J. Phytol. Res. **33**(1):71-91, 2020 **ISSN 0970-5767**

DIVERSITY AND DISTRIBUTION OF LICHENS IN RELATION TO CLIMATIC CONDITIONSIN KODAIKANAL HILLS OF TAMIL NADU, INDIA

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The objective of the present study highlights the diversity distribution of lichen species and effect of climatic factors on species diversity in Kodaikanal hills belong to Western Ghats of Tamil Nadu. Thin layer chromatography and microcrystal test were used for identification of lichen species. The Kodaikanal hills has good number of lichens represented by foliose 66.67%, followed by fruticose and crustose 23.80% and 4.76% respectively. The Park view area of Kodaikanal hills of Western Ghats in Tamil Nadu has the highest number of lichens with 65% followed by Guna cave 15%. The lichens recorded from Madhiketancholai and Berijem lake showed very few species. The article enumerates the occurrence of 21 lichen species in Kodaikanal hills of Western Ghats in Tamil Nadu. Form diversity distribution showed that majority of them are corticolous (76%), followed by saxicolous (14%), muscicolous (5%) and both corticolous and terricolous showed 5% diversity. Parmeliaceae (57%) and Physciaceae (9%) families dominated the region. The article also discusses the detailed information with reference to diversity, distribution and ecology of 21 species from different regions of Kodaikanal hills in Western Ghats. The influence of sunshine, rainfall, temperature, relative humidity, evaporation rate and wind speed on lichen diversity was investigated by historical data using response surface methodology and concluded that sunshine played crucial role.

Key words: Form diversity, family diversity, lichen substances, microcrystal test, response surface methodology and thin layer chromatography

Introduction

The Kodaikanal hills, in Tamil Nadu, the part of Western Ghats, stretches over an expanse of 736 km^2 with elevation ranges from 900 to 2,130 m. Mean annual rainfall is between 500 and 4617 mm and major reservoirs are fed by two service reservoirs such as Pambar river and Masonry Dam in Manoranjitham Shola upstream of earthen dam and nine ground level reservoirs such as observatory compound, Chellapuram, Levenge road, Naidupuram, Anna Salai, Anandhagiri, KurinjiAndavarKovil and Srinivasapuram which originate from theBeriiam lake and Keelgundar River. The importance of selecting Kodaikanal goes beyond our interest in lichen abundance of Tamil Nadu.

Lichens are the fungal parasites that live symbiotically on algae for its survival. Two are the organisms live in one single house. One such is a Mycobiont (fungi) and the other refers to photobiont (algae)[1]. The lichens are known to exhibit substrate specificity, form diversity and altitude diversity. On the basis of size and shape,

they are categorized into three major groups (a) crustose (b) foliose and (c) fruticose[2]. There is a great species diversity of lichen, occurring naturally in the temperate regions and tropical rainforests, where about 22000 species are distributed all over the World. On the basis of attachment of lichens to the specific substratum, they are classified into saxicolous (attached to rock), corticolous (growing on the surface of bark of plants), muscicolous (attached to moss plant) terricolous (attached to soil).

India has a rich diversity of lichens represented by more than 2714 species[3]. Information about species of Indian lichens was brought together by Linnaeus who reported the occurrence of two taxa, *Lichen fuciformis*(L.) D.C. and *Rocella montagnei* Bél. in his magnum opus "Species Plantarum"[4].The earliest lichen study was carried out by European lichenologist Belanger (1838) who reported about forty taxa in Pondicherry and Coromandel Coast of Western Ghats and later by Montagne (1842) who identified *P. perlata* (Huds.) Ach., *P. phvsodes* (L.)Ach., *P. physodes*f. *Nlgrovittata* Mont*, P. sinuosa*from Western Ghats[5]. Nylander (1860, 1867, 1869, 1873) identified lichen diversity in Calcutta, Andaman and Bengalensis[6]. Műll Arg. (1892) reported lichens from Manipur. Jatta (1902, 1905, 1911) investigated lichens from different parts of India and Malabar. Smith (1931) introduced new family Cryptotheciaeae and comprised about 76 lichen taxa from India[7]. The first lichen survey in Kodaikanal hills was initiated by Awasthi[8]and reported a new foliose lichen *Parmelia pseudobilteriana*. Awasthi[7], the first Indian lichenologist who reported the occurrence of 1310 lichen species from Nepal, Ceylon, Sri Lanka and Pakistan. Singh added a total of 225 taxa to lichen species of Western Ghats during 1966 – 1977[9]. Identification keys of 1850 species of lichens comprising 700 macrolichens and 1150 microlichens that belonged to foliose to fruticose forms and crustose to squamulose forms were prepared during 1988 and 1991 respectively[10,11]. Study on the floral diversity and distribution of lichens in India flourished extensively since the last three decades. Rather extensive accounts on the lichens of Himalayas as well as those of tropical, temperate and alpine regions of India were documented[12]. Except Nilgiris hills in the Western Ghats of Tamil Nadu, most of the other places are under explored for lichens[9].

Altitude and humidity were the putative key factors controlling the diversity and distribution of lichens within the Sanctuary. The mid altitude range 1400–1600 m had the greatest lichen diversity, which showed a unimodal pattern in relation to altitude[13]. Nayaka *et al*.[9] have studied the status of lichen diversity in Western Ghats as well. Abe*et al*.[14] identified new species of lichens in Yercaud hills of Eastern Ghats, Tamil Nadu. A total of 10 new additions of lichens to India were reported from the Nilgiri Hills of the Western Ghats area around Silent Valley National Park and Mahabaleshwar[15]. Ramya and Thirunalasundhari[16] recorded interesting lichen species belonged to following genera were such as *Caloplaca*, *Parmotrema*, *Ramalina* and *Usnea* etc. Evidence from the literature shows that accelerated climate change in recent years has a direct impact on lichen biodiversity, imposing species to either migrate or adapt new traits. Moreover, the study on the effect of climatic conditions on the survival of lichen is more difficult because complex interpretation is required. To answer more specific questions about the health of forest environment, researchers often examine lichen species richness. In this present study, detailed information with reference to the effect of climatic factors on lichen biodiversity was recorded in different elevation gradients to list out the lichen flora of the Kodaikanal hills.

The study was carried out to identify the localities with rich lichen biodiversity and occurrence of various form, substrate and family diversity. The information on ethnolichenological features was collected by interacting with the indigenous tribal people. The ecological lichen diversity distribution studies involving the assessment of occurrence and species richness will throw light in understanding the composition and structure of lichen species in the Kodaikanal hills. These changes in climatic conditions or lichen species can be used in formulating effective future conservation strategies. While relating the concepts of lichen diversity, parameters such as sun shine, temperature, rainfall, relative humidity and evaporation rate have also been taken to consider the needs of environmental approach and also to outline the response of lichen species to climate change we used response surface methodology model. In preparation of taxonomic identification of lichens, we referred many papers, visited herbarium centers (BSA, Allahabad and NBRI, Lucknow) and had open discussions with university scholars and several resource persons. The state of Tamil Nadu, the part of Western Ghats has high construction activities, illegal exploitation of lichen species, deforestation in the fragile ecosystem of Western Ghats. Moreover, quantitative and qualitative assessment of lichen floral diversity and the rapid changes in the vegetation patterns in the present study area is very essential prior to designing the conservation strategies for effective management of existing biodiversity in the Kodaikanal hills. Therefore, it is highly imperative to have a detailed account on the various ecosystems in the study area based on voucher collections of lichens through extensive and intensive explorations. Hence, the primary objective of the study was to integrate lichen survey data with outside sources of climate conditions data. Similar lichen survey has not been attempted in recent years to comprehensively document the existing lichen flora of Kdoaikanal hills, which possesses unique climatic conditions in its wide range of natural habitats. The present study will not be a duplication of any on-going or existing projects.

Materials and Methods

Study Area

The Kodaikanal hills is located in Dindigul district, Western Ghats of Tamil Nadu, India at the geographic location of 10°14'17.21"N 77°29'21.06"E. The lichen biodiversity was recorded during 2016 July to October 2018. The height of hills is about 2130 meters. Moreover, of selected region climatic factors decided the specific kind of lichens to habituate on the particular region. Specimen samples were collected from different altitude across the hills: Mother Teresa University – 2100 m, Park view – 2130 m, Guna cave area – 2197 m, Mathiketancholai – 2230 m, Silent valley – 2300 m. Physiographically, Kodaikanal is a hilly region with elevation ranging from 1000 m at the foothill to 2343 m at solar observatory. Geologically, the study area occupied by the hills that slope down into Vilpatti and Pallani on North, Palani on the east, on the south slopes down into Cumbum valley and Manjampatti, Anamalai hills, Indira Gandhi National park on the west. The study site showed optimum humidity, temperature, wind breeze direction, annual precipitation, pollution free environment and altitude of height above 2000 m with abundant lichens. The habitat information of species level showed differences. The survey requirement in the expanded range gained support from Tribals, Hill Guard, forest conservation department guards and tools for lichens.

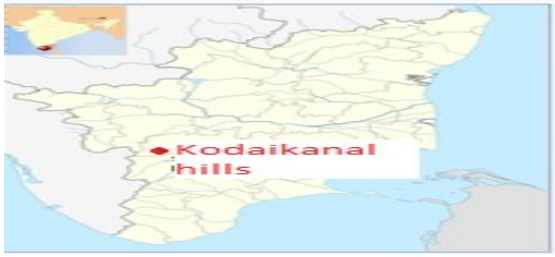
Plot design

Lichen species richness surveys were conducted on a circular, 0.20-ha plot centered on plot 1 (Moir point) in our plot

design. Similarly, we collected lichen species from all other three plots. Accordingly, plot 2 (Mother Teresa University), 3 (Guna cave) and 4 (Berijem lake) were about 13.6 km, 2 km and 12. 6 km away from Plot 1. The lichen survey was conducted by using belt transect method. The different localities of study area were laid with transects measuring 50 x 10 m and totally 25 transects were laid in each plots of the study area. Relative density of lichen species was investigated according to the procedure of Phillips[17]. The number of lichen species in the study area is represented as species diversity. Surveys lasted for 2 hours once in a month in each plot during which we collected a representative voucher specimen of all prevailing lichen species. The corticolous lichen specimenswere collected from the trunks of all trees, above 0.5 m from the surface, the terricolous lichen specimensattached with the soil surface were brought to lab as intact along with the substratum, and saxicolous lichens found firmly attached to rocks were collected with the help of hammer and chisel.

Identification of lichen samples collected from Kodaikanal hills

The representative lichen specimens were identified by studying their external morphology, reproductive structures, anatomical features, and chemical profile referred from the manual[10,18]. The map (Figure 1) shows the study area.



Coordinates :10°14'17.21"N, 77°29'21.06"E
Name of District and State: Dindugul, Tamil Nadu
Nearest Forest Dept office : Kodaikanal 1 km
Total Area :736.87 km^2
Height :2130 m above MSL
Average Rainfall : 4,617 mm
Temperature during Winter season and Summer season : 5.3 °C and 17 °C

Figure 1: Showing location Map

Identification of lichen substances

The lichen chemistry was analyzed according to Culberson and Kristinson[19]. The colour test was done on medulla of thallus using test reagents of potassium hydroxide (K), Calcium hypochlorite (C) and Paraphenylene di amine (PD). The lichen compounds extracted from thallus using solvent acetone by soxhalet method[20]. The extracts were applied directly to silica gel precoated TLC plate (Merck 60 F_{254}, Germany). The mobile phase was solvent system A, Toluene:1, 4, Dioxane: Acetic acid (180: 45: 5). The R_f class of unknown lichen secondary metabolite was matched with the standard reference chart[21] for the identification of the lichen compound. In 1936, Asahina was the first to develop microcrystallization[6] test procedure for tentative identification of lichen metabolite on a micro scale. The test has been extensively used for lichen chemical identification in connection with systematic work. The procedure that employs the controlled recrystallization of extracted lichen metabolite and identification of these crystals under compound microscope and can differentiate closely related lichen compounds more accurately. A few drops of each lichen extract were introduced on a glass slide and added crystallizing solvent mixture Glycerol: ethanol: water (GEW, 1:1:1). After gently heating of glass slide, crystals were appeared under 40 X lens of compound microscope (Olympus, bx 2 bright filed microscope, japan) and observed images were compared with previous literature[19,21–23].

The shape of the crystals appeared under 40 X objective lens of compound microscope were photographed. The specimens of a few species have been deposited in the herbarium of CSIR National Botanical Research Institute, Lucknow and Bharathiar University, Bio Medical Research Lab, Coimbatore. The diversity distribution of lichen species is presented in graphical representations using R program.

Survey analysis by Response surface methodology

The lichen diversity survey was done at three consecutive years including 2016, 2017 and 2018. The survey was conducted based on the sunshine, rainfall, temperature, relative humidity, evaporation rate and wind speed against lichen diversity. The meteorological source data was collected from India Meteorological Department(IMD). The survey result was analyzed by response surface methodology (Design Expert® 7.0).

Results and Discussion

The survey of lichen samples collected from five different sites of Kodaikanal resulted in identification of 21 species belonged to 13 genera (Table 1). The study area showed predominant number of (16) lichen species were corticolous which represented (76%) followed by 3 lichen species were saxicolous accounted 14%, one species was muscicolous with 5% and (Figure 2 A). One species *Parmotremareticulatum* which represented 5% showed specificity to two substrata was interesting to note on both the bark of the tree as well as on rocks. Two species such as *Graphis* spp., and*Bulbothrix* spp., and*Cladonia* spp. were identified up to genus level.

Lichen abundance has been generally used as a health indicator of forest ecosystem and the reasons for excess or less in number of lichen species are due to air pollution, forest fire, climatic conditions and plant diversity. The major vegetation in Kodaikanal hills comprises of Eucalyptus trees, Pine trees, *Azadirachtaindica*, Palm trees, *Prunus salcinia*, etc., In pine tree forest around Guna cave, the branches of pine tree barks (corticolous) were regularly colonized by *Parmotremareticulatum* while the bark of Eucalyptus tree was generally free from lichens. In one of earlier study[24]Tectona and Eucalyptus bark did not support the growth of lichens in Katarnigaht reserve forest. This was due to the flakiness of the bark. Nayaka *et al*.[24] reasoned that the exfoliating nature of Eucalyptus tree bark is one of the major reasons for their poor growth and establishment of lichens. This contrasts sharply with Neem tree (*Azadirachtaindica*) which facilitates a less appropriate substrate due to the unknown reasons of the bark. The variation in tree bark nature is one of the major reasons for such lack of lichen growth. The reason for heavy lichen growth of *Parmotremaaustrosinense* on the bark of *Prunus salcinia* was due to rough surface of its tree branches. The results of the present study are in agreement with the findings of Ramya and Thirunalasundari[16] who reported that foliose and fruticose lichens such as *Ramalinaconduplicans, R.subpusilla, R.sinensis, Usnea cineraria, Parmotremareticulatum, Canoparmeliatexana,* and*P.cristiferum*etc were luxuriantly present all over the *Prunus persica* tree branches and trunk in Kodaikanal hills. The pictorial representation of some of the lichens in the study area are presented in figure 3 and 4.

Primarily rock inhabiting lichen species are found to survive under intensely tough conditions of colonization. The lichen species *Parmotremadefectum* for example, are found growing on the chernokite rocks in blocks of building foundation, around Mother Teresa University, Kodaikanal. On the other hand, few species of *Graphis* spp*.,* colonized the rocks not on the vertical side, but of some regions, it was found exposed under direct sunlight. This indicates the conducive environment of tropical dry ever green and tropical semi evergreen forests present in Kodaikanal hills. The species on the rough sandy surfaces comprised a few lichen communities quite different from that on rough and fissured bark. It was found that *Parmotremareticulatum* not only colonized on bark of the tree, but also

Table 1 The list of different species of Lichen, its locality, latitude longitude with forms and chemistry.

Sl.No	Lichen	Altitude (m)	Family	Longitude	Bark/Rock	Location	Forms	Chemistry and spot test
1	*Bulbothrix*spp.	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Atranorin, Salazinic acid, K+ cortex
2	*Chrysothrixchlorina*	2300 m	Chrysothricaceae	10.188166 N , 77.425143 E	Corticolous	Bericham lake	Leprose	Vulpinic acid K-;C-KC-;PD -
3	*Cladonia*spp.	2230 m	Cladoniaceae	10.2107"N 77.46" E	Muscicolous	Madhiketancholai	Foliose	Psoromic acid, PD+
4	*Everniastrumcirrhatum*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Atranorin Salazinic acid, K+
5	*Graphis spp.*	2130 m	Graphidaceae	10°14'17.21"N 77°29'21.06"E	Saxicolous	Park view	Crustose	Norstictic acid, K+, PD+
6	*Heterodermiadiademata*	2197 m	Physciaceae	10.2107" N, 77.46" E	Corticolous	Guna Cave	Foliose	Zeorin, K+; PD+
7	*Heterodermialeucomelos*	2130 m	Physciaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Zeorin, Salazinic acid, K+
8	*Hypotrachynacostaricensis*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Atranorin, K+ Cortex
9	*Leptogiumcyanascens*	2130 m	Collemataceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	No substance

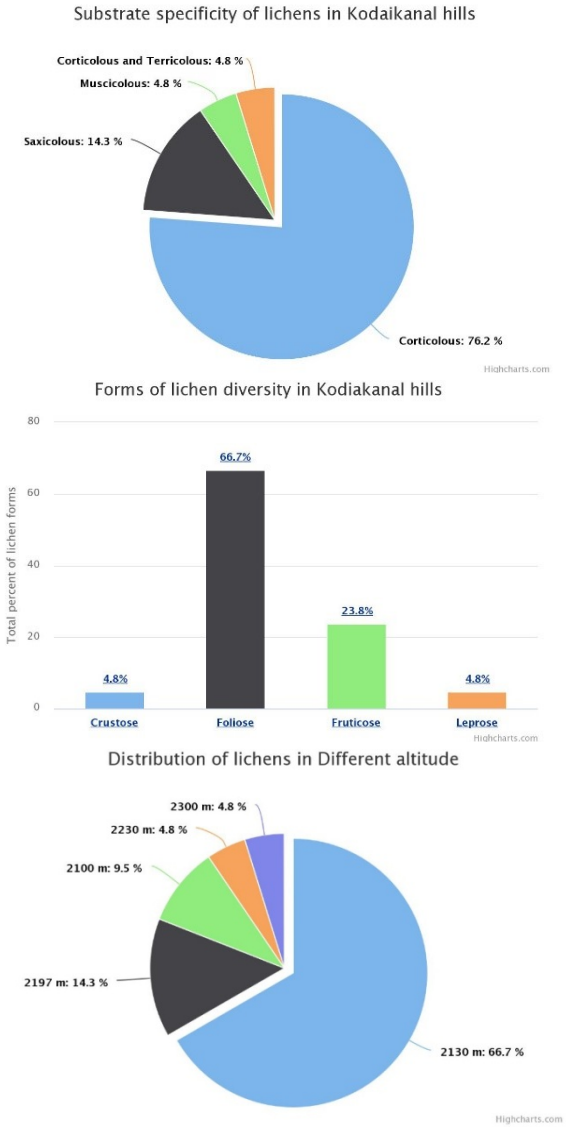
10	*Parmotremaaustrosinense*	2100 m	Parmeliaceae	10.188166”N 77.425143”E	Corticolous	Mother Terasa University	Foliose	Lecanoric acid, K+, KC+
11	*Parmotremadefectum*	2100 m	Parmeliaceae	10.188166”N 77.425144”E	Saxicolous	Mother Terasa University	Foliose	Lecanoric acid, K+, KC+
12	*Parmotremagrayanam*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Saxicolous	Park view	Foliose	Atranorin, K+
13	*Parmotremareticulatum*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous/ Terricolous	Pine forest	Foliose	Atranorin, Salazinic acid, K+
14	*Parmotremaravam*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Atranorin Protocetraric acid All Spot tests negative
15	*Parmotremastuppeum*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Salazinic, consalazinic acid, K+
16	*Parmotrematinctorum*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Lecanoric acid, K+, KC+
17	*Pseudocyphalariaaurata*	2130 m	Lobariaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Foliose	Triterpenoids
18	*Ramalinaconduplicans*	2130 m	Ramalinaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Fruticose lichen	SalazinicSekikaicacid, K+
19	*Teloschistes flavicans*	2197 m	Teloschistaceae	10.2107"N N, 77.46" E	Corticolous	Guna Cave	Fruticose lichen	Parietin, K+
20	*Usnea stigmatoides*	2130 m	Parmeliaceae	10°14'17.21"N 77°29'21.06"E	Corticolous	Park view	Fruticose lichen	Sticitc acid, K+, P+
21	*Usnea complanata*	2197 m	Parmeliaceae	10.2107"N N, 77.46" E	Corticolous	Guna Cave	Fruticose lichen	Usnic acid KC+, PD+

distributed it more diversely and made it prevalent to red sand areas. Instability of sand texture was the important factor for other lichens that failed to grow in the sand vegetation.

The most outstanding characteristic of lichens is their form diversity. Being comprised like a leathery texture the foliose lichens are more or less firmly attached to the support. Few examples of this type of lichen are *Parmotrema austrosinense*, *Dirinaria species*, *Heterodermia leucomelos* etc. The distribution of form diversity of lichen study suggested that the foliose lichens were found to be predominant to about 66.67% followed by fruticose (23.80%), crustose (4.76%), and leprose lichens (4.76%) (Figure 2 B).

Abundant growth of the lichens depends on latitude. The majority of lichen (66.7%) were observed to survive at 2130 m MSL park view in Kodaikanal hills. Figure 2 C showed that 14.3 per cent of lichen diversity were from Guna cave (2197 m MSL) and 9 per cent were from Mother Teressa University (2100 m MSL). The luxuriant growth of the leprose lichen *Chrysothrixchlorina* was recorded on *Acacia* spp., trees of the Berijem lake. The lowest point at which corticolous*P. austrosinense* and *P. stuppeum*lichen have been detected was at 2100 m around Mother Teresa University. The higher the altitude fewer were the species that occurred. Baral[25] confirmed similar observations having found lichens up to certain high elevation mostly with either foliose or fruticose forms. According to him the crustose forms prevail at low altitude. Similarly, in the high hills of Kodaikanal, the occurrence of foliose and fruticose lichen was most abundant at 2130 m followed 2197 m (Figure 2). Reason behind this is high altitude exceeding the optimum elevation, limits the survival of lichen species. Similar study was conducted by Bhat *et al.*[26]showed that the altitude ranges between 1000 – 2400 m was optimum for lichens of Western Ghats. Highest diversity was found in the park view.

Among diversified lichen families, the study showed that the Parmeliaceae was leading by 54.5% of total lichen species (Figure 2 D). The second largest collection of lichens was Physciaceae (9%). Taxonomically, well represented families include Chrysothricaceae, Cladoniaceae, Collemataceae, Graphidaceae, Lobariaceae, Ramalinaceae and Teloschistaceae represented 1 species each.



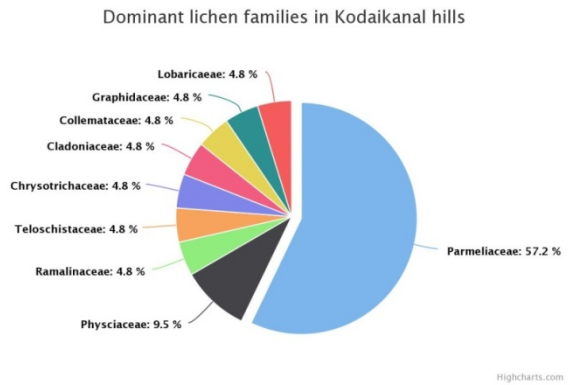
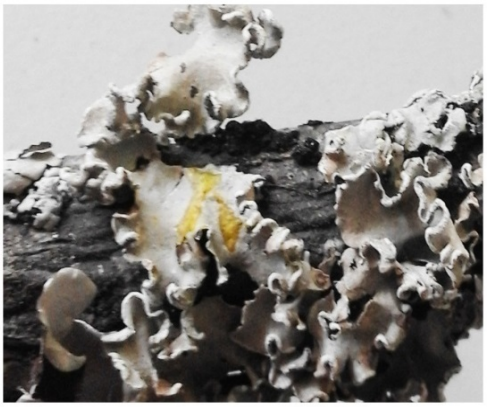


Figure 2: Diversity distribution of lichen species in Kodaikanal hills A) The substrate specificity of Lichens in Kodaikanal, B) Forms of Lichen diversity in Kodaikanal, C) Distribution of lichens in different altitude, D) Dominant Lichen families in Kodaikanal hills

The reasons for the markedly predominant lichen species richness belonged to Parmeliaceae family was due to the fact that it is the largest family classified with 79 genera and 2,726 species placed under 5 major clades such as Alectorioid, Cetrarioid, Hypogymnioid, Parmelioid and Usneoid. Similar result was observed by Thell*et al.*[27]. The foliose lichen genera belonging to family Parmeliaceae were *Bulbothrix sp., Everneastrum cirrhatum., Hypotrachyna costaricens, Parmotrema defectum, P.grayanum, P.reticulatum, P.tinctorum, P. austrosinense, P. stuppeum, P.ravum*and *Usnea stigmatoides and U. complanata,* In the high hills of Kodaikanal hills of Tamil Nadu the occurrence of Parmeliaceae family remains high. Results are in agreement with the findings of Thell*et al.*[27]. Photographs of lichens found in Kodaikanal region are presented in (Figures 3 and 4). The crystals of some selected lichen compounds were given in Figure 5. Test analysis of microcrystal and TLC results are in accordance with the findings of Parizadeh and Garampalli[28]. TLC identification test results are presented in Figure 6. Several authors described the TLC of lichen substances[19,21]. Lichen compounds were identified by their relative position and colour in TLC plate. Atranorin is found to be more prevalent in most of the lichen samples tested. Lecanoric acid was prevalent in *Parmotrema austrosinense*, *P.defectum* and *P.tinctorum*. Salazinic acid was reported from *Parmotrema reticulatum*, *P.stuppeum*, *Bulbothrix* spp., *Heterodermia leucomelos*, and *Everniastrum cirrhatum* etc. Renner and Gerstner[29] standardized the TLC technique of lichen substances. White and James[30] slightly modified the TLC method for the separation and identification of lichen compounds[31] Microcrystal test distinguished the lichen substances. Lecanoric acid showed clusters of long crystals in GEW solvent. Atranorin produced short crystals. Salazinic acid appeared fine crystals around spherical dark structures. Protocetraric acid produced brush like appearance. Usnic acid showed sharp edged golden yellowish long crystals. Earlier report showed that the form of crystals depends on the concentration of the lichen compound and type of solvent used[21]. For extensive work, more than one solvent such as GE (glycerol and glacial acetic acid 3:1), An (Anyline glycerol and ethanol, 1:2:2) can be used to show dissimilar form characteristics in GAE solvent. Schumm and Elix,[32] used HPTLC to identify lichen compounds.

The effect of sunshine, rainfall, temperature, relative humidity, evaporation rate and wind speed on lichen diversity was analyzed by Historical data using Design Expert (Table 2,4,6, Figure 7). The analysis shows that among the various contributing factors sunshine was found to be the major factor which has great impact on lichen diversity in the survey area. The year wise analysis was done.Table 3 shows the ANOVA result for the effect of independent variables for the year 2016. The Model F-value of 61.83 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A (Sunshine), C (Temperature), D (RH-

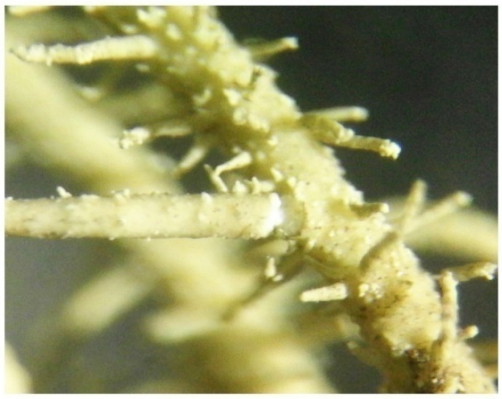
Teloschistes flavicans (Sw.) Norm.



Parmotrema austrosinense (Zahlbr.) Hale

Pseudocypellaria aurata (Sm. *ex* Ach.) Vain

Ramalina conduplicans Vain.



Usnea complanata (Mull. Arg.)Mot.

Usnea stigmatioides G. Awasthi

Figure 3: Pictorial representation of few lichens in the study area - I

Parmotrema reticulatum (Taylor) Choisy

Chrysothrix chlorina (Ach.) J.R. Laundon

Cladonia sps.

Graphis sps.

Heterodermia leucomela (L.) Poelt

Heterodermia diademata (Taylor) D. O. Awsthi

Figure 4 Pictorial representation of few lichens in the study area - II

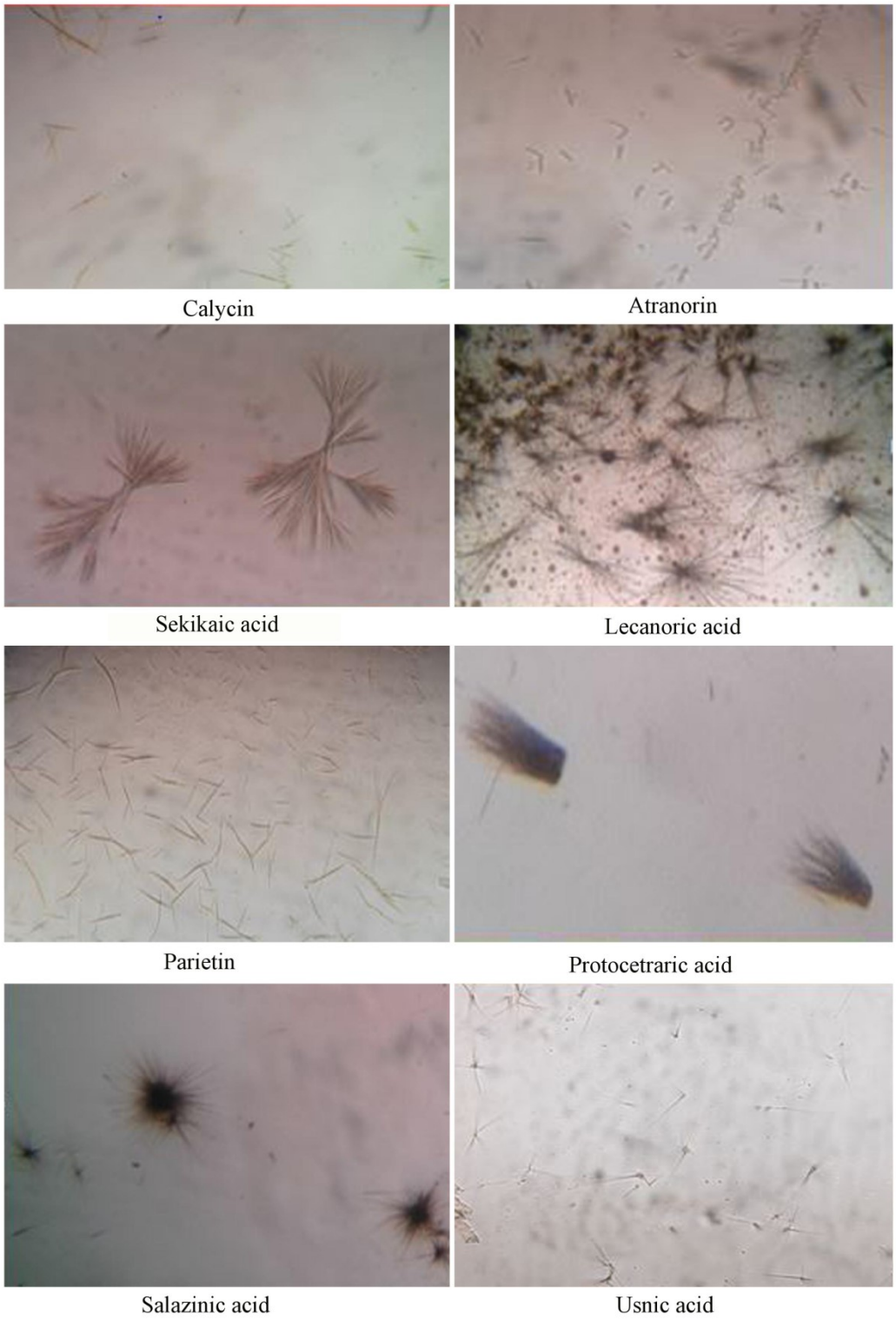


Figure 5 Micro crystallization of lichen compounds in GAE solvent

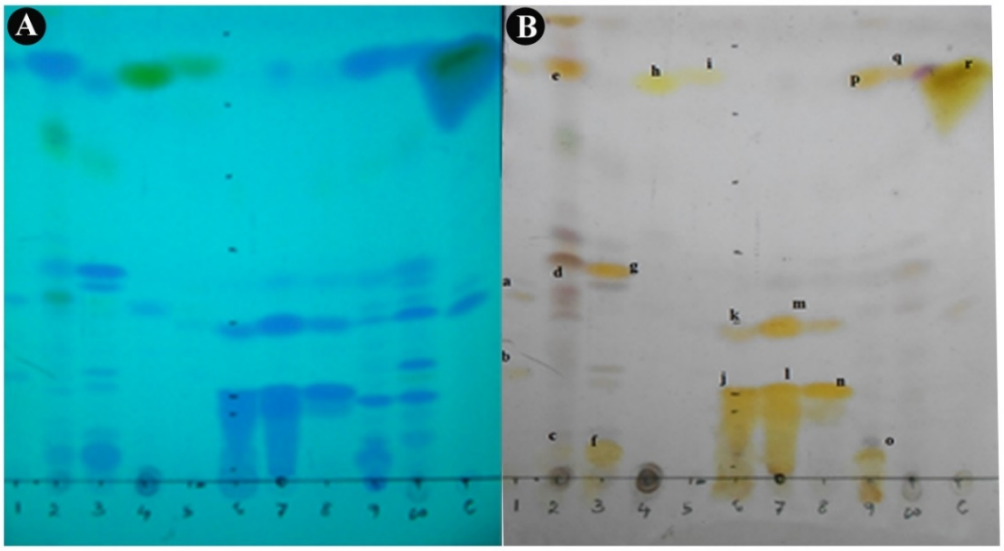


Figure 6 Thin layer chromatography profile of lichen species in solvent system.
A) TLC plate under UV before acid spray B) TLC plate after acid spray
1, *Pseudocyphellariaaurata.* with triterpenoids, (a), unknown (b), 2 *Heterodermialeucomela* with salazinic acid (c), Zeorin (d), atranorin, (e); 3, *Ramalinaconduplicans* with Salazinic acid (f), sekikaic acid (g); 4,*Chrysothrixchlorina* with vulpinic acid (h), 5, *Teloschistesflavicans* with parietin (i), 6, *P. austrosinense* with lecanoric acid (j) unknown (k); 7, *P. tinctorum* with lecanoric acid (l) unknown (m); 8, *P. defectum* with lecanoric acid (n); 9, *P. reticulatum* with salazinic acid (o), atranorin (p); 10, *P. nilgherrense* with atranorin (q); 11, C, control Usnic acid

Table 2 Screening of significant climatic factors with respect to lichen diversity - 2016

Months	A: Sunshine (hr)	B: Rainfall (mm)	C: Temperature (Deg.Cel)	D: RH (%)	E: Evaporation (mm)	F: Wind speed (km/h)	Lichen diversity (Nos.)
Jan	7.25	0	19	75	2.39	2	32
Feb	7.06	5	19.5	73	2.7	1	32
Mar	7.46	2	21	73	2.94	1	31
Apr	5.08	5	22	85	2.2	1	30
May	4.48	2	22.8	83	2.38	1	29
Jun	1.44	26	21	91	1.26	1	28
Jul	0.83	23	20.8	93	1.12	0	28
Aug	0.81	27	21	93	1.15	1	28
Sep	3.11	15	21	90	1.62	2	30
Oct	4.53	11	22	88	2	1	31
Nov	5.03	5	21	84	1.92	1	31
Dec	5.87	0.4	20	81	2.39	0.3	32

Table 3: ANOVA for Response Surface Reduced Linear Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	26.52285	3	8.840949	61.8346	< 0.0001
A-Sunshine	8.365553	1	8.365553	58.50963	< 0.0001
C-Temperature	2.91842	1	2.91842	20.41176	0.0020
D-RH	2.766806	1	2.766806	19.35135	0.0023
Residual	1.143819	8	0.142977		
Cor Total	27.66667	11			
R-Squared	0.9587				
Adj R-Squared	0.9432				
Pred R-Squared	0.8989				
Adeq Precision	21.056				

Table 4 :Screening significant climatic factors with respect to lichen diversity – 2017

Months	A:Sunshine (hr)	B:Rainfall (mm)	C:Temperature (Deg.Cel)	D:RH (%)	E:Evaporation (mm)	F:Wind speed (km/h)	Lichen diversity (Nos.)
Jan	7.98	0.02	18.3	76	2.2	2	35
Feb	7.98	0.814	20.15	71	2.7	1	34
Mar	6.84	0.86	21.8	74	2.8	1	32
Apr	5.27	5.66	22.5	56	1.8	1	31
May	4.23	5.58	22.4	86.5	1.7	1	31
Jun	1.66	17.53	20.95	90.5	1.3	0	30
Jul	0.49	18.34	20.75	90.5	1.2	0	31
Aug	1.97	21.43	20.3	91.5	1.3	0	31
Sep	3.21	9.8	20.7	88	1.5	0	32
Oct	4.41	9.65	21.3	86	1.6	1	32
Nov	6.73	3.42	19.75	83	1.8	1	33
Dec	6.22	0.084	19.6	76	2.2	1	35

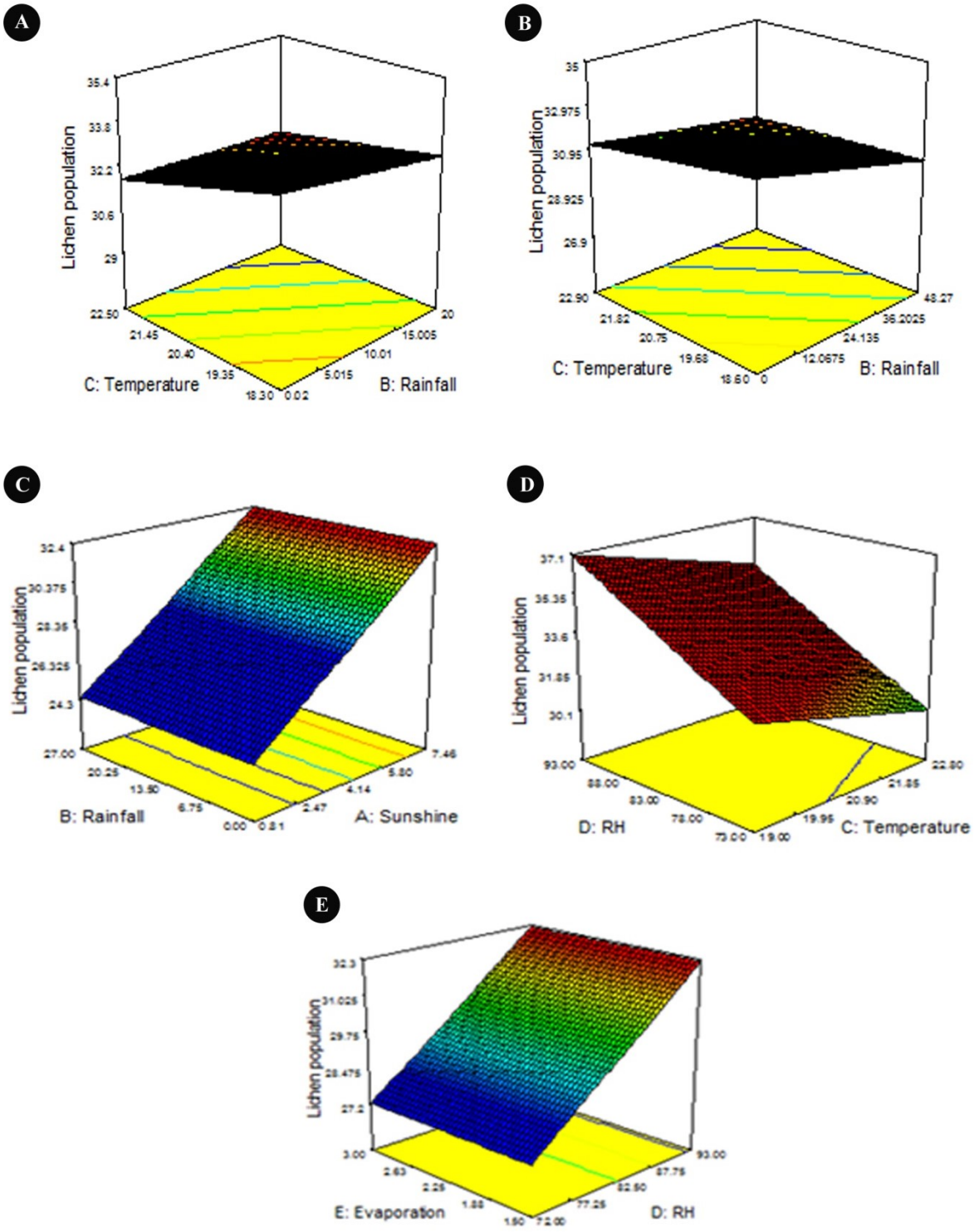


Figure 7 Surface plot of significant factors A) Temperature and Rainfall B) Temperature and Rainfall C) Sunshine D) Temperature E) Relative Humidity

Relative humidity) are significant model terms. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 21.056 indicates an adequate signal. This model can be used to navigate the design space. The "Pred R-Squared" of 0.8989 is in reasonable agreement with the "Adj R-Squared" of 0.9432.

Final Equation in Terms of Actual Factors:
Lichen diversity =16.82586 +1.20164 * Sunshine -0.57311 * Temperature +0.23823 * RH

Table 5 shows the ANOVA result for the effect of independent variables for the year 2017. The Model F-value of 56.95 implies the model is significant. In this case B (Rainfall) and C (Temperature) are significant model terms. The "Pred R-Squared" of 0.8728 is in reasonable agreement with the "Adj R-Squared" of 0.9105. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.747 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors:
Lichen diversity = +50.92137 -0.13690* Rainfall -0.85030 * Temperature

Table 7 shows the ANOVA result for the effect of independent variables for the year 2018. The Model F-value of 82.00 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Final Equation in Terms of Actual Factors:
Lichen diversity = +50.53854 -0.089869 * Rainfall -0.84017 * Temperature

According to Bidussi*et al*.[33] the occurrence of lichen species was controlled primarily by temperature, sunshine, rainfall and also subsequently by a combination of other physical factors such as the wind speed and relative humidity on availability of snow, affecting negatively on terricolous lichens. Schwierz*et al*.[34] reported that increase in temperature, wind speed and growth of vascular plants could depress lichens under the effect of climate change. The effect of increase in wind speed could cause warming effect on vascular plants and enable the growth of terricolous lichen[35].

The monthly temperature varied from 19 ºC in Jan to 22.8 ºC in May 2016. The monthly temperature varied from18.3 ºC in the month of January to 22.5 ºC in April and 18.6 in the month of Dec 2018 to 22.9 in April 2018. May was the hottest month in the year 2016 and April in 2018 and 2019.

The monthly rainfall varied from 0 in Jan to 26 in June 2016. The monthly rainfall varied from 0.02 in the month of Jan to 21.43 ºC in August and 0 in the month of Jan to 48.27 in June 2018. May was the hottest month in the year 2016 and April in 2018 and 2019. Highest maximum rainfall recorded for 3 years was 48.27 mm in June 2018 and no rainfall recorded was in January 2016. The total rainfall recorded during Jan to Dec 2016, 2017 and 2018 at Kodaikanal was 216 mm, 93.88 mm and 154.93 mm respectively. The result also implies that an increase in evaporation rate would reduce the richness of species. Accordingly, an increase in sun shine or relative humidity or other variables would not increase species diversity.

Table 5: ANOVA for Response Surface Reduced Linear Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	28.03	2	14.02	56.95	< 0.0001
B-Rainfall	11.68	1	11.68	47.46	< 0.0001
C-Temperature	11.25	1	11.25	45.72	< 0.0001
Residual	2.22	9	0.25		
Cor Total	30.25	11			
R-Squared	0.9268				
Adj R-Squared	0.9105				
Pred R-Squared	0.8728				
Adeq Precision	18.747				

Table 6: Screening significant climatic factors with respect to lichen diversity - 2018

Months	A:Sunshine (hr)	B:Rainfall (mm)	C:Temperature (Deg. Cel)	D:RH (%)	E:Evaporation (mm)	F:Wind speed (km/h)	Lichen diversity (Nos.)
Jan	7.72	0	18.8	73	2.4	1	34
Feb	7.7	2	20	73	2.4	1	34
Mar	5.7	3.14	21.4	79	2.2	1	32
Apr	9.99	4.88	22.9	82	2.2	1	31
May	3.21	1.85	22.85	83.5	1.9	1	31
Jun	0.41	48.27	20	90	1.6	1	29
Jul	0.17	38.03	19.6	90.4	1.2	0	31
Aug	0.84	26.63	19.6	89	1.5	0	32
Sep	2.74	18	19.8	87.5	2	0	32
Oct	4.31	7.45	20	82.5	2.2	1	33
Nov	6.7	4.52	19.9	84.5	1	1	34
Dec	6.33	0.16	18.6	80	2.3	1	35

Table 7: ANOVA for Response Surface Reduced Linear Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	30.96731	2	15.48366	82.00343	< 0.0001
B-Rainfall	22.65115	1	22.65115	119.9634	< 0.0001
C-Temperature	14.39054	1	14.39054	76.21414	< 0.0001
Residual	1.699355	9	0.188817		
Cor Total	32.66667	11			
R-Squared	0.947979				
Adj R-Squared	0.936419				
Pred R-Squared	0.901078				
Adeq Precision	25.314				

Relative humidity influenced lichen species richness similar to other variables. The highest maximum relative humidity of 93.0 %, 91.5 % and 90.4 % were recorded in 2016, 2017 and 2018 respectively.

Because lichen species richness was less, optimum conditions for diversity in numbers were identified at maximum temperatures below 20.15°C, 7.98 hr sun shine, 0.16 mm rainfall, below 2.3 mm evaporation and relative humidity below 80.0%. Effect of some climatic factor variables on diversity of lichen study was conducted to find out the optimum conditions such as 6.3 to 7.98 hr sun shine, 0.084 to 0.16 mm rainfall, 18.6 to 20.15 ℃ temperature, 76 to 80 % relative humidity, 2.2 to 2.3 mm evaporation rate, 1 to 2 km/hr wind speed and lichen species richness total number 35 for better growth of lichen.

Response surface methodology (RSM) was first used by Box and Wilson[36] and described the relationships between explanatory variables and response variables. The RSM helps to find an optimal response surface. RSM in the present study is used with 8 factors in 12 months. The abundance of various lichen species (crustose, foliose and fruticose) were examined under various factors (viz., Sunshine, Rainfall, Temperature, Relative Humidity, Evaporation, Wind speed, and lichen(diversity) population in various months viz., Jan, Feb, Mar, Apr, up to Dec. The results show that the models of RSM are statistically significant on the basis of values denoted as highest F and lowest P for all the models. This result indicates that climatic conditions play an important role in the lichen species diversity. ANOVA showed that the climatic variables have significant effect on lichen diversity. Padalia*et al*.[37] used the Generalized Linear Model (GLM) with Poisson distribution to correlate remote sense based environmental variables such as landscape, climate and topographic variables with vegetation richness and found that environmental

parameters such as elevation, moisture, plant species richness and canopy vegetation affect alpine richness in the Western Himalayan region. Chitale*et al.*[38] observed huge variation between vegetation indices and plant diversity in open canopy vegetation classes.

Conclusion

The present study reveals the occurrence of 21 lichen species which indicates the most commonly available lichen species of the study area and there is massive future scope for lichenological research. It is desirable to study shape of crystals using microcrystallization test to distinguish closely related lichen compounds of same Rf value, class and colour of the spot in TLC. The present study used response surface methodology and found that effect of sunshine had an impact on lichen diversity. The RSM model is one of the tools that can help researchers to investigate forest health in the model's geographic regions. It is concluded that climate change can repress lichen biodiversity.

Acknowledgement

The authors wish to acknowledge the UGC-SAP and DST-FIST, Government of India for their funding towards this project. Authors are immensely grateful to Dr. K.S. Vinayaka, Principal, Kumadvathi First Grade College, Shikaripura, Karnataka and Dr. G. Ayyappadasan KSR College of Technology, Tiruchengode, Tamil Nadu for their extensive support in lichen identification and authors wish to extend their sincere gratitude to Dr. Sanjeeva Nayaka, National Botanical Research Institute, Lucknow for his noble guidance in identification of lichen. Authors express their gratitude to Dr. A. Rajendran, Professor & Head, Department of Botany, Bharathiar University, Coimbatore for providing excellent help throughout this research work.

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