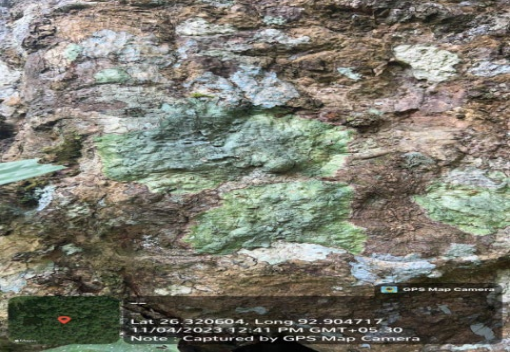
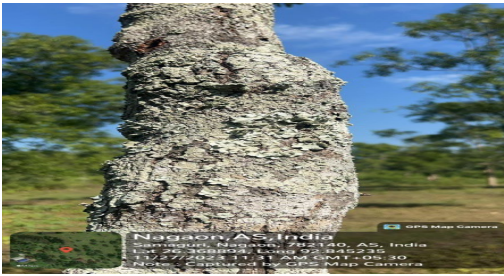
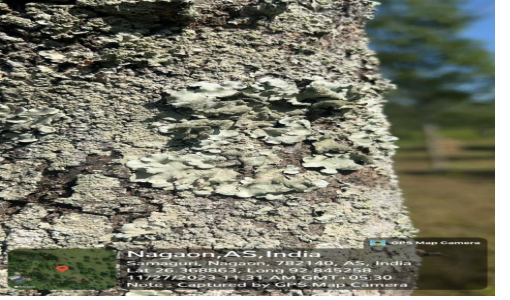
Figure 36: Sample no. 29 is a wood decaying fungus



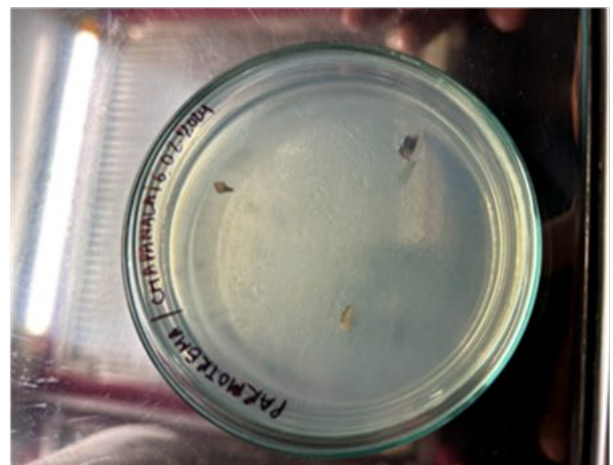
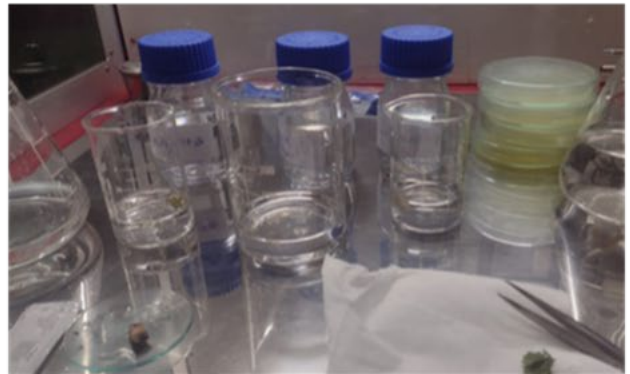
Figure 37: Is a Fruticose Lichen species

Total identified=29

➤ **Photoplate 6: Location wise collection of Lichens**

L1	 Dhing, AS, India Dhing, Dhing, 782123, AS, India Lat 26.457140, Long 92.487541 11/01/2023 01:44 PM GMT+05:30 Note : Captured by GPS Map Camera	 Dhing, AS, India SH 47, Dhing, Dhing, 782123, AS, India Lat 26.457747, Long 92.485705 11/01/2023 01:55 PM GMT+05:30 Note : Captured by GPS Map Camera
L2	 Nagaon, AS, India Simasari, Nagaon, 782140, AS, India Lat 26.339437, Long 92.894914 11/04/2023 11:41 AM GMT+05:30 Note : Captured by GPS Map Camera	 Nagaon, AS, India Lat 26.320604, Long 92.904717 11/04/2023 12:41 PM GMT+05:30 Note : Captured by GPS Map Camera
L3	 Nagaon, AS, India Simasari, Nagaon, 782140, AS, India Lat 26.339437, Long 92.894914 11/04/2023 11:41 AM GMT+05:30 Note : Captured by GPS Map Camera	 Nagaon, AS, India Simasari, Nagaon, 782140, AS, India Lat 26.339437, Long 92.894914 11/04/2023 11:41 AM GMT+05:30 Note : Captured by GPS Map Camera
L4&L5	 Nagaon, AS, India Simasari, Nagaon, 782140, AS, India Lat 26.225750, Long 92.808167 12/04/2023 12:30 PM GMT+05:30 Note : Captured by GPS Map Camera	 Nagaon, AS, India Simasari, Nagaon, 782140, AS, India Lat 26.225742, Long 92.808169 12/04/2023 12:27 PM GMT+05:30 Note : Captured by GPS Map Camera

➤ **Photo Plate: 7 Collection and Surface Sterilization of collected Species:**



➤ Photo Plate 8: Surface Sterilization.



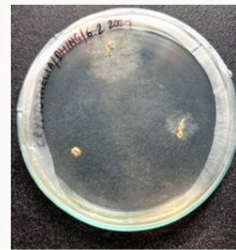
Parmotrema sp.



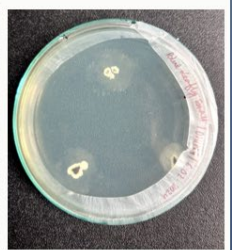
Oxineriopsis sp.



Cryptothecia sp.



Unidentified sample



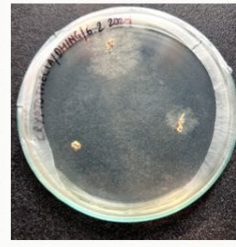
Parmotrema sp.



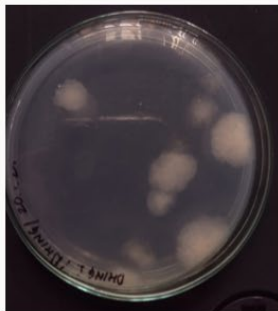
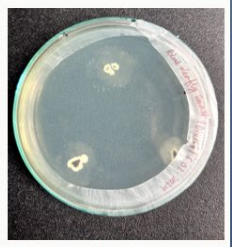
Oxineriopsis sp.



Cryptothecia sp.



Unidentified sample



Parmotrema sp.



Oxineriopsis sp.



Cryptothecia sp.



Unidentified sample



9. CONCLUSIONS SUMMARIZING THE ACHIEVEMENTS:

- ❑ A total of 29 lichen species were collected from 5 sampling stations namely Dhing, Kothiatoli, Nonoi, Podumoni, Chapanala area. A dominance of *corticolous* lichen was observed during the collection process.
- ❑ Corresponding to lichens species about 10 ELF were identified from five sample stations. The highest number of ELF was isolated from genus *permotrema* and *pyxine*.
- ❑ All the collected samples have been identified with the help of lichenology laboratory of Nowgowng University, Nagaon and ICFRE- Rain Forest Research Institute, Jorhat, Assam.
- ❑ The collected samples were surface sterilized, *sporulated endolichens* were isolated by streaking and pure colonies were isolated and stored.
- ❑ Biochemical and growth patterns of the endolichens showed similar results. All the ELFs are catalase and oxidase positive.
- ❑ Further the antimicrobial activity & MIC of the lichen extracts was evaluated against two bacterial pathogens, *Ralstonia solanacearum* (MTCC 13168) and *Pseudomonas syringae* (MTCC 673), and two fungal pathogens, *Fusarium oxysporum* (MTCC 12994) and *Alternaria alternata* (MTCC 13407), using agar well diffusion assay and recording their Optical Density(OD₆₀₀) using UV-VIS Spectrophotometer . The highest antibacterial activity and percent(%) inhibition was observed against *R. solanacearum* and *P. syringae*, with petroleum ether and acetone extracts producing comparatively larger zones of inhibition. In contrast, the fungal pathogens exhibited lower sensitivity to the

lichen extracts. Only weak to moderate inhibition was observed against *F. oxysporum* and *A. alternata*, irrespective of the solvent used for extraction.

- ❑ The comparatively lower inhibition observed against the fungal pathogens may be attributed to differences in cell wall composition and inherent resistance mechanisms.
- ❑ The results indicate that the lichen extracts possess promising antibacterial activity, particularly against phytopathogenic bacteria. The variation in antimicrobial activity among the solvent extracts suggests that the extraction efficiency of bioactive secondary metabolites depends on the polarity of the solvent employed.
- ❑ To conclude the project has yielded significant insights into the local lichen diversity and the associated endolichenic fungal community leading to the successful isolation and storage of pure colonies for further analysis. To enhance understanding and distinction among various ELF's further study is required paving the way for future potential applications in ecological research and biotechnology.

10. SUMMARY (IN 200 WORDS ONLY):

The findings of this study offer valuable insights into the lichen diversity and endolichenic fungal community in the Nagaon district of Assam. The isolation and taxonomic classification of ELF will laid the foundation for further investigations into the ecological roles and potential applications of these lichens. The successful isolation and storage of pure colonies of sporulated endolichens signify a crucial step towards understanding the endolichenic fungal community associated with these lichens. The consistent positive

delayed catalase and oxidase activity exhibited by all endolichenic fungi (ELFs) suggest metabolic activity indicative of potential symbiotic or parasitic relationships with their lichen hosts. These findings corroborate previous studies highlighting the metabolic diversity of endolichenic fungi and their potential ecological significance.

The study of antimicrobial activity of the lichen extracts evaluated against bacterial and fungal pathogens, is an indicate that the lichen extracts possess promising antibacterial activity, particularly against phytopathogenic bacteria, while exhibiting limited antifungal potential. The variation in antimicrobial activity among the solvent extracts suggests that the extraction efficiency of bioactive secondary metabolites depends on the polarity of the solvent employed. The planned implementation of additional biochemical and molecular tests in future will elucidate the diversity and genetic makeup of the endolichenic fungal community further. These tests will provide valuable insights into the functional roles of ELFs within lichen symbiotic associations and their potential biotechnological applications. Overall, this study contributes to our understanding of lichen diversity and endolichenic fungal communities in the Nagaon district of Assam. The data generated pave the way for future research endeavors aimed at exploring the ecological, evolutionary, and biotechnological significance of these fascinating organisms in natural ecosystems.

11. Any new product/ process developed : Nil

12. Any new lead: Nil

13. Any technology developed: Nil

14. Any patent taken: Yes

[01 Design Patent granted and 01 Design patent applied for]

1. Design Patent Certificate: Glass Chamber for Surface Sterilization



2. Patent applied: Portable Herbarium Preservation Chamber for Lichens

FORM 1

Application for Registration of Design

[See section 5 and 44]

You are requested to register the accompanying design in:

Sr.	Class	Sub Class	Articles
1	24-00-Medical and laboratory equipment	24-01-APPARTUS EQUIPMENT FOR DOCTORS, HOSPITALS AND LABORATORIES	PORTABLE HERBARIUM PRESERVATION CHAMBER FOR LICHENS

In the name of :

Sr.	Name	Nationality	Address
1	Dr. Sanjeeb Kumar Nath	India	Co-PI, Advanced Institutional Level Biotech Hub (Phase II) & Associate Professor & HOD, Department of Botany, Dhing College, Dhing, Nagaon, Assam 782123
2	Dr. Monoj Kumar Saikia	India	PI, Advanced Institutional Level Biotech Hub (Phase II) & Rtd Associate Professor Department of Botany, Dhing College, Dhing, Nagaon, Assam 782123
3	Chandamita Goswami	India	Project Associate , Advanced Institutional Level Biotech Hub (Phase II), Dhing College Dhing, Nagaon, Assam 782123
4	Uddhab Talukdar	India	Laboratory Assistant , Department: Advanced Institutional Level Biotech Hub (Phase II), Dhing College, Dhing, Nagaon, Assam 782123
5	Mousumi Terangpi	India	Assistant Professor, Department of Botany, Dhing College, Dhing, Nagaon, Assam 782123

who claim(s) to be the proprietor(s) thereof.

Four exactly similar representations of the design accompanying this request.

The design is to be applied to **PORTABLE HERBARIUM PRESERVATION CHAMBER FOR LICHENS**,

**INTELLECTUAL
PROPERTY INDIA**
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

**GOVERNMENT OF INDIA**

Controller General of Patents, Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Design Application Details

Application Number:

454312-001

CBR Number:

207280

CBR Date:

04/04/2025 17:57:45

Applicant Name:

1. Dr. Sanjeeb Kumar Nath
2. Dr. Monoj Kumar Saikia
3. Chandamita Goswami
4. Uddhab Talukdar
5. Mousumi Terangpi

Design Application Status

Application Status:

Application Under Process(awaiting for Technical Examination)

Back

➤ **Journal Publication:**

1]. Title: Lichens of Dhing, Nagaon District, Assam

Authors: Sanjeeb Kumar Nath, Manoj Kumar Saikia, Uddhab Talukdar,
Mousumi Terangpi

Published in December 2025

Name of Journal: Himalayan Journal of Basic and Applied Sciences (ISSN
3107-9113) Vol. 1, Issue 2 Page 133-138

[An open-access, peer-reviewed Journal]

2]. Applied for Publication on Cryptogam Biodiversity and Assessment.
(Receive letter is enclosed)

➤ **PUBLICATION AND CONFERENCE PRESENTATION**

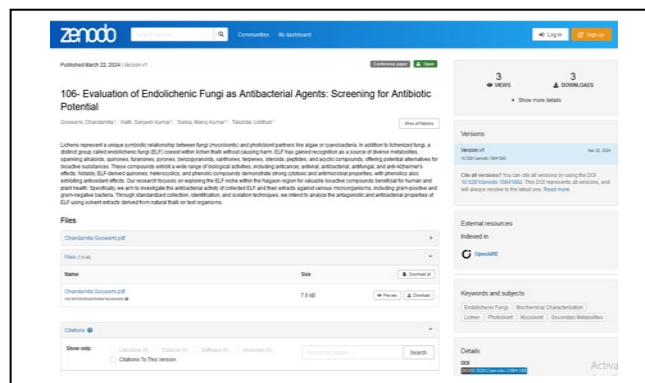
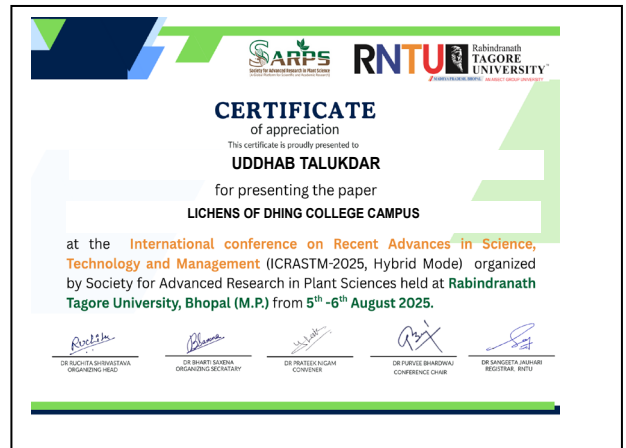
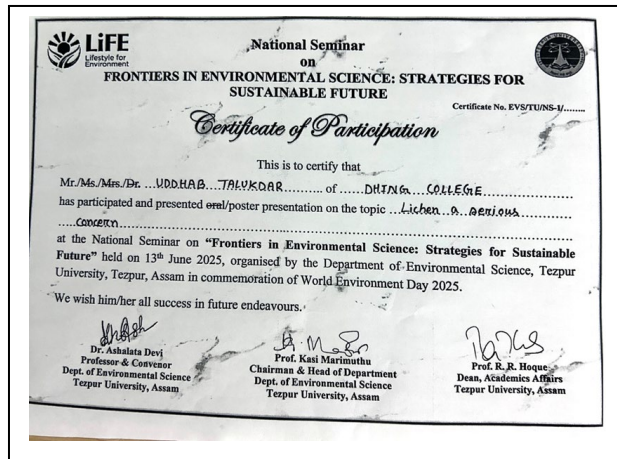
1. Abstract titled "Screening of antimicrobial properties of endolichenic fungi against certain bacterial species" was presented in International conference "International Conference on Plant science & Sustainable Agriculture" organized by SARPS.

2. Abstract titled "Evaluation of Endolichenic Fungi as Antibacterial Agents: Screening for Antibiotic Potential" was presented in International conference "International Conference on Traditional Knowledge Exploring Traditional Knowledge: Bridging Past and Future" organized by Internal Quality Assurance Cell, Nowgong College (Autonomous) In Association with Research and Development Cell (RDC), Nowgong College (Autonomous)

3. Talukdar, U. (2024) "107- Survey of Lichens of Nagaon, Assam". International Conference on Traditional Knowledge (Exploring Traditional Knowledge: Bridging Past and Future), Zenodo. doi: 10.5281/zenodo.10841908.

4. Goswami, C. (2024) "106- Evaluation of Endolichenic Fungi as Antibacterial Agents: Screening for Antibiotic Potential". International Conference on Traditional Knowledge (Exploring Traditional Knowledge: Bridging Past and Future), Zenodo. doi: 0.5281/zenodo.10841883x.

Certificates of participation and presentation:



16. Financial progress. Viz. equipments purchased, manpower, copies of UCs/SEs of various financial year, etc.

[Enclosed all financial documents]

☐ List of Enclosers:

- ✓ 1.UC & SOE [2023-2026]
- ✓ 2.Capital assets certificate [2023-2026]
- ✓ 3.Consolidated Statement of Accounts [2023-2026]
- ✓ 4.Complete Progress report [2023-2026]
- ✓ 5.Format of final completion Report [2023-2026]
- ✓ 6.Target & Achievement for completed report [2023-2026]
- ✓ 7.Objective wise achievement table [2023-2026]
- ✓ 8 Format of manpower and staffing [2023-2026]
- ✓ Letter of retention of equipments.

Sanjeeb Kr. Nath

(Dr. Sanjeeb Kumar Nath CO-PI [PI-In-charge]
(Dr. Manoj Kumar Saikia, PI (Retd)

Coordinator & PI
Biotech Hub
Dhing College



(Dr. Biman Hazarika)
Principal

Principal
Dhing College

RNeog

(Ranjon Neog, Accountant)
UDA cum Accountant
Dhing College
Dhing: Nagaon(Assam)